






RESEARCH NOTE

REVISED **Detection of *Mycoplasma pneumoniae* in hospitalized children with pneumonia in Laos**

[version 5; peer review: 1 approved, 1 approved with reservations, 4 not approved]

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Abstract

Mycoplasma pneumoniae has been described worldwide as an important cause of community-acquired pneumonia. From December 2013 to December 2014, 461 children admitted to Mahosot Hospital, Vientiane, Laos, with acute respiratory infection were investigated for upper respiratory microorganisms using probe-based real-time polymerase chain reaction (PCR) (FTD33). *M. pneumoniae* was detected by FTD33 in the upper respiratory tract of three patients, two girls and one boy, 5.7 and 3.9 years old and 13.6 years old, respectively. They presented with clinical features compatible with *M. pneumoniae* infection. They improved without *M. pneumoniae* directed therapy. The two girls were also positive for other potential pathogens. The boy had abnormal pulmonary auscultation, and one of the girls had significant anaemia. These results suggest that enhancement of diagnostic systems for *M. pneumoniae* detection is needed to improve understanding of the epidemiology of *M. pneumoniae* infection in Laos.


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REVISED Amendments from Version 4

In the introduction we included information on the *Mycoplasma pneumoniae* detection rates from the the PERCH and GABRIEL studies. We better contextualised the epidemiology by mentioning and citing references about the periodicity (3 to 7 years) of *M. pneumoniae* epidemics. We have modified one sentence in the last two paragraphs of the introduction to avoid redundancy. We added the reference of our previously unpublished paper (published this year) we are referring to. We removed the patient ID number in the Table 1. We detailed a bit more the paragraph on the study limitation in the discussion section.

Any further responses from the reviewers can be found at the end of the article

Introduction

Mycoplasma spp., the smallest self-replicating microorganism, is associated with both upper and lower respiratory tract infections, most commonly tracheobronchitis, and diverse other clinical manifestations, especially of the skin and central nervous system. *M. pneumoniae* infection, which occurs worldwide with both endemic and epidemic patterns, may have been responsible for up to 20–40% of community-acquired pneumonia before the coronavirus disease 2019 (COVID-19) pandemic, especially in children aged 5–15 years old^{1,2}. However, two large multicenter case-country studies, PERCH and GABRIEL, conducted between 2010 and 2014 in hospitalized children under 5 years of age with pneumonia, reported a detection rate of less than 2%^{3,4}. High incidence rates tend to fluctuate in epidemiological waves, typically occurring every 3 to 7 years^{5–7}. Only limited published data on *M. pneumoniae* infection in low and middle income country are available^{8–11}. As far as is known *M. pneumoniae* only infects humans^{12–14}. However, limited surveillance data hinder estimation of the true burden of *M. pneumoniae* infection. Similarities in presentation with other respiratory pathogens, and the absence of reliable point-of-care diagnostic tests, make it likely that the incidence of this infection is underestimated. Diagnosis is important as conventional treatment of respiratory tract infections with β -lactam antibiotics will not be efficacious for *M. pneumoniae*. *M. pneumoniae* has been described from adjoining Cambodia, Vietnam and Thailand, but not, as far as we aware, from Myanmar/Burma^{15,16}. Little is known about the diversity of etiologies of acute respiratory infection (ARI) and incidence of *M. pneumoniae* in Laos. Currently prompt clinical diagnosis remains elusive as only one laboratory in Laos has demonstrated capability of testing for this pathogen. Few studies have been conducted, mainly on viruses^{17–19}; *M. pneumoniae* has been reported in only one study, detected in 12 people with influenza-like illness from a community cohort study²⁰. Therefore there is a need for more information on the burden of *M. pneumoniae* infection in Laos. We describe here three patients positive for *M. pneumoniae*, to highlight the occurrence of this pathogen in Laos in children with ARI.

Methods

From December 2013 to December 2014 we investigated the etiologies of ARI in hospitalized children at Mahosot

Hospital, Vientiane^{21,22}. This is an approximately 400-bed hospital providing primary, secondary, and tertiary care and receiving \approx 2,000 inpatients/month.

Children aged <15-years-old admitted to paediatric wards with a clinical presentation compatible with ARI were included as previously described²¹. Demographic, medical history, clinical and environmental data were collected by research physicians using a questionnaire, by interviews, physical examination and consulting medical charts²².

Written informed consent from the legal guardians of all patients was obtained before recruitment to the study. Ethical clearance was granted by the National Ethics Committee for Health Research, Ministry of Health, Vientiane, Laos, and the Oxford Tropical Research Ethics Committee (Oxford, UK).

