PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Global prevalence of nontuberculous mycobacteria in adults with non-cystic fibrosis bronchiectasis 2006-2021: a systematic review and meta-analysis
AUTHORS	Zhou, Yunchun; Mu, Wei; Zhang, Jihua; Wen, Shi Wu; Pakhale, Smita

VERSION 1 – REVIEW

REVIEWER	Masoud Shamaei Shahid Beheshti University of Medical Sciences, Chronic Respiratory Disease Research Center
REVIEW RETURNED	15-Sep-2021

GENERAL COMMENTS	Dear Editor, Thank you for inviting me to review this review article, I try to explain step by step my reason to reject the manuscript. This is valuable work, but not completed, I believe this team is not qualified for this review; it seems they are more statistics specialists; they need to add expert clinicians and microbiologists to this work. 1-ABSTRACT is well structured, according to the paper, but in conclusion or everywhere, when they say" The global prevalence of NTM in adults with non-CF bronchiectasis is 10%, which has been on the rise in recent years", "is" is not correct, it is just estimation. 2-INTRODUCTION is weak. They can't show the different issues which impact the prevalence of NTM, also the difference in global prevalence. They never talk about the impact of HIV, smoking, age-related condition in different settings. When they correctly discuss overestimation because of the registry/referral center, they forget that the diagnosis of NTM is complicated, it needs an expert team of clinicians, radiologists, and laboratory setting, so I am sure that all centers that they have included in their studies are referral centers, so they must consider this fact that this is the only estimation and we can compare it with the previous study. Also, they don't consider the difference between prevalence and incidence, with improvement in a long-term health facility and medical care, the prevalence absolutely has increased, but we must also talk about incidence, this is important. Considering, nowadays, the most center uses molecular methods for diagnosis the NTM, that is more sensitive, but it more overestimates the pathogens, also knowledge and concern about the NTM has increased, the molecular methods have improved, considering aged population, absolutely the prevalence has increased. So, there is not any new finding, any well-defined situation, and also the exclusion criteria for the centers are not well designed or perfect. 3-METHODS:

	They must talk a little about diagnosis, it is very important, the first as a reader I need to realize that they have enough knowledge and concern about diagnosis dilemma in NTM, the second, how much it has been impacted in the results of different centers, which center more report with molecular methods, or culture or is it any other methods like HPLC to confirm the results? 4-RESULTS: 4-1-AS I told previously, there is no impact of HIV, age adjusting, smoking history in the results. 4-2-They have excluded the co-infection of TB and NTB, but it is a significant part of this issue, so they must report this co-infection separately. 4-3-There is no result about mixed infection (isolation) of NTM or just single isolation of NTM 5-Discussion: 5-1-There is no important finding for this job, we already knew that prevalence is increasing, MAC is more common, and the author could not give us any explanation, discussion is really poor. 5-2-They can't explain or suggest why there is some difference in the geographical study, they didn't discuss or evaluate the impact of population age, smoking habits, HIV, and about availability of long-term health facilities for elderly people. 5-3-They must compare with the literature, but with respect, my sense is they want to show that previous job by Chu et al is very weak, and we are very accurate, they mention much time it, that paper doesn't need, they must more respect to other scientists. In general, I reject this manuscript, and I don't accept major revisions, because I believe they are not eligible for this job, also there is no important finding for publication in your valuable journal. With Respect, Masoud Shamaei, MD, PhD

REVIEWER	Rajesh Bhagat University of Mississippi, Pulmonary and Critical Care
REVIEW RETURNED	05-Nov-2021

GENERAL COMMENTS	Title: Global prevalence of nontuberculous mycobacteria in adults with non-cystic fibrosis bronchiectasis 2006-2020: a systematic review and meta-analysis. Zhou Y; Mu W; Zhang J et al Objective: Accurately estimate the global prevalence of NTM in adults with non-CF bronchiectasis from 2006-2020 and to determine the proportion of NTM species and subspecies in clinical patients General comments: The authors should be commended for looking occurrence of NTM in bronchiectasis. It is a timely paper. The field is about to gain wider recognition, it will be the focus of investigation since some academic societies now recommend screening for NTM in all patients with bronchiectasis. The manuscript is generally alright. However, needs to be edited and made more succinct. This will help sharpen the message. I think the major limitation of data is very limited data from Asia and Africa, as the authors have highlighted. Questions for the authors – they focused on data above 18 years of age. I am an adult pulmonologist (not a statistician) so quite comfortable with "above 18 years of age" but wonder: 1) How much data on the topic of the manuscript is there in children?

