Author’s response to reviews

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

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Author's response to reviews:

Jann-Yuan Wang, Ph.D. (Reviewer 1):

Comment 1: The major limitation comes from the original data itself. It contains only isolate numbers, rather than case numbers. Presence of NTM in respiratory samples does not equal to NTM disease. In addition, increase in isolate numbers may probably due to multiple sampling during one's disease course. If treatment for NTM is not necessary, the patient's number of isolates tends to be larger than that in NTM patients whose NTM was successfully treated. However, the latter should be the one that doctors need to pay more attention to.

Response: The reviewer is correct, report/isolate does not equate to disease. However, the “report” unit was used because that was how the pre-existing data (at least in the U.S.) was reported. In order to make comparisons to the previous data, “like” had to be compared to “like” as much as possible. Patient data from the states has already been published (citation below). To look at broader overview of NTM epidemiology or pathogenesis in relation to time (e.g. decades), it was necessary for the author to use the number of reports as the quantifiable unit.
Comment 2: Is laboratory reporting for NTM obligatory in the four States?

Response: There are laws within these states that require the reporting of NTM positive cultures typically within two weeks of the confirmed result. These are laws at a state-level only. Note: as of 2016, the state of Ohio passed a law removing NTM as a reportable microorganism from Ohio’s reportable disease list.

Comment 3: The NTM rate seems different in the four States. Do the authors have any idea about that?

Response: The NTM rates are different in the four states. The states had different underlying demographics, hydrology, and climates. This paper does not allow for the statistical comparison across the rates. Yet, analysis of these states patient prevalence rates by time was performed and reported in the paper (Donohue and Wymer, 2016). Result of the analysis showed that there was no statistical significance between the patient rates observed for each state.

Comment 4: Line 146: The sentence is redundant because it has been described in Line 131-132.
Response: The sentence was removed.

Comment 5: Line 152-153 and Line 227: M. fortuitum rarely has clinical significance.

Response: Although M. fortuitum infections do not typically require treatment, M. fortuitum is identified in ATS/IDSA NTM document where it is listed as an NTM species of clinically relevance or significance. It is in this context that the term “significance” is being used. Text in lines 155-156 have been included to include context.

Comment 6: Some numbers in Table 2 are different from Figure 1. For example, MAC rate in 2014 is 8.53 in Figure 1 but 10.61 in Table 2.

Response: The error has been corrected.

Comment 7: M. chelonae-abscessus Group plus M. mucogenicum-phocaicum are combined in Figure 1 but separated in Table 2. Why?

Response: M. mucogenicum and M. phocacium were not recognized NTM species until 1995 and 2006, respectively. Prior to their current designation, they were known as “chelonae-like”. Therefore, data prior to 1996, as well as past identification strategies (e.g. Runyon’s or biochemical) typically grouped these isolates with M. cheloneae. To compare “like” to “like”, the 2014 data need to be combined these two groups to provide the most accurate comparison to the 1994 data set.
In Table 2 these species were separated because the data came from 2014 only and no comparisons to 1994 was made. Additionally, the M. mucogenicum species are now being identified separately in laboratory report.

Troels Lillebæk, MD, DMSc, DTM&H (Reviewer 3):

Comment 8: 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 nontuberculous mycobacterium. Science 2016; 354(6313):751-757.

Response: The Bryant JM et al. citation was added to the report and the text in line 62 was altered to include the new citation.

Comment 9: 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."

Response: The sentence in old L-75-76 has been adjusted in light of the reviewer’s comment. The paper has been reorganized to make it more relevant to an audience with clinical interests.
Comment 10: 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.

Response: The author understands the reviewer's comments. Text has been added in lines 88-91. The author does disclose in new Table 1 the techniques were considered valid by each State's health department in 2014.

Comment 11: 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 correctly, 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.

Response: In response to “If I understand correctly, you compare four states with total numbers for all of US. Can you justify this comparison?” The reviewer’s assumption that the comparison applied to the entire U.S. is incorrect. In the CDC’s 1999 document, NTM reports are reported by state and by year (1994-1996). The author extracted the 1994 data for each of the four states in this study; obtained the U.S. census populations for each state and made the projections for only the four states. This distinction is clearly stated in Lines 102-104.

Comment 12: 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 are 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

Response: An error was made. “40 years” should have been “20 years”. The adoption of the MGIT system in the 1990’s helped automate recovery of NTM from human specimens. Due to re-structuring this area of the paper, this text is no longer present in the paper.