Nasal and throat swab specimens were collected from included patients using Sigma Virocult® (Medical Wire & Equipment), then 100 μ L of each were pooled, extracted and tested by real-time polymerase chain reaction (RT-PCR) for 33 pathogens using the FTD® respiratory pathogens 33 kit (FTD33, Fast-track Diagnostics), as previously described²¹. A PCR assay was considered as positive if the Cq value was <35. FTD33 targets the Adhesin P1 gene of *M. pneumoniae* and validation by the manufacturer showed a detection limit of 100 copies/mL of plasmid and no reaction with *M. pneumoniae*-negative samples by real-time PCR reference methods (communication from Fast-track Diagnostics). No cross-reaction was observed when clinical samples containing 66 different bacterial, viral or parasitic pathogens were tested with FTD33 (communication from Fast-track Diagnostics).

Results

From December 2013 to December 2014, of 472 patients eligible and consenting to the study, 461 hospitalized with an ARI presentation were included (11 patients had missing clinical or laboratory data). One or multiple potential pathogens were detected in respiratory specimens of 447/461 (97.0%) patients²². *M. pneumoniae* was detected in three patients, two girls and one boy, 5.7, 3.9 and 13.6 years old, respectively (Table 1). All lived in Vientiane City and all had fever and cough. One patient had low haemoglobin (4.2g/dL), low haematocrit (13.3%) and high platelet count ($8.43 \times 10^5/\text{mm}^3$) on presentation, without other respiratory or gastroenteric signs or symptoms. The two other patients presented with runny nose, sore throat, and vomiting, in addition to cough. One patient had abnormal pulmonary auscultation on presentation and was hypoxic (O_2 saturation of 90% on room air). None of these three patients had chest radiography performed.

For the patient with abnormal pulmonary auscultation, *M. pneumoniae* was the only one of the 33 potential pathogens detected in the upper respiratory tract. Influenza B virus, *Streptococcus pneumoniae* and *Moraxella catarrhalis* were also detected in the two other patients. In addition, human cytomegalovirus and *Staphylococcus aureus* were also detected from one patient. Serology, culture and cold-agglutination assays were not available.

Table 1. Characteristics of the three patients found PCR positive for *Mycoplasma pneumoniae* in the upper respiratory tract. All three patients were of Lao Loum ethnicity, all had documented fever and cough at enrolment, but none reported difficulty breathing, diarrhoea, chest indrawing, rash, cyanosis, respiratory distress, wheeze, stridor, nasal flaring, grunting, convulsion, conjunctival suffusion, lymphadenopathy, inability to drink, prostration or lethargy. None had known comorbidities at the time of presentation, or clinical evidence of pneumonia (WHO criteria²³), none required ICU admission and all were discharged alive with full recovery after two weeks. All symptoms for the three patients (rigors, sputum, runny nose, sore throat, vomiting, nausea) are consistent with *M. pneumoniae* infection.

Patient	<i>M. pneumoniae</i> patients		
	#1	#2	#3
Admission date	Feb 2014	Feb 2014	Sept 2014
Age (years)	5.7	13.6	3.9
Gender	female	male	female
Birth weight (g)	2,500	2,800	2,800
Ward	General Paediatrics	Infectious Disease Paediatric	Infectious Disease Paediatric
Clinical presentation			
Duration of illness prior to hospitalization (days)	2	6	4
Rigors	no	no	yes
Sputum	no	yes	no
Runny nose	no	yes	yes
Sore throat	no	yes	yes
Vomiting	no	yes	yes
Nausea	unknown	yes	no
Physical examination			
Abnormal pulmonary auscultation	no	yes	no
Oxygen saturation in room air (%)	99	90	unknown
Respiratory rate (breaths/min)	33	28	25
Duration of hospitalisation (days)	4	5	4
Other organisms detected by FTD33			
Influenza B virus	yes	no	yes
Human cytomegalovirus	no	no	yes
<i>Staphylococcus aureus</i>	no	no	yes
<i>Streptococcus pneumoniae</i>	yes	no	yes
<i>Moraxella catarrhalis</i>	yes	no	yes
CBC Test of peripherhal blood			
White blood cells (10 ³ cells/mm ³)	7.10	3.32	8.92
Lymphocytes (10 ³ cells/mm ³)	1.04	1.55	1.98