	2) Is it worth the time and effort to explore the data in children with this manuscript? Title: OK Abstract: OK Strengths and limitations: 1) I would suggest delete “provided by a previous study with patient data from 1990 to 2006” 2) This study provides a…. instead of double number of studies approx. 10 times more patients would suggest give the actual number of studies 3) OK Just want strengths and limitations to stay positive and focused on current work. The previous estimates and limitations will be in the discussion Introduction: is OK, it needs editing Methods and Data: Acceptable Results: - Liked Table 1 and limitations - Of course, referral centers will have higher prevalence I guess Conclusion is OK References: OK Tables and Forest Plot: OK No Ethical Concerns

REVIEWER	Stanton Glantz University of California, San Francisco, Center for Tobacco Control Research and Education
REVIEW RETURNED	18-Dec-2021

GENERAL COMMENTS	My review is limited to the statistical aspects of this paper. Report p values to no more than three decimal places and statistics to 2-3 significant digits. Reporting more decimal places implies greater precision than is present in the results. Indicate what do the asterisks and boldface type in Table 1 indicate in the table itself. They appear to be the studies the authors identified as “outliers” that were excluded from the analysis. Since they were excluded, it would probably be clearer to segregate them into a separate section in the table. The sensitivity (subgroup) analyses should include formal tests for the hypothesis of no difference. This can be done using a meta-regression with a dummy variable to identify the different subgroups. (All the authors do not is qualitatively compare the point estimates and 95% CIs.) The justification for pooling the American and African data in the geographic analysis (section 3.2) is weak. The difference in prevalence between the prospective and retrospective analyses (section 3.3.) is weak and probably not anywhere near statistically significant. The authors need to do a formal test of this difference and revise the text accordingly. How was statistical significance assessed in section 3.4 (method of specimen assessment)? Page 19, line 10 (and Table 3): This is another place where the

	results are reported to too many significant digits. The figures report pooled estimates for fixed effects models. The methods say only random effects models were used, which is appropriate. The fixed effects results should be deleted from the figures. The scales on the Forest plots needs to extend down to include the estimates for the low prevalence estimates.

VERSION 1 – AUTHOR RESPONSE

Responses to Reviewer 1

1. ABSTRACT is well structured, according to the paper, but in conclusion or everywhere, when they say" The global prevalence of NTM in adults with non-CF bronchiectasis is 10%, which has been on the rise in recent years", "is" is not correct, it is just estimation.

Response: We agree with the reviewer and have rephrased the sentence as "The global prevalence of NTM in adults with non-CF bronchiectasis from 2006 to 2021 was estimated to be approximately 10%".

2. INTRODUCION is weak. They can't show the different issues which impact the prevalence of NTM, also the difference in global prevalence. They never talk about the impact of HIV, smoking, age-related condition in different settings. When they correctly discuss overestimation because of the registry/referral center, they forget that the diagnosis of NTM is complicated, it needs an expert team of clinicians, radiologists, and laboratory setting, so I am sure that all centers that they have included in their studies are referral centers, so they must consider this fact that this is the only estimation and we can compare it with the previous study. Also, they don't consider the difference between prevalence and incidence, with improvement in a long-term health facility and medical care, the prevalence absolutely has increased, but we must also talk about incidence, this is important. Considering, nowadays, the most center uses molecular methods for diagnosis the NTM, that is more sensitive, but it more overestimates the pathogens, also knowledge and concern about the NTM has increased, the molecular methods have improved, considering aged population, absolutely the prevalence has increased. So, there is not any new finding, any well-defined situation, and also the exclusion criteria for the centers are not well designed or perfect.
Response: In the revised manuscript, we have added information to significantly strengthen the introduction section.

1) We added the impacts of several factors, including age, sex, cigarette smoking, HIV infection, low socioeconomic status, and underlying health conditions, to the susceptibility to NTM infection.