Comment 13: 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.

Response: The 1999 Butler and Crawford et al. report did not identify the identification methods were used by each state. The author is not comfortable on speculating on which methods were “in use” for each of the states at the time.

Comment 14: 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
Response: In the U.S., the general public is not generally aware of NTM as a common cause of illnesses. NTM effects manifest themselves among the population in subtle ways as ailments affecting the elderly, or as a compounding of HIV and COPD. NTM are not like other bacteria (Legionella, E. coli or Listeria) that can cause acute outbreaks that can capture the attention of the media (TV/Radio/Newspaper). In the past forty-years there has not been an incidence that has caused the public to be more aware of NTMs and their potential impact on their daily lives. It has been the authors experience that public awareness tends to drive the most efforts to remedy causative conditions/circumstances. No text was added. Disclosers are already included in text lines 200-204.

The author agree that the reviewer’s concern is reasonable for the 1980 data. Therefore, comparisons to this dataset have been removed from the paper and Figure 2.

Comment 15: 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.

Response: Although it is possible to have more than one report per person, the reported 16.0 positive reports per 100,000 persons is consistent with the results from other studies based on laboratory reports such as Smith et al, 2015: 15.9 /100,000 persons or 13.7/100,000 persons excluding M. gordonae (North Carolina). Cassidy et al., 2009; 17.5/100,000 person (Oregon). The Smith et al. study examined the number of reports per person. It found that 94 % of all reports represented one person with 1 report. Only 4% of the laboratory reports came from individuals who had generated two reports. Since the possibility does exist that there was more than one report per person it could lead to over inflation of the prevalence rate. This fact is disclosed in text Lines 185-187.
However, the reality is that the 16.0 reports/100,000 persons probably is an underestimation of infection episodes in the U.S.

Comment 16: 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.

Response: Alteration in the text has been made new line 42 and line 132. Although, it would be nice to say something about disease, those assertions cannot be made in this paper. The quantifiable unit used report the way to examine NTM epidemiology in U.S because the CDC reports used it. In order to compare the present to the past the units for quantification have to be the same. Report does not indicate disease. A report can only indicate a respiratory concern by either a patient or doctor.

Although clinically, M. gordonae is irrelevant. It does have the second highest frequency of recovery from human specimens (for the reasons stated above by the reviewer). From an environmental perspective M. gordonae is not isolated often from water. I hope to at one point in these series of papers to examine both the clinical/environmental detection frequency.

Comment 17: 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.

Response: The author understands the reviewer’s perspective about “what does two times higher” really represents. It is the author stance that two time higher reflexes the number of reports of possible respiratory related concern, as stated in the text. The 16.0 reports/100,000 persons is not an overinflated number and it is in-line with other studies such as Smith et al. (15.9/100,000 person) and Cassidy et al. (17.5/100,000 persons). These values have been inserted into the text lines 185-187. The author discloses the other factors (in Lines 204-208) that could have influence the accounting. However, there is no way to quantify their impact because past reports (pre-existing data) did not include this information. No text was added.

Comment 18: 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)).

Response: The purpose of the paper was not to better quantify the prevalence of NTM diseases in the U.S. The reviewer is correct that by overlaying more stringent definition of either
microbiological infection and or disease would increase the ability to assess the levels of infection in the U.S. However, the point of this analysis study was to use laboratory reports (as they did 20 years ago) to see if the same NTM occurrence trend as describe by Adjemian et al. (2012) and Donohue et al. (2016) would be relevant over a lager time frame (20 years) using “report” as a quantifiable unit. No text was added.

In the Henkle et al. (2015) paper, 60% of MAC reports, 70% of abscessus/chelonae reports and 30% of fortuitum related reports meet the ATS/ISDA NTM microbiological definition. This data along with that from other studies (O’Brien et al. (1985), Winthrop et al. (2011), etc.) demonstrated that these species have a higher potential of progressing to a disease state than other NTM species (e.g. M. gordonae). Although these species do not affect human health equally, they are clinically significant as described by the ATS/IDSA document.

Comment 19: P9, L179-181: 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 the both the young and elderly population as well as the poor and the uninsured" How does state reports capture illness. In line with section just above, how do you know the reports reflect illness?

Response: The sentence has been re-written for better clarity new line 177-179.

Comment 20: P10, L185-188: You write "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.
Response: The data in the paper does support other research efforts that has shown an increase in NTM infection not only at a patient-level but also applying ATS/ISDA criteria; Cassidy et al. (2009), Winthrop et al. (2011), and Henkle et al. (2015). For these reasons, the author is confident in concluding an increase in NTM reporting.