	<i>M. pneumoniae</i> patients		
Patient	#1	#2	#3
Monocytes (10 ³ cells/mm ³)	0.50	0.18	0.26
Granulocytes (10 ³ cells/mm ³)	5.55	1.59	6.67
Lymphocytes (%)	14.7	46.8	22.2
Monocytes (%)	7.1	5.3	3
Granulocytes (%)	78.2	47.9	74.8
Hemoglobin (g/dL)	4.2	14.2	9.9
Mean corpuscular volume (fL)	59	83	79
Platelet count (10 ³ cells/mm ³)	843	112	151
Treatment			
	Paracetamol 250mg pro re nata	Paracetamol 500 mg pro re nata	Paracetamol 120mg x4 times/day
	Folic acid 5mg 1 time per day for 30 days	IV fluid	IV fluid
	Blood transfusion 2 times during admission (21 & 24 Feb 2014)	Vitamin C 500mg x3 times/day	Cefixime 50mg x 2 times/day, 1 day in hospital and continue at home
		Penicillin G 400,000 UI x 3 times/day for 5 days in hospital and continue for 5 days at home	

All patients received paracetamol to reduce fever. The patient with low haematocrit received a two-unit blood transfusion, whereas the two other patients received penicillin G and cefixime, respectively, during hospitalization. None received a macrolide, tetracycline or fluoroquinolone. After 4–5 days of hospitalization, all three patients improved and were discharged and 2 weeks later the three patients had fully recovered.

Discussion

We describe three Lao children with *M. pneumoniae* infection, a pathogen that was first detected in Laos in 2019²⁰. In the USA, the proportion of community-acquired pneumonia caused by *M. pneumoniae* increased following the introduction of pneumococcal vaccination^{12–14}. The roll-out of pneumococcal vaccination in Laos since 2013²⁴ therefore suggests that *M. pneumoniae* is a respiratory risk that warrants attention. However, there has been a very striking reduction in *M. pneumoniae* incidence in many countries during the COVID pandemic until recent resurgence in 2023^{25,26}.

Our study has important limitations, including that patients were recruited over only one year, and do not include recent data, and that we only collected upper respiratory tract samples. A pooled sample of nasal and throat swabs was used for *M. pneumoniae* detection by PCR, rather than the commonly

accepted nasopharyngeal swab. However, we believe this did not significantly impact our results, as a recent study found higher sensitivity for *M. pneumoniae* detection in throat swabs compared to nasopharyngeal samples²⁷. No serology was performed and no published independent evaluation of the accuracy of the commonly used diagnostic kit used is available. The detection test used was PCR which is highly specific and sensitive; *M. pneumoniae* isolates of patient samples were not handled in the PCR laboratory, and therefore it is very unlikely that those results could be false positives due to contamination. While good laboratory practices were followed, contamination cannot be 100% ruled out. Results are likely to correspond to the co-detection of several micro-organisms (co-infections and/or carriage) in the upper respiratory tract as has been reported in other studies: more than one micro-organism was detected in 73% of patients with ARI in the study in Laos by Phommason *et al.* 2022²⁸ and in 93% of cases (hospitalized children with severe pneumonia) in multiple countries in the PERCH study⁸. All children improved without *M. pneumoniae* directed therapies, suggesting the infection may have been self-limiting. However, the possibility remains that *M. pneumoniae* was not the primary cause of illness in these cases.

Attributing causality of *M. pneumoniae* infection to disease is complicated, as with so many other respiratory pathogens,

as a result of its occurrence in asymptomatic people. The three patients described here all had illnesses consistent with the described clinical features of *M. pneumoniae*^{12,14}, but two were also PCR-positive for other potential respiratory pathogens. In addition, one of the girls had significant anaemia, raising the possibility of *M. pneumoniae* cold-agglutinin associated haemolytic anaemia, although other potential causes of anaemia were not investigated and cannot be excluded¹⁴.

Further investigation of the importance of *M. pneumoniae* infection in Laos, and elsewhere where diagnostic services need reinforcing, is important for three key reasons. First, only one hospital in Laos has diagnostic facilities for *M. pneumoniae*, and these are not generally available for routine diagnosis in children with respiratory infection. Second, lacking a cell wall, *M. pneumoniae* is not susceptible to β -lactam antibiotics that would most commonly be used for respiratory tract infections²³. Macrolides, tetracyclines and fluoroquinolones are most commonly used to treat *M. pneumoniae* infection but with a poor evidence-base for efficacy^{12,13}. More information is needed to understand which patients should receive empirical macrolide therapy in addition to β -lactam antibiotics. The revised 2020 Lao Pediatric Antimicrobial Prescribing Guidelines recommend oral amoxicillin and intravenous ampicillin as first line therapy for pneumonia not needing and requiring hospital admission, respectively, with option for oral switch to azithromycin, erythromycin or clarithromycin²⁹. When to add these anti-*Mycoplasma* antibiotics is difficult to judge given the paucity of local data and the fact that all three children described here recovered without receiving such agents. Third, the percentage of *M. pneumoniae* infection with macrolide-resistant *M. pneumoniae* is increasing worldwide, probably owing to the widespread use of this antibiotic class. Resistance frequencies in Asia are rising with reports that they represent the majority of isolates in some countries^{13,30}. The resistance rate continues to be high following

the COVID-19 pandemic³¹. There are no data on the antibiotic resistance of *M. pneumoniae* from Laos in the public domain³².

