PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form ([http://bmjopen.bmj.com/site/about/resources/checklist.pdf](http://bmjopen.bmj.com/site/about/resources/checklist.pdf)) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

| TITLE (PROVISIONAL) | Prevalence of atypical pathogens in patients with severe pneumonia: a systematic review and meta-analysis |
| AUTHOR(S) | Wang, Sidan; Tang, Jiaoqi; Tan, Yurong; Song, Zhi; Qin, Ling |

VERSION 1 – REVIEW

| REVIEWER | Kitsios, Georgios |
| University of Pittsburgh Medical Center |
| REVIEW RETURNED | 09-Oct-2022 |

GENERAL COMMENTS

This is an important clinical question with vast amounts of primary data, thus amenable to systematic review and meta-analysis, where appropriate. The authors have conducted thorough review of the literature and employed appropriate synthesis methods in their meta-analysis. However, I have a series of comments/criticisms which need to be addressed before the results of this report can be adopted on face value.

1. Search strategy time limitation: systematic search was conducted through Sep 2019. this is already 3 years old and has missed the large wave of the literature during the pandemic examining atypical pathogen co-infections in the context of covid-19. I understand that this clinical context differs from the primary objective of the literature through 2019, but I do not think that the authors excluded any studies (or that the primary studies did so) if there was co-infection either. Unless there is a scientific reason to justify the limitation to 2019, the search needs to be updated.

2. Selection of studies: the patient population selection is rather confusing and conflicting with subsequent analyses. Why exclude elderly, post-transplant or mechanically ventilated patients when these represent the most seriously ill patients with severe pneumonia (the scope of this review)? Furthermore, the authors then perform a sensitivity analysis of studies including vs. excluding immunocompromised patients, when by design they had already excluded the immunosuppressed studies upfront. This comparison is thus ill defined, and an appropriate comparison should include the studies in immunocompromised populations.

3. Exclusion of studies that did not report on mycoplasma, chlamydia or legionella separately. I think that pooled estimates on combined atypical pathogen prevalence are still important, and would consider a pooled analysis of all 3 pathogens, including these studies.

4. The decision on quality assessment is rather atypical, when there are consensus and established tools for methodological quality assessment in observational studies, e.g. from Cochrane.

5. The most important variable with regards to atypical pathogen prevalence is the diagnostic method used for detection in my view.
This variable has not been factored into the analysis, although reported in Table 1, the sensitivity/specificity performance of serology vs. PCR vs. culture tests is markedly different, and we expect higher detection rates in studies using PCR methods, especially with the use of sensitive panels in the market in recent years (e.g. Filmarray). This is an important piece of the analysis that needs to be included.

6. Analyses of severe vs. non-severe pneumonia cannot provide reliable estimates when most severe cases of pneumonia have been excluded by the PICOD criteria.

7. I am concerned about missed studies from the literature review or study selection. Another meta-analysis for Legionella pneumonia only included 219 studies. 

8. Several of the studies in Table 1 included HAP cases. The results then do not necessarily apply to CAP, and if the authors aim to report estimates on severe pneumonia, then their PICOD strategy needs to be revised.

REVIEWER
Messika, Jonathan
AP-HP, Service de Réanimation Médico-Chirurgicale

REVIEW RETURNED
10-Oct-2022

GENERAL COMMENTS
I read with interest this systematic review on the prevalence of what the author call "atypical pathogens" in severe pneumonia.
In this systematic review, the authors determine the prevalence of Mycoplasma, Chlamydia and Legionella in severe pneumonia. After an exhaustive literature search, they included 31 studies.
They therefore provide prevalences in adult/children/mixed populations for atypical pathogens, Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila.
Their findings are quite important. I nevertheless have few comments.

MAJOR:
1/ My main comment is on the huge heterogeneity on the microbiological means of diagnosis of the atypical causative pathogen. One of the major bias is the means for aetiological diagnosis. This systematic review includes studies performed on a 46 years period (from 1972 to 2018). The microbiological methods have constantly evolved and improved, with continuous higher performance.
The authors use the "year of publication" as a proxy for assessing this heterogeneity. I think the analysis should
* take into account the type of methodology for the diagnosis of the causative pathogen
* be done again in excluding papers who base their diagnostic strategy on poorly reliable techniques (or with a high number of non-documented pneumonias?)
* this point should be a major point addressed in the discussion

2/ what is the PROSPERO registration number of this systematic review?

3/ The authors should provide details on the severity diagnosis of each study included

4/ I don't get the comparison SCAP/nonSCAP: did the papers include only SCAPs? It does not seem so, as some included HAP and HCAP. This point should be made clear.