Comment 21: P11, L 211-216: You write "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.

Response: Please see the Comment 20 response.

The report data only gives an indication that NTM illness are increasing in the U.S. However, with multiply lines of evidence (including the 4-state data) that demonstrate the same trend, it strengthens the assertions (made by many physicians) that NTM related infections are increasing among the U.S. population.

Brian Kendall (Reviewer 4):

Comment 22: line 80 "gage" instead of "gauge".
Response: The correction has been made.

Comment 23: use of the word "almost" in line 150

Response: The correction has been made.

Comment 24: The first paragraph in the background (starting line 49) is confusing and should be revised

Response: The text has been revised to achieve better clarity.

Comment 25: There are some repetitive sections, e.g. lines 146-147.

Response: The text in old line 146-147 has been removed.

Comment 26: The terms "media" and "medium" may be technically correct but are confusing to clinicians and laboratorians in this context. Suggest using alternative terms such as "source".

Response: Changes to the text have been made. The use of the terms “media” and “medium” has been changed to “source”.

Comment 27: The writing would benefit from editing by a coauthor with a clinical background. There are also two inaccurate statements.

Response: The author agrees, the author had the (revised) paper reviewed by a clinician who once worked for the Texas state TB laboratories.

Comment 28: Lines 71-73 describe NTM as "slow-growers"; true for some but many of the isolates described are categorized as "rapid-growers" (M. chelonae, M. abscessus, M. fortuitum).

Response 29: It was not the intent of the author to suggest that M. chelonae, M. abscessus and M. fortuitum are slow-growers. These species are rapid-growers. This text was removed during the revisions to the Introduction.

Comment 30: Line 109 reports no significant change in isolation procedures for NTM in the last 40 years. The increasing use of liquid media in the last 20 years has increased isolation rates and certainly has a significant impact on the primary finding of this manuscript.

Response: The reviewer is correct. However, due to re-structuring this area of the paper, this text is no longer present in the paper.

Rogerio Ruas (Reviewer 5):

Comment 31: Page 3, line 56: NTM's natural habitat…
Response: Correction to the text has been made.

Comment 32: Page 3, line 57: of all NTM isolates are pulmonary…

Response: Correction to the text has been made.

Comment 33: Page 4, line 75: suggests that exposure to these NTM species is potentially increasing

Response: Correction to the text has been made.

Comment 34: Page 5, line 110: not changed dramatically, unlike the procedures used for identification

Response: Correction to the text has been made.

Comment 35: Page 5, line 112: introduced in the 1990's were the methods…

Response: Correction to the text has been made.

Comment 36: Page 7, line 148: NTM reports have increased over
Response: Correction to the text has been made.

Comment 37: Page 9, line 189: why are the state's reports numbers lower than the numbers reported by Medicare part B? The reasons provided (state including more population and more than one specimen being collected) make us expect the inverse results - the state's total should be bigger.

Response: The reviewer has an interesting point. The author does not know the answer. The researchers that used Medicare part B extrapolated from only 5% of the data. We don’t know if that there was a bias in the sampling or if the underlying assumptions in the sample extraction did not hold true. Despite the limitations in these two different data sources, the outcome is in agreement with each other. The prevalence reported is also of the same order of magnitude as Stollo et al. (2016) who used Medicare part B data for their estimates.

Comment 38: Page 9, line 197: these results don't demonstrate, they suggest that NTMs have universal, yearly impact on human health in the U.S. and that the case load appears to be increasing.

Response: Due to reorganization and revision, this line is no long is present in the text.

Comment 39: Page 10, line 212: literature to support these statements Page 10, line 219 and page 11, line 231 contradict themselves: The species responsible for NTM illnesses has shifted moderately over time/The species of NTM causing most infections has not changed much over time.
Response: The text on old lines 231 has been removed.

Comment 40: Page 12, line 254: the line "The lack of seasonality suggests that routes of illness and or disease occur year-round and may not be influenced by seasonal changes in activities of the infected population" is better placed at the beginning of the paragraph when it is stated that "The lack of a seasonal affect is probably due to the fact that pulmonary NTM lung infection and/or diseases are not acute illnesses."

Response: The section on seasonality has been revised.

Comment 41: The phrase: "This mean that doctors and clinicians must be aware year-round to the possibility that a patient may have acquire an NTM infection" is not needed in my opinion.

Response: The sentence has been removed.