This study shows that *M. pneumoniae* existed in Laos in 2013–2014, without clarity on how often it causes infection. Surveillance research into the local prevalence of disease and antibiotic resistance patterns would assist in better understanding the epidemiology of *M. pneumoniae* infection and to inform treatment guidelines. More research on the current burden of *M. pneumoniae* in Laos is important in light of resurgence of *M. pneumoniae* infections post COVID-19 and recent data indicating high incidence of *M. pneumoniae* infection in young children in China in 2023³³.

Data availability

All data underlying the results are available as part of the article and no additional source data are required.

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This paper is a useful first step in documenting the presence of *M. pneumoniae* in Lao children with ARI, but its impact is limited by the very small number of cases, potential confounding by co-infections, and lack of comprehensive diagnostics. The study's main value is in highlighting the need for larger, more current surveillance studies and improved diagnostic capacity in Laos to inform clinical management and public health policy.

The authors should provide detail on other non-*M. pneumoniae* isolates and their clinical findings. Two of the three positive cases also had other respiratory pathogens detected. This raises questions about whether *M. pneumoniae* was the primary cause of illness or an incidental finding.

The data are from 2013–2014, and the authors acknowledge that epidemiology may have changed, especially post-COVID-19. This reduces the current relevance of the findings.

Authors should describe why a *M. pneumoniae* patient did not receive antibiotics.

Overall, this paper should be combined with Dubot-Pérès *et al.* unpublished.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 31 Jul 2025

Audrey Dubot-Pérès

Reviewer comment: The authors should provide detail on other non-*M. pneumoniae* isolates and their clinical findings.

Response: We appreciate the reviewer's suggestion. The details regarding non-*M. pneumoniae* isolates and their associated clinical findings have already been published in two other publications, the references for which are provided in our revised version. (Nguyen et al. 2017, Dubot-Pérès et al., 2025).

Reviewer comment: Two of the three positive cases also had other respiratory pathogens detected. This raises questions about whether *M. pneumoniae* was the primary cause of illness or an incidental finding.

Response: We agree that the detection of co-infecting respiratory pathogens raises the possibility that *M. pneumoniae* may have been an incidental finding rather than the primary cause of illness. We have addressed this point in the manuscript, stating: "Attributing causality of *M. pneumoniae* infection to disease is complicated, as with so many other respiratory pathogens, due to its occurrence in asymptomatic individuals. The three patients described here all had illnesses consistent with the clinical features of *M. pneumoniae* [7,9], but two were also PCR-positive for other potential respiratory pathogens."

Reviewer comment: The data are from 2013–2014, and the authors acknowledge that epidemiology may have changed, especially post-COVID-19. This reduces the current relevance of the findings.

Response: We acknowledge that the epidemiology of *Mycoplasma pneumoniae* may have evolved since 2014, particularly in the post-COVID-19 context. However, the lack of other published data on this topic in Laos highlights the importance of our study. We believe that sharing these findings remains valuable, as it is crucial to provide data on the circulation of

M. pneumoniae in Laos.

Reviewer comment: Authors should describe why a *M. pneumoniae* patient did not receive antibiotics.

Response: We regret that we are unable to answer this as these decisions were made by the ward doctors and not by the study team. Moreover, the reason for not prescribing antibiotics was not captured in our study questionnaire, and we therefore do not have access to this information.

Reviewer comment: Overall, this paper should be combined with Dubot-Pérès *et al.* unpublished

Response: We would like to clarify that the manuscript by Dubot-Pérès *et al.* has already been published. That study focused specifically on the co-detection of *Streptococcus pneumoniae* and *Haemophilus influenzae* with RSV and influenza viruses and did not include any discussion of *Mycoplasma pneumoniae*. Including *M. pneumoniae* in that context would not have aligned with the scope or objectives of the publication and would have appeared out of place. For this reason, we believe it is appropriate to present the *M. pneumoniae* findings in a separate manuscript.