MINOR
1/ some formulations should be reworded
2/ what does "pilgrim" (p5 L42) stand for?
I’d be happy to review the revised version of the manuscript if the Editor deems it necessary.

REVIEWER
Vaisman, Alon
University Health Network

REVIEW RETURNED
19-Oct-2022

GENERAL COMMENTS

Overall Comments
The study examines the prevalence of atypical organisms as a cause for severe community acquired pneumonia based on prior literature. The methodology and results are well put together. The data description is extensive and the tables are detailed. The main draw back of the paper is the introduction and discussion. The Introduction does not sufficiently address the existing gap in the literature that current exists. The discussion is more or less a re-iteration of the results without any in depth analysis. Limited discussion about the implications of the findings and how it affects practice.

Introduction
- Page 2 Line 12 – what do you mean by causing “zoonosis” – which kind of infection are you referring to here?
- Page 2 Line 20 – what is meant by “linked” – a risk factor? A cause?
- Page 3 Second last paragraph – have there been any prior reviews or meta-analyses of this question? If yes, please reference. If not, please mention this. Is there a gap in the literature?
- What do some of the more commonly used guidelines say about empiric antibiotic coverage for CAP or SCAP? Most would say that for SCAP, patients should empirically be given atypical coverage, because even if rare, these patients are in severe distress. So how does this work hope to influence treatment?
- Page 3 Line 49 – add “empiric antibiotic use”

Methods
- Page 5 Line 48 – exclusion of a study should go in the Results, not the Methods section

Results
- Page 12, Line 18 – how many studies were included in this analysis of categories of SCAP?
- Can you comment on why and how these continents were chosen?

Discussion
- Page 13, first paragraph – You describe the geographical differences. However, there is no discussion about why there are these differences – can you propose an explanation? Anything from prior literature to explain this? There is some explanation at the top of page 15; they should be merged together
- Page 13 Line 27 – If you recommend caution to be taken interpreting your main results (the pooled estimates), what do you propose is the main takeaway from your findings? How can we use your findings if the literature contains such a wide variety of prevalence estimates?
- Page 13, line 31 – What do you mean by “several possible factors” – several possible factors for what?
- Page 13, Line 37 – What does the severity of illness due to Chlamydia have to do with whether the infection has been implicated in HAP?
- Page 13 Line 57 – avoid using double negative “not a rare” – do you mean common?
- Page 14 Line 4 – What is meant by “certain proportion” – what is the proportion exactly? I would expect that Legionella rarely causes HAP as compared to CAP.
- Page 14 Line 39-45 – Why is there a discrepancy between your findings and previous meta-analyses? Did your analyses use the same primary papers? Were those reviews much older?
- Page 14 Line 54 – What is the relevance of childhood infections in this paragraph? It does not address the previous sentences discussing prior work in this area.
- Page 16 Line 32 – Avoid using double negative “not uncommon”
- Again, getting back to the issue of empiric therapy, how do the results influence treatment? What can clinicians take away from this study? How do your findings compare to recommendations in the current guidelines?

**VERSION 1 – AUTHOR RESPONSE**

Comments of reviewer 1:
Thank you very much for spending time in reviewing our manuscript. We also appreciate your clear and detailed feedback and hope that the explanation has fully addressed all of your concerns. In the remainder of this letter, we discuss each of your comments individually along with our corresponding responses.

Response to comments:

1. Search strategy time limitation: systematic search was conducted through Sep 2019. This is already 3 years old and has missed the large wave of the literature during the pandemic examining atypical pathogen co-infections in the context of covid-19. I understand that this clinical context differs from the primary objective of the literature through 2019, but I do not think that the authors excluded any studies (or that the primary studies did so) if there was co-infection either. Unless there is a scientific reason to justify the limitation to 2019, the search needs to be updated.

Response: It is really true as Reviewer suggested that the time limitation of our previous analysis. We have redone our data extraction and analysis, as well as updated our search to the studies published through November 2022.

2. Selection of studies: the patient population selection is rather confusing and conflicting with subsequent analyses. Why exclude elderly, post-transplant or mechanically ventilated patients when these represent the most seriously ill patients with severe pneumonia (the scope of this review)? Furthermore, the authors then perform a sensitivity analysis of studies including vs. excluding immunocompromised patients, when by design they had already excluded the immunosuppressed studies upfront. This comparison is thus ill defined, and an appropriate comparison should include the studies in immunocompromised populations.