Ettie M. Lipner (Reviewer 6):

Comment 42: Figure 1 How was Percent Change calculated? It is unclear how the author arrived at the percent changes reported. This should be explained in more detail in the methods section. I tried to calculate the Percent Changes, but I wasn't able to come up with the same estimates. Additionally, the denominators that were used to calculate the Rate should be provided in this figure.
Response: The percent change was calculated based on the “rate” value normalized to 100,000 persons. The denominator was added to the figure legend.

Comment 43: Figure 2 also needs to be explained in more detail, or perhaps refer the reader to the tables where the numbers are coming from that determine how the rates are reported in this figure.

Response: The numbers are in the supplementary file sent with the manuscript.

Comment 44: Conclusion, Line 260

The sentence needs further justification. Why does identifying NTM species most frequently associated with disease help to identify likely sources of exposure?

Response: See the revised text (lines 257-261).

Comment 45: Background, Lines 64-65, This is an awkward sentence, as it is not clear what the ranges of percentages are referring to. M. avium 3 to 26% is a range of what? Same for M. abscessus.

Response: This sentence is no longer in the document.

Comment 46: Background, Line 71: "Additionally, NTMs are classified as slow growers…". Not all NTMs are classified as slow growers. Please consider rewriting as "NTMs that are classified as slow growers take 7 days…".
Response: The sentence has been removed, due to the re-focusing of the introduction.

Comment 47: Background, Line 77, Please delete the comma that falls in between "human-specimen" and "reports".

Response: The comma has been removed from line 77.

Comment 48: Historical Data Comparison, Line 110, Change "what has changed as the procedures" to "what has changed are the procedures"

Response: Correction to the text has been made.

Comment 49: Discussion, Line 180

Please delete the word "the" in the sentence and change to "State NTM reports capture illness in the both…” to "State NTM reports capture illness in both…”

Response: Correction to the text has been made.

Comment 50: Discussion, Line 232 Remove the "s" from "populations".

Response: Correction to the text has been made.
Comment 51: Discussion, Line 247 Please change "affect" to "effect".

Response: Correction to the text has been made.

Comment 52: Discussion, Line 253 Please change "file" to "filed".

Response: Correction to the text has been made.

Comment 53: Discussion, Line 256 Please change "This mean" to "This means".

Response: Correction to the text has been made.

Comment 54: Discussion, Line 257 Please change "a patient may have acquire" to "a patient may have acquired".

Response: Correction to the text has been made.

Takeshi Kinjo, M.D., Ph.D. (Reviewer 7)

Comment 55: Firstly, the author described that the aim of this study was "to determine if these trends by species were mirrored in human infections, …". I expected that the author compared
the frequency of NTM isolation by species between potable tap water and human samples, however, I cannot find any results in terms of this point in the result section. Thus, I think the hypothesis and results do not link at all.

Response: The introduction has been rewritten to better align the objectives and results L52-78.

Comment 56: The most weak point of this manuscript is the lack of clinical information. Since NTMs are ubiquitous bacteria, isolation of NTMs from human specimens does not always mean infection. (a)The author should not use "NTM illnesses (line 219)" and (b)"The species of NTM causing most infections (line 231)" because the data from four states contains contamination. (c) Additionally, the data is not distinguished by the organs of sample collection. Since isolation of NTMs from respiratory, skin, and other organs was mixed up in the data, the focus of study seems unclear.

Response: Unfortunately, laboratory reports in the U.S. do not contain clinical data.

(a) Text has been change in old L219, new line 211.

(b) This sentence has been removed.

(c) The author was not able to include this information (specimen type) because not all health departments had the time or resources to tabulate the results. Two of the four states identified sputum and bronchial washes as the specimen type most often positive for NTM. This is in-line with other studies that have used laboratory reports as their data source Smith et al. (2016) and Cassidy et al. (2009).

Comment 57: where samples were collected.
Response: The samples came from physicians located in hospitals, private practices, and institutional settings, etc.

Comment 58: organ of the sample collection

Response: The author was not able to include this information (specimen type) because not all health department had the time nor the resources to commit to tabulating these results. Two of the four states identified sputum and bronchial washes as the specimen types most often found positive for NTM. This is in-line with other studies that have used laboratory report as their data source.

Comment 59: data used in this study does not have contains samples author did not distinguish the mix-up data by organ used in this study was mixed up and does not distinguished by organs.

Response: The author does not have the type of data that would allow for the investigation of organ/specimen mix-up.