2) Unequivocally, just as the peer reviewer mentioned, the diagnosis of NTM is complicated and it needs an expert team of clinicians, radiologists, and laboratory setting. However, we believe that: **a)** the data reported in the literature are peer reviewed results with scientific significance, and they are valid since there were publicly reported for the references of other researchers. Thus, we can synthesize the eligible studies to provide readers a comprehensive concept on the prevalence of NTM in adults with non-CF bronchiectasis from 2006 to 2021. **b)** We noticed that the clinical patients in many studies were not randomly selected, such as those from registry/referral centers, inevitably

resulting in overestimation and thus, we excluded 10 studies for an accurate estimation; whereas the clinical patients in the other 14 studies were randomly selected, and that may truly reflect the prevalence of NTM in adults with non-CF bronchiectasis in the population. Fortunately, the science community has developed rules such as the Cochrane Collaboration's tool to discreetly evaluate the risk of bias (Supplementary Table S3: Ratings of the quality of the evidence and Risk of Bias assessment). Therefore, we could exclude those studies with selection/source bias, making an accurate estimation possible. **c)** In the revised manuscript, we did consider the difference between prevalence and incidence. We noticed that prevalence is the measure of a condition in a population at a given point in time; whereas incidence is the rate of new cases in a population over a particular period of time. In this article, the goal is to estimate the prevalence. Therefore, we discussed this issue in section 2.2 and excluded a study specifically on incidence (Kwak N, et al. BMC Pulm Med 2020; 20: 293). **d)** we strengthened the introduction section by adding related references on the impacts of detection methods to the increasing trends. Moreover, in Table 2, we added a column specific on detection methods, including PCR and MBC. Unfortunately, due to the selection criteria associated with the objective of this study, there was only one eligible study on PCR with a small sample size, making the comparison between PCR and MBC impossible. We believe that the comparison between PCR and MBC is an interesting topic, and it is better to make selection criteria specifically focusing on comparison between detection methods, regardless of diseases. By this way, more data on PCR could be searched and included for a good comparison. However, when the topic is defined as the prevalence of NTM in adults with non-CF bronchiectasis from 2006 to 2021, only one study used PCR and it is not appropriate to draw a conclusion for the comparison between methods.

3) As per new findings, many clinicians have noticed the increasing trend of the prevalence of NTM, but it has never been quantitated. The only available reference on this issue was Chu's study in 2014. However, in the study by Chu et al, all clinical data was collected prior to 2006, which could not reflect the trend in most recent years. Thus, we believe that it is significant to update this information. In this study, we quantitated the percentage to reflect the increasing trend. Moreover, our in-depth investigation found that the prevalence of NTM before 2006 estimated by Chu et al in 2014 was in fact only 5%, and this percentage was found to have been increased to ~10% during the past 15 years in our study. Furthermore, the most common subspecies were also significantly changed. We believe that our results could provide clinicians a comprehensive and quantitative concept on the issue, thereby guiding clinical treatments and monitoring prognosis.

4) As per new well-defined situation, compared to Chu's study in 2014, in which all the clinical data were between 1990 and 2006, the clinical data in our study were from 2006 to 2021. Therefore, our study provided an updated estimate of the NTM prevalence in adults with non-cystic fibrosis bronchiectasis. Therefore, there were no data and time overlaps between our study and Chu's study in 2014. Moreover, within the same amount of time, the number of studies tripled from 8 to 24, indicating an increasing interest in the prevalence of NTM in patients with non-CF bronchiectasis during the past 15 years.

5) As per the exclusion criteria for the referral centers, many professionals in this field, such as Shteinberg and Aksamit, believed that the estimates of NTM prevalence from bronchiectasis referral centers were exaggerated, and including studies from medical/registry/referral centers would inevitably overestimate the prevalence of NTM. Therefore, these studies should be excluded (Shteinberg, et al. Upper airway involvement in bronchiectasis is marked by early onset and allergic features. ERS Monogr 2018;4 (1); Aksamit, et al. United states bronchiectasis registry longitudinal follow up at two years. Am J Respir Crit care Med Conf Am Thorac Soc Int Conf ATS; 2017;195).