Response: It makes sense as Reviewer suggests that our exclusion criteria may have some limitations. The reason why we excluded the elderly, post-transplant, and mechanically ventilated patients, and the immunocompromised is that in these populations, the etiological distribution of severe pneumonia is significantly different from that of the general population. Different etiological spectrum between the special populations will result in biased results. If necessary, we will do a separate analysis focusing on these populations.
3. Exclusion of studies that did not report on mycoplasma, chlamydia or legionella separately. I think that pooled estimates on combined atypical pathogen prevalence are still important, and would consider a pooled analysis of all 3 pathogens, including these studies.

Response: In our revised search strategies, we have included the studies that report the prevalence of mycoplasma, chlamydia, or legionella separately, as well as the studies reporting the pooled prevalence of atypical pathogens. We also added the pooled estimate and subgroup analysis of atypical pathogen in the Results.

4. The decision on quality assessment is rather atypical, when there are consensus and established tools for methodological quality assessment in observational studies, e.g. from Cochrane.

Response: We are sorry for our negligence in a concrete quality assessment in our previous study. We have improved our quality assessment method by using an 11-item checklist, which was recommended by Agency for Healthcare Research and Quality (AHRQ). And we use this checklist to assess and score the methodological quality of all the studies included. The details of the assessment of each study are presented in our supplementary materials.

5. The most important variable with regards to atypical pathogen prevalence is the diagnostic method used for detection in my view. This variable has not been factored into the analysis, although reported in Table 1. the sensitivity/specificity performance of serology vs. PCR vs. culture tests is markedly different, and we expect higher detection rates in studies using PCR methods, especially with the use of sensitive panels in the market in recent years (e.g. Filmarray). This is an important piece of the analysis that needs to be included.

Response: We have handled the variable of the diagnostic method carefully. We have grouped the diagnostic method into two groups, and redone our meta-regression analysis to find their contribution to the heterogeneity. We have also added more explanations in the Discussion. And this is a major point of our limitation discussed in the revised manuscript.

6. Analyses of severe vs. non-severe pneumonia cannot provide reliable estimates when most severe cases of pneumonia have been excluded by the PICOD criteria.

Response: We have improved our PICOD criteria according to this comment, in which we included studies focusing on the severe subgroup of pneumonia, instead of severe pneumonia only, to get more reliable results.

7. I am concerned about missed studies from the literature review or study selection. Another meta-analysis for Legionella pneumonia only included 219 studies.

Response: As Reviewer suggested that our previous research may miss some studies, we have made several changes to address this problem. We have expanded the scope of search by adding a large database, Web of Science, to our data sources. We also improved our search strategies. Moreover, we included the studies that enrolled cases of patients in the severe subgroup of pneumonia, rather than severe pneumonia only. After revision, we finally included 376 studies in the final review and included 75 studies in our final meta-analysis.

We have read the linked study and made a comprehensive comparison. The study mentioned focuses on Legionella prevalence in CAP, and our study limits pneumonia type only in severe cases. As many studies didn't present the prevalence rate in severe pneumonia separately, a large number of pneumonia research is not applicable to our study.
8. Several of the studies in Table 1 included HAP cases. The results then do not necessarily apply to CAP, and if the authors aim to report estimates on severe pneumonia, then their PICOD strategy needs to be revised.

Response: Considering this suggestion, we updated our PICOD strategy. In the inclusion of our study, we didn’t limit the pneumonia categories to CAP. Instead, we aim to study the prevalence in all severe pneumonia cases to offer statistical reference to the empirical antibiotic use. We also performed a subgroup analysis based on the pneumonia categories to show the different prevalence of different types of pneumonia.

Special thanks to you for your good comments.

Comments of reviewer 2

Thank you very much for your dedication in reviewing our manuscript and providing us with a list of constructive comments. We have taken all the comments and suggestion carefully and detailed all the changed made to the manuscript.

Response to comments:

MAJOR:

1/ My main comment is on the huge heterogeneity on the microbiological means of diagnosis of the atypical causative pathogen. One of the major bias is the means for aetiological diagnosis. This systematic review includes studies performed on a 46 years period (from 1972 to 2018). The microbiological methods have constantly evolved and improved, with continuous higher performance.

The authors use the "year of publication" as a proxy for assessing this heterogeneity. I think the analysis should

* take into account the type of methodology for the diagnosis of the causative pathogen
* be done again in excluding papers who base their diagnostic strategy on poorly reliable techniques (or with a high number of non-documented pneumonias?)
* this point should be a major point adressed in the discussion

Response: Thank you very much for this constructive suggestion. Concerning the methodology for the diagnosis of pathogens, we have redone the meta-regression analysis and discussed the means for etiological diagnosis and the heterogeneity. Our explanation is also mentioned in Question 5 of Reviewer 1, and we have also addressed this point in the Discussion of our revised manuscript.