Competing Interests: No

Reviewer Report 09 July 2025

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Daniel E Park 

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The authors have done a commendable job of addressing the peer reviews to date. I support that the information presented in the manuscript has limitations, but would be a helpful contribution to the literature as the region has a dearth of data on the subject matter. However, I believe given the substantial limitations, this should be written more similarly to a case report and thus have fewer population level conclusions. I have some minor comments for consideration and some general comments.

General comments:

- It would be important to contextualize findings with the understanding that *Mycoplasma pneumoniae* is known to have longer periodicity (3-7 years), with the study possibly occurring during a quiescent period.
- The authors should consider whether portions of the discussion are directly linked with the

findings of the study, which is essentially a case report of 3 children. More directly, it would not be prudent to inform population level perceptions of *M. pneumoniae* diagnosis, treatment, and antimicrobial susceptibility based on 3 patients. Thus, some of the information could either be omitted or shortened.

- Results: The authors may want to consider whether a supplemental table comparing demographic and clinical characteristics of patients with and without *M. pneumoniae*. This is not essential as the $n=3$ is quite small, but it could give some indication as to the representativeness and characteristics of the source/total population.

Minor comments:

- Introduction: The estimate of 20-40% is both within epidemic periods and among specific age groups. Large standardized etiology studies such as PERCH, GABRIEL, and EPIC have demonstrated that the attributable fraction is much lower across children, likely in the <5% range. The nuances are important when framing the expected fraction in the study.

- Introduction: While the revision to note the one hospital is helpful, it is now repeated in consecutive sentences towards the end of the first paragraph and the first sentence of the second paragraph. This construction seems unnecessarily repetitive.

- Methods: What is the reference to the unpublished study at the end of the first sentence? The contents of the sentence do not seem to require a citation beyond the existing reference (#16).

- Methods, paragraph 4: The authors should confirm that these are indeed nasal swabs. Nasal swabs potentially have lower sensitivity for *M. pneumoniae* detection compared with nasopharyngeal swab.

- Results, Table 1: Adding the patient ID appears to not add any useful information to the table while also risking patient confidentiality.

- Discussion, paragraph 1: As a stylistic choice, the first paragraph in the discussion typically summarizes the primary findings of the study. Including the false positive discussion here appears more of a response to reviewer questions rather than the main findings, and that line may be better suited for the limitations or later in the discussion.

- Discussion, paragraph 1: The paragraph concludes that *M. pneumoniae* incidence was greatly reduced post-COVID. However, this is untrue, and is even contradicted by the concluding paragraph in the paper which points out the large outbreaks in China in 2023.

- Discussion, paragraph 2: Many *M. pneumoniae* infections are self-limiting without *M. pneumoniae* directed therapies. This does not particularly address the question of whether *M. pneumoniae* was the primary etiologic cause or not. Note, pneumoniae is also mis-spelled in this sentence.

- Discussion, paragraph 4: I believe "cell well" is a typo and should be "cell wall"

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 31 Jul 2025

Audrey Dubot-Pérès

We have prepared a new version of the manuscript taking into account the reviewers' comments.

General comments:

-We have modified the manuscript to contextualize the findings by adding information about the periodicity in the introduction: "High incidence rates tend to fluctuate in epidemiological waves, typically occurring every 3 to 7 years (Yamazaki et al. 2016; Brown et al. 2016 ; Merişescu et al. 2025)".

-We have removed the portions of the discussion which are not directly linked with our findings.

-Given the low number of *M. pneumoniae* patients, a comparison of demographic and clinical characteristics between patients with and without *M. pneumoniae* would not be much informative. In addition, the characteristics of the study patients are included in the published article Nguyen et al. 2017. Therefore, we don't think it is necessary to add that information in supplemental table.

Minor comments:

-We included to the introduction the *Mycoplasma pneumoniae* detection rates from the the PERCH and GABRIEL studies. We did not cite the EPIC study as its detection rate of 19% in children over 5 year old aligns with the previously mentioned estimate of 20-40%.

- We have modified the end of the first paragraph and the beginning of the second paragraph to avoid the repetition.

-The unpublished study we were refereing to has now been published, so we have added the corresponding reference.

- As described in the method section, both nasal and throat swabs (not nasopharyngeal

swab) were collected and then pooled. A recent study showed that sensitivity of *M. pneumoniae* detection was significantly higher in oropharyngeal samples than in nasopharyngeal samples. However, this is probably not widely acknowledged, therefore we have added the sentence below to the limitations paragraph in the discussion section. "A pooled sample of nasal and throat swabs was used for *M. pneumoniae* detection by PCR, rather than the commonly accepted nasopharyngeal swab. However, we believe this did not significantly impact our results, as a recent study found higher sensitivity for *M. pneumoniae* detection in throat swabs compared to nasopharyngeal samples (Kitagawa et al. 2025)." -We have removed the patient ID from Table 1.