Whereas for other professionals, such as this reviewer, may believe that the estimate should include all studies, including those from medical/registry/ referral centers. To deal with this controversy, we provided two sets of estimations for the references of clinicians in this study. One set of estimation was without taking the source bias into consideration. Chu's study in 2014 estimated that the prevalence of NTM from 1990 to 2006 was 9.3%. In contrast, our initial analysis estimated that the prevalence of NTM from 2006 to 2021 was 13.76%, suggesting an increasing trend of NTM infections in patients with non-CF bronchiectasis. Another set of estimation was with taking the source bias into consideration, we re-calculated the prevalence of NTM in adult with bronchiectasis from 1990 to 2006 in Chu's study in 2014, and it was modified to be 5% (Supplementary Figure S1). In contrast, our study estimated that the global prevalence of NTM from 2006 to 2021 was approximately 10%, also suggesting the increasing trend, but the percentage increased from 48% (increased (13.76%-9.3%)/9.3% x 100% = 48%) to 95% (9.75%-5%)/5% x 100% = 95%), suggesting a more prominent increasing trend once the source bias was considered.

3. METHOD: They must talk a little about diagnosis, it is very important, the first as a reader I need to realize that they have enough knowledge and concern about diagnosis dilemma in NTM, the second, how much it has been impacted in the results of different centers, which center more report with molecular methods, or culture or is it any other methods like HPLC to confirm the results?

Response: We agree with the reviewer that the diagnosis is important in NTM. Since this is a systematic review and meta-analysis, we have to follow the Prisma 2020 Checklist and thus the method section has to focus on how the data was searched, selected, and analyzed. As per how much it has been impacted on the results of different centers, it is visible in Figure 2 during our initial analysis, and we added section 2.2 to exclusively discuss this issue. As per molecular methods, due to the selection criteria associated with the specific objective of this study, there was only one eligible study on PCR with a small sample size, making the comparison between PCR and MBC impossible. We have disclosed this limitation in the discussion section. As per other the methods to confirm the results, in the revised manuscript, we added a paragraph to discuss the impact of diagnosis in the strengths and limitations section: The American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) Statement (2007) recommended that NTM positive culture results should be from at least two separate expectorated sputum samples. Accordingly, we performed a statistics calculation on all the included 14 studies (Page 19-20), to highlight the limitation of current studies on the NTM detections.

4. As I told previously, there is no impact of HIV, age adjusting, smoking history in the results.

Response: We believe that many factors, including HIV, age and sex, smoking, may impact the prevalence of NTM infection. However, this study was an overall estimate of the prevalence of NTM infection, regardless of underlying health condition except TB infection. We have added this information to the results section 2.3.

5. They have excluded the co-infection of TB and NTB, but it is a significant part of this issue, so they must report this co-infection separately.

Response: Our position is that the impact of TB co-infection is overwhelming, to the consequence of bronchiectasis, detection methods, diagnosis, and even the prognosis. Thus, the topic associated with TB infection should be studied separately. Metaphorically, one of the consequences of HIV infection is that the patients are vulnerable to cancers. If the cancer patient was found to have HIV infections simultaneously, the diagnosis, pathogenesis, facilities for detection, therapy, and the prognosis including severity has to be changed, resulting in completely different studies. Similarly,

bronchiectasis might be a consequence of post-infections, including TB infection. However, the bronchiectasis with TB infection has its own pathogenesis, detection methods, diagnosis, therapy, and prognosis, which are quite different from the non-CF bronchiectasis we mentioned herein. Therefore, in this study, we excluded all the studies on the co-infection of TB and NTB, but focused exclusively on non-CF bronchiectasis patients with NTM infections.

6. There is no result about mixed infection (isolation) of NTM or just single isolation of NTM

Response: The information on mixed infections and single isolation of NTM were discussed with details in section 4. Moreover, the interplay between subspecies in mixed infection was very complicated and can be studied independently. We believe that the most common subspecies of NTM in the population is more clinically significant and thus, we summarized the frequency of each subspecies in the included 14 studies into Table 3 in the manuscript.

7. DISCUSSION: There is no important finding for this job, we already knew that prevalence is increasing, MAC is more common, and the author could not give us any explanation, discussion is really poor.