Moreover, we have revised our search strategies and done the meta-analysis again. We excluded papers without reliable diagnostic techniques or with vague diagnostic methods. And we have added more explanations in the Methods of our revised manuscript.

2/ what is the PROSPERO registration number of this systematic review?

Response: Our review was registered in PROSPERO (International Prospective Register for Systematic Reviews) with the registration number CRD42022373950. And we have added this in the Methods part in our revised manuscript.

3/ The authors should provide details on the severity diagnosis of each study included

Response: We have rechecked the diagnostic criteria of severe pneumonia in each study included and made a summary. We have attached the details in the Supplementary Materials. Methods 3. We also summarized these diagnosis criteria in the Table 1 of our paper.
4/ I don't get the comparison SCAP/nonSCAP: did the papers include only SCAPs? It does not seem so, as some included HAP and HCAP. This point should be made clear.
Response: We aimed to study all the severe pneumonia, including SCAP, SHAP, and other types of severe pneumonia. We are sorry for the misunderstandings of SCAP and non-SCAP in our previous abstract. As our study focus on severe pneumonia, we didn’t make a comparison with SCAP and non-SCAP in our analysis, and we have revised our abstract.

MINOR
1/ some formulations should be reworded
Response: We have checked the formulations carefully and made the correction according to the Reviewer’s comments.

2/ what does "pilgrim" (p5 L42) stand for?
Response: Previously we meant the studies focusing on people with religious beliefs. But after our screening in our revised analysis, we found only one paper in this group, and it was excluded based on the reason of lacking etiological analysis. So, we deleted this word to avoid ambiguity.

I’d be happy to review the revised version of the manuscript if the Editor deems it necessary.

Special thanks to you for your good comments.

Comments of reviewer 3
Thank you very much for spending time in reviewing our manuscript and providing us with a list of constructive comments. We have addressed all the comments and detailed all the changed made to the manuscript.

Response to comments:

Overall Comments

The study examines the prevalence of atypical organisms as a cause for severe community acquired pneumonia based on prior literature. The methodology and results are well put together. The data description is extensive and the tables are detailed. The main drawback of the paper is the introduction and discussion. The Introduction does not sufficiently address the existing gap in the literature that current exists. The discussion is more or less a re-iteration of the results without any in depth analysis. Limited discussion about the implications of the findings and how it affects practice.

Response: Thank you very much for your constructive comments. We have made the following revisions and updated some epidemiological data. For the Discussion, we have revised the main findings, limitation, and the clinical importance of our research.

Introduction
- Page 2 Line 12 – what do you mean by causing “zoonosis” – which kind of infection are you referring to here?
Response: We meant the Chlamydiaceae infection from non-human beings, like infected from livestock or other animals. And we have changed this word in the revised edition.

- Page 2 Line 20 – what is meant by “linked” – a risk factor? A cause?
Response: It means “a risk factor”, and we have changed the expression.
- Page 3 Second last paragraph – have there been any prior reviews or meta-analyses of this question? If yes, please reference. If not, please mention this. Is there a gap in the literature? 
Response: There haven’t been any prior reviews or meta-analyses of this question. And we have added some explanation in our Introduction to show the gap in the current literature and the importance of our study.

- What do some of the more commonly used guidelines say about empiric antibiotic coverage for CAP or SCAP? Most would say that for SCAP, patients should empirically be given atypical coverage, because even if rare, these patients are in severe distress. So how does this work hope to influence treatment?
Response: We consider it a very constructive suggestion, and we have added some explanation about the empiric antibiotic treatment in the Discussion.

- Page 3 Line 49 – add “empiric antibiotic use”
Response: “Empiric antibiotic use” was added.

Methods
- Page 5 Line 48 – exclusion of a study should go in the Results, not the Methods section
Response: We have made the correction according to this comment.

Results
- Page 12, Line 18 – how many studies were included in this analysis of categories of SCAP?
Response: We have checked the data again, and we included 40 SCAP studies of 46 adult studies in the Legionella prevalence. We have also added this figure to our revised manuscript.

- Can you comment on why and how these continents were chosen?
Response: Previously why we chose these continents is because we included more studies from these regions. After we updated our search strategies and redid the subgroup analysis, we finally identified studies covering 6 continents, compared with the 4 continents previously. This change can provide more comprehensive and universal results for our analysis.