-We have moved the sentence about false positive discussion to the paragraph on limitations. -We have modified the sentence to: "However, there has been a very striking reduction in *M. pneumoniae* incidence in many countries during the COVID pandemic until recent resurgence in 2023".

- We have modified the sentence as below: "All children improved without *M. pneumoniae* directed therapies, suggesting the infection may have been self-limiting. However, the possibility remains that *M. pneumoniae* was not the primary cause of illness in these cases".

-We have corrected the typo "cell wall"

Competing Interests: No

Reviewer Report 13 June 2025

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Kristina Krohn 

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The manuscript is okay to be indexed. I have no further points to add.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 3

Reviewer Report 25 March 2025

<https://doi.org/10.21956/wellcomeopenres.26279.r120203>

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**Kristina Krohn**

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Thank you for your thorough response to my previous review. You have responded to all of my prior comments. My comments regarding the introduction and rephrasing or providing citations have been addressed by the changes in removing the Asia prevalence. This makes my related comment about the dating of that paper moot.

Introduction:

I appreciated your description in your response of the knowledge about *Mycoplasma pneumoniae* in Laos. You stated, "There is no citation for this information, it comes from healthcare professional working in Laos and the fact that only one lab in the whole country can and has tested for this pathogen and it has only been described in Lao once before." I would suggest that rather than stating one person's opinion about *Mycoplasma pneumoniae* knowledge as is done with the phrase "of which there is little apparent awareness in Laos and adjoining countries", it would be more accurate, and impactful to state the known facts that demonstrate that even if providers know that *M. pneumoniae* exists in theory, they are unable to identify it in clinical practice as there is only one lab in the country that has demonstrated capability to identify this pathogen.

Consider rephrasing to something along the lines of "Diagnosis is important as conventional treatment of respiratory tract infections with β -lactam antibiotics will not be efficacious for *M. pneumoniae*. Currently prompt clinical diagnosis remains elusive as only one laboratory in Laos has demonstrated capability of testing for this pathogen."

Conclusion:

- Similarly, the sentence about "awareness" of the diagnosis is an opinion statement. Providing a factual sentence on testing capabilities would be more compelling. You provide that later in the discussion, so I would delete this sentence.

- Thank you for moving away from the prior overstatements in the conclusion regarding that this study shows the need for studying antibiotic resistance in *Mycoplasma pneumoniae* infections. As currently stated, I am uncertain of the significance of resistance for *Mycoplasma pneumoniae* for Laos. I think the discussion of regional outbreaks and cases with resistance in China recently is more compelling on why it is important to include resistance patterns when working to add surveillance for *Mycoplasma pneumoniae* in Laos.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 May 2025

Audrey Dubot-Pérès

Introduction: Thank you for the suggestion, we have modify the text as suggested.

Conclusion: Thank you for the suggestion, we have removed the sentence about "awareness". We are already mention the recent increase in resistance in Asia and cite the recent review "New Insights into the Epidemiological Characteristics of Mycoplasma pneumoniae Infection before and after the COVID-19 Pandemic". Therefore we are not sure if there is a specific request from the reviewer.

Competing Interests: No competing interests were disclosed.

Version 2

Reviewer Report 16 January 2025

<https://doi.org/10.21956/wellcomeopenres.24901.r115279>

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Yan Chen 

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This study addresses an important topic by investigating the role of *Mycoplasma pneumoniae* in community-acquired pneumonia among pediatric inpatients in Laos. The use of PCR for pathogen detection is a strength, highlighting the study's methodological foundation. However, the

manuscript has significant limitations that undermine its impact and reliability. The extremely low positivity rate for *M. pneumoniae* raises concerns about the diagnostic accuracy or methodological limitations, while the analysis and interpretation of the results are insufficiently developed. The study does not provide robust evidence to support the conclusions drawn, limiting its contribution to understanding the epidemiology of *M. pneumoniae*