Response: Although most clinicians have already known that prevalence is increasing, and MAC is more common than before, this information has never been quantitated and expressed explicitly before our study. In the revised manuscript, we summarized 3 important findings to justify this study: **a)** this study provided an updated estimate of the NTM prevalence in adults with non-cystic fibrosis bronchiectasis with two quantitative results. Without taking the source bias into consideration, Chu's study in 2014 estimated that the prevalence of NTM from 1990 to 2006 was 9.3%. In contrast, our initial analysis estimated that the prevalence of NTM from 1990 to 2006 was 13.76%, suggesting an increasing trend of NTM infections in patients with non-CF bronchiectasis (increased (13.76%-9.3%)/9.3% x 100% = 48%). If we took the source bias into consideration, the prevalence of NTM from 1990 to 2006 should be adjusted to be 5%; and the global prevalence of NTM from 2006 to 2021 was 9.75% (increased (9.75%-5%)/5% x 100% = 95%), suggesting once the source bias was considered, the increasing trend was more prominent (48% vs 95%). **b)** We discovered that from 2006 to 2021 the three most prevalent NTM subspecies have changed to be MAC, M. simiae, and M. gordonae. We quantitated the percentage on how common these subspecies were, and this information is an essential reference for clinical doctors. **c)** We explored the factors which may cause the overestimation of the prevalence of NTM in adults with non-cystic fibrosis bronchiectasis. Thus, when the other researchers are updating the estimates in the future, based on our analysis, they may take more factors into consideration to provide a more accurate estimation, just as we take more factors into consideration in this most recent study than Chu's study in 2014 to provide a more accurate estimation. **d)** We significantly strengthened the discussion section as indicated in "Comparison with literature".

8. They can't explain or suggest why there is some difference in the geographical study, they didn't discuss or evaluate the impact of population age, smoking habits, HIV, and about availability of long-term health facilities for elderly people.

Response: As per why there is some difference in the geographical study, it is a big scientific question and currently remained elusive. We have mentioned this in the introduction, and emphasized that further studies on regional differences, such as climate, environments, genetics, and cultures, may

help to understand the pathogenesis of NTM infection in adults with non-CF bronchiectasis in the result section. The objective of this study is to provide an overall estimate of the global prevalence of NTM in adults with non-cystic fibrosis bronchiectasis, regardless of age, smoking, and other underlying health conditions except TB infections. As per the impacts of population age, smoking habits, HIV, and about availability of long-term health facilities for elderly people, each of the topics can be studied as a separate paper. We believe that other researchers may focus on each of these interesting topics and gain unprecedented insights into the pathogenesis of NTM infections.

9. They must compare with the literature, but with respect, my sense is they want to show that previous job by Chu et al is very weak, and we are very accurate, they mention much time it, that paper doesn't need, they must more respect to other scientists.

Response: Just as the peer reviewer requested, we have significantly strengthened the 'Comparison with literature' section. This study is based on Chu's study in 2014, and the objective of our study is to provide an update after 15 years. Moreover, compared to Chu's study in 2014, we improved the study on the same topic by taking source bias into consideration. We hope that in the future, other researchers may update this study and take into considerations on factors that our study has not been able to consider. We believe that scientific results were not static, but will progress with ongoing updates and improvements.

Responses to Reviewer 2

I think the major limitation of data is very limited data from Asia and Africa, as the authors have highlighted. Questions for the authors – they focused on data above 18 years of age. I am an adult pulmonologist (not a statistician) so quite comfortable with "above 18 years of age" but wonder:

1) How much data on the topic of the manuscript is there in children?

Response: NTM is rarely isolated in children and adolescence with Non-CF bronchiectasis (Chang, et al. European Respiratory Society guidelines for the management of children and adolescents with bronchiectasis. Eur Respir J 2021; 58: 2002990), especially in those under 15 years old (Pierre-Audigier, et al. Age-Related Prevalence and Distribution of Nontuberculous Mycobacterial Species among Patients with Cystic Fibrosis. J Clin Microbiol 2005; 43:3467–70). Therefore, this study focused exclusively on the adult population. This reasons for including only adults have been added to the first paragraph of the introduction section in the revised manuscript (Page 4 in red).