Discussion
- Page 13, first paragraph – You describe the geographical differences. However, there is no discussion about why there are these differences – can you propose an explanation? Anything from prior literature to explain this? There is some explanation at the top of page 15; they should be merged together
Response: We have merged the discussion about regional differences together according to this suggestion. Furthermore, based on our updated analysis, we added some new findings and explanations concerning the geographical differences in the Discussion.

- Page 13 Line 27 – If you recommend caution to be taken interpreting your main results (the pooled estimates), what do you propose is the main takeaway from your findings? How can we use your findings if the literature contains such a wide variety of prevalence estimates?
Response: This comment is very productive, and we have reconsidered the clinical significance of our findings. We expected to offer a reliable reference for people in treating severe pneumonia, and we have added our main takeaway in the Discussion, which mainly focuses on the clinical importance of the empiric antibiotic use in severe pneumonia.

- Page 13, line 31 – What do you mean by “several possible factors” – several possible factors for what?
Response: Here we meant several possible factors for the heterogeneity, and we have added it to the sentence.
- Page 13, Line 37 – What does the severity of illness due to Chlamydia have to do with whether the infection has been implicated in HAP?
Response: Our focus is on prevalence. Analyzing and exploring severity between subgroups was not performed. Based on our findings that Chlamydia infection almost exclusively occurred in SCAP, its infection in severe HAP is rare.

- Page 13 Line 57 – avoid using double negative “not a rare” – do you mean common?
Response: Many thanks for this correction. We have changed this word to “common”.

- Page 14 Line 4 – What is meant by “certain proportion” – what is the proportion exactly? I would expect that Legionella rarely causes HAP as compared to CAP.
Response: We have summarized the proportion in the Results. The results of our analysis displayed a higher rate in other types of pneumonia than CAP. This may be due to the small sample size of the group of HAP and other pneumonia, as well as the aquatic properties of Legionella, which may cause HAP more easily.

- Page 14 Line 39-45 – Why is there a discrepancy between your findings and previous meta-analyses? Did your analyses use the same primary papers? Were those reviews much older?
Response: No previous article focused on the same topic as what we discussed, so there is no discrepancy. And the difference from the common incidence of pneumonia has been analyzed in the Discussion.

- Page 14 Line 54 – What is the relevance of childhood infections in this paragraph? It does not address the previous sentences discussing prior work in this area.
Response: According to this suggestion, we deleted the childhood infections as it is irrelevant to the main idea of the paragraph.

- Page 16 Line 32 – Avoid using double negative “not uncommon”
Response: We are sorry for this inappropriate expression here. We have changed it to “common”.

Again, getting back to the issue of empiric therapy, how do the results influence treatment? What can clinicians take away from this study? How do your findings compare to recommendations in the current guidelines?
Response: Our findings will bring new insights into the empirical antibiotic treatment in severe pneumonia. We have added our main takeaway for clinicians in the Discussion.

Special thank you for your good comments.

VERSION 2 – REVIEW

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Kitsios, Georgios</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Pittsburgh Medical Center</td>
<td></td>
</tr>
<tr>
<td>REVIEW RETURNED</td>
<td>23-Dec-2022</td>
</tr>
</tbody>
</table>

| GENERAL COMMENTS | The authors have responded to my criticisms. no further comments. |

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Messika, Jonathan</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP-HP, Service de Réanimation Médico-Chirurgicale</td>
<td></td>
</tr>
<tr>
<td>REVIEW RETURNED</td>
<td>21-Dec-2022</td>
</tr>
</tbody>
</table>
I read with interest the revision of this paper, and the huge efforts made to answer the reviewer’s comment. The authors should be commended for their important work. The names of bacterial species showed be written in throughout the whole manuscript. In my opinion, the paper is suitable for publication.

Reviewer: 1
Dr. Georgios Kitsios, University of Pittsburgh Medical Center
Comments to the Author:
The authors have responded to my criticisms. No further comments.

R: Thanks for reading our paper again and all the constructive comments you made, which greatly improved the quality of our paper. Thank you very much for your comment again.

Reviewer: 2
Dr. Jonathan Messika, AP-HP
Comments to the Author:
I read with interest the revision of this paper, and the huge efforts made to answer the reviewer’s comment. The authors should be commended for their important work. The names of bacterial species showed be written in throughout the whole manuscript. In my opinion, the paper is suitable for publication.

R: Thank you very much for your recognition. We have made further revisions to make our paper more suitable for publication. Many thanks to your dedication in revising our paper.

We have tried our best to improve the manuscript. And here we did not list all the changes but marked them in red in the revised paper. We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the revision will meet with approval.

Once again, thank you very much for your comments and suggestions.