1. This study is a retrospective analysis of *Mycoplasma pneumoniae* detection and clinical information collection from pediatric inpatients at Mahosot Hospital between 2013 and 2014 using PCR. However, concerns arise regarding the accuracy of the data and results. While the study mentions 461 children as subjects, the focus on only three positive cases is not adequately explained. It is unclear whether the sample size is statistically representative. The low *M. pneumoniae* positivity rate compared to other reports raises questions about the reliability of the detection method used, yet the authors do not discuss this potential limitation.
2. The results section lacks sufficient detail, providing only clinical information about the patients. It does not describe the methods of data analysis or include comparisons or statistical discussions among case groups. Even with a small sample size, some preliminary statistical analysis should be provided. Furthermore, there is a disconnect between the results and the discussion sections, which undermines the cohesion of the study.
3. The conclusion suggests enhancing diagnostic systems and analyzing antibiotic resistance for *M. pneumoniae*. However, it fails to substantiate why these measures are necessary, especially considering the study reports an exceptionally low *M. pneumoniae* positivity rate in Laos. This discrepancy should be addressed and supported by the findings.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: infectious diseases; virulence; resistance mechanism

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 16 Feb 2025

Audrey Dubot-Pérés

We are unclear about the reviewer's comment suggesting that our finding (low positive rate) is inconsistent with other reports. It is possible that in Laos, *M. pneumoniae* infection plays a smaller role in community-acquired pneumonia, or that the samples we collected were taken outside of epidemic periods. We utilized a real-time PCR assay, recognized as the gold standard for sensitive pathogen detection, using the FTD33 kit, which has been widely employed in numerous studies, including the PERCH study, which includes a control population. Only one study on *Mycoplasma pneumoniae* has been published in Laos, where the positive rate was higher than in our study (14/548). However, the study design differed, even though the same assay was used for detection.

This is a descriptive study, and given that only 3 patients tested positive for *M. pneumoniae*, I'm unsure what reliable statistical analysis could have been performed. Moreover, the purpose of this paper is not to estimate the incidence of *M. pneumoniae* or analyze risk factors, but rather to highlight its presence in Laos and emphasize the need for increased surveillance and research to better understand its epidemiology and ensure the availability of appropriate diagnosis and treatment. The aim of this article is to demonstrate the presence of *M. pneumoniae* in order to raise awareness about the need for further research to better understand its epidemiology in Laos, as there has been no specific research conducted on this pathogen and no laboratories currently perform its diagnosis. Given the public health significance of this pathogen in other countries, it seems crucial to publish even limited findings in an effort to increase awareness and highlight the need for more research.

Competing Interests: No competing interests were disclosed.

Reviewer Report 14 October 2024

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Chao Shi 

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The authors mentioned in the abstract that is needed to raise awareness and improve understanding of the epidemiology of *M. pneumoniae* infection in Laos, enable targeted therapy. The authors highlighted the need for better diagnostic tools and the understanding of antibiotic resistance to improve treatment and management of *M. pneumoniae* infection in Laos.

The authors investigated at Mahosot Hospital in Vientiane, Laos, examined 461 children with acute respiratory infections from December 2013 to December 2014. Using a PCR test, *M. pneumoniae* was found in the upper respiratory tracts of three children.

1. The positive results of *M. pneumoniae* detection by PCR only accounted for 0.6% of total number. In fact, this set of data could not explain the importance of diagnosis of *M. pneumoniae*. Besides, only the specificity of FTD33 kit has been verified, but have not been checked by other methods, such as serology. We suggest amending this.
2. And the results of this survey are inconsistent with introduction that *M. pneumoniae* infection, which occurs worldwide with both endemic and epidemic patterns, may have been responsible for up to 20–40% of community-acquired pneumonia before the coronavirus disease 2019 (COVID-19) pandemic.
3. This article aims to improve the treatment of *M. pneumoniae* infection in Laos by improving the understanding of the epidemiology of *M. pneumoniae* infection in Laos. Therefore, we suggest analyzing the importance of distinguishing *M. pneumoniae* infection from other respiratory infections with the same symptoms from the serious consequences such as drug-resistant infection caused by antibiotic abuse, and then clarify the significance of *M. pneumoniae* diagnosis.

References

1. Atkinson TP, Balish MF, Waites KB: Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections. *FEMS Microbiol Rev.* 2008; **32** (6): 956-73 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Kakiuchi T, Miyata I, Kimura R, Shimomura G, et al.: Clinical Evaluation of a Novel Point-of-Care Assay To Detect Mycoplasma pneumoniae and Associated Macrolide-Resistant Mutations. *J Clin Microbiol.* 2021; **59** (7): e0324520 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Merida-Vieyra J, Aquino-Andrade A, Palacios-Reyes D, Murata C, et al.: Detection of Mycoplasma pneumoniae in Mexican children with community-acquired pneumonia: experience in a tertiary care hospital. *Infect Drug Resist.* 2019; **12**: 925-935 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular biology ; Nucleic acid testing ; POCT ; Biosensor

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 16 Feb 2025

Audrey Dubot-Pérés

We agree that our data cannot explain the importance of *M. pneumoniae* in Laos, and this is not what we state, we just report the detection of *M. pneumoniae* which as been described in Laos only once before. We clearly said in the discussion that "Further investigation of the importance of *M. pneumoniae* infection in Laos is important".