2) Is it worth the time and effort to explore the data in children with this manuscript?

Response: In the revised version, we explained why our study is focused on adults (Page 4 in red).

Strengths and limitations:

1. I would suggest delete “provided by a previous study with patient data from 1990 to 2006”

Response: We agree with the reviewer and have deleted this sentence.

2. This study provides a…. instead of double number of studies approx. 10 times more patients would suggest give the actual number of studies

Response: We agree with the reviewer and have deleted this sentence. Instead, we have provided the actual number of included studies (14 studies) and patients (21,056 patients) in the results section.

3. OK Just want strengths and limitations to stay positive and focused on current work. The previous estimates and limitations will be in the discussion.

Response: We agree with the reviewer and in this revised manuscript, we stayed positive and focused more on current work. In addition, we moved the previous estimates and its limitations into the discussion section.

4. Introduction: is OK, it needs editing.

Response: Thank you for the comments. We significantly edited the introduction section.

5. Results: - Liked Table 1 and limitations - Of course, referral centers will have higher prevalence. I guess. No Ethical Concerns.

Response: Thank you for the comments.

Responses to Reviewer 3

1. Report p values to no more than three decimal places and statistics to 2-3 significant digits. Reporting more decimal places implies greater precision than is present in the results.

Response: We agree with the reviewer and have updated manuscript with more decimal places in p values and statistics.

2. Page 19, line 10 (and Table 3): This is another place where the results are reported to too many significant digits.

Response: Thanks for your comments. The p values have been reported to 3 significant digits in the revised manuscript.

3. Indicate what do the asterisks and boldface type in Table 1 indicate in the table itself. They appear to be the studies the authors identified as “outliers” that were excluded from the analysis. Since they were excluded, it would probably be clearer to segregate them into a separate section in the table.

Response: In the revised manuscript, no outlier was excluded. As a result, asterisks and boldface type were no longer needed. To make the manuscript more concise, we moved Table 1 to Supplementary File 2 Table S2, which included 24 studies after our updates. It is noteworthy to mention that, according to the editor’s advice, we re-reviewed the latest data, and 24 studies (vs 15 studies in the previous manuscript) were included in the initial estimation, and 10 studies (vs 4 studies in the previous manuscript) were excluded due to source bias. All these information was provided in the Supplementary Table S2, and the excluded 10 studies were highlighted with asterisks and boldface type with a clear note in the supplementary table legend.

4. The sensitivity (subgroup) analyses should include formal tests for the hypothesis of no difference. This can be done using a meta-regression with a dummy variable to identify the different subgroups. (All the authors do not is qualitatively compare the point estimates and 95% CIs.) The justification for pooling the American and African data in the geographic analysis (section 3.2) is weak. The difference in prevalence between the prospective and retrospective analyses (section 3.3.) is weak and probably not anywhere near statistically significant. The authors need to do a formal test of this difference and revise the text accordingly.
Response: We agree with the reviewer. We have performed meta-regression analyses as suggested to assess potential moderators for further subgroup analysis. The texts have been revised accordingly. According to the results of meta-regression analyses, in the revised manuscript, a subgroup analysis was conducted by stratifying studies into East Asian countries and other countries.

5. How was statistical significance assessed in section 3.4 (method of specimen assessment)?

Response: After performing meta-regression analyses to assess potential moderators for further subgroup analysis as the reviewer suggested, we found that test of moderators p=0.802, as a result, the moderating variables of the method of NTM specimen examination failed to explain the heterogeneity among studies. As a result, we delete this section.

6. The figures report pooled estimates for fixed effects models. The methods say only random effects models were used, which is appropriate. The fixed effects results should be deleted from the figures.

Response: We agree with the reviewer and have deleted the fixed effect results.

7. The scales on the Forest plots needs to extend down to include the estimates for the low

prevalence estimates.

Response: We agree with the reviewer and have extended the scale of the forest plots.

VERSION 2 – REVIEW

REVIEWER	Stanton Glantz University of California, San Francisco, Center for Tobacco Control Research and Education
REVIEW RETURNED	19-Apr-2022

GENERAL COMMENTS	No further comments