We do not understand why this is inconsistent. Perhaps in Laos *M. pneumoniae* infection is responsible for less community-acquired pneumonia, or the sample collected were outside epidemics.

No study has been conducted to estimate this, and this is why our manuscript is important to enhance awarness on the need for research on *M. pneumoniae* to understand the epidemiology and estimate the burden in Laos. Our motivation for writing this paper is not to over-, or underestimate, the incidence of *M. pneumoniae* but to point out that it is in Lao and that more surveillance and research is needed to better understand its epidemiology and ensure that appropriate diagnosis and treatment is available. The aim of the article is to enhance awarness on the need for more research to better understand the epidemiomogy of *M. pneumoniae* in Laos and to inform treatment guidelines (additionnal research that would be conducted in the future would permit to inform treatment guidelines not this current article).

Competing Interests: No competing interests were disclosed.

Reviewer Report 15 August 2024

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Overall comments:

- I will still defer to the editors on questions from my previous review.
- Again, I think noting the *M. pneumonia* was present in Laos is appropriate and worth indexing. The current version still overstates the findings. Toning this down to only stating what can be determined from this current study and citing anything that is from other information is important for accuracy.

Abstract:

- It looks like you have re-written the abstract but have left the prior abstract still there. Please delete the first paragraph of the abstract.
- "Other germs were also detected in the girls." This is too colloquial for a scientific paper. Please re-write back to "The two girls were also positive for other potential pathogens."
- Because it is very important in your study that these children improved without *M pneumoniae* directed therapy, please add that to the abstract.
- Your study primarily suggests that clinicians should think of *M. pneumonia* as a potential pathogen and consider testing for it. Your study doesn't actually look at resistance patterns, so while important, I would not include that in the abstract. Therefore, add the need to improve diagnosis and detection (not just improved tools) of *M. pneumonia* to the last line, as in "The study highlights the need for improving the diagnosis and understanding of *M. pneumoniae* infection in Laos."

Introduction:

- This sentence "For reasons that are not understood, *M. pneumoniae* appears to be more common in Asia than elsewhere." Should be referenced.
- Also, it may be relevant to state "*M. pneumoniae* appeared to be more common in Asia than elsewhere before the COVID-19 pandemic" if you are unable to find more recent epidemiologic data.
- This statement "of which there is little awareness in Laos and adjoining countries." Should

be cited.

- The last line of the introduction overstates what can be accurately interpreted from this study. Please consider rewording, such as “We describe here three patients positive for *M. pneumoniae* to highlight that this pathogen can occur in Laos.”

Methods:

- The parenthetical phrase that Reference 16 is unpublished contradicts the citation in the references
- Please rephrase to “This is an approximately 400 bed hospital...”
- Please confirm that the Dubot-Pérès cited at the end of the second paragraph is indeed unpublished, or if it too is reference 16.

Table 1:

- The following information should not be in the table, but in the text/limitations section.

“Contamination cannot be 100% ruled out, however the test were performed following good laboratory practice using quality controls. Those results are likely to correspond to co-detection of several micro-organism (co-infections and/or carriage) in upper respiratory tract as it is reported in other studies: more than one microorganism was detected in 73% of patients with ARI in the study in Lao by Phommasone et al. 2022 and in 93% of cases (hospitalized children with severe pneumonia) in the PERCH study.”

Results:

- 843×10^3 is not accurate scientific notation. Please change to 8.43×10^5 .

Discussion:

- The information provided does not support the sentence “The detection test used was PCR which is highly specific and sensitive; *M. pneumoniae* isolates of patient samples were not handled in the PCR laboratory, and therefore it is very unlikely that those results could be false positives due to contamination.” There is not adequate information in this study to assess the specificity and sensitivity. Nor is the fact that the samples were done outside of the PCR lab make it less likely to be contaminated, even the very best labs sometimes have contamination. In any event, this sentence is unnecessary, and I’d suggest simply deleting it.
- The statement of little awareness would benefit from some sort of citation or explanation.
- Limitations paragraph. The best studies are very transparent about their limitations. Please expand on this section. The paragraph from the Table should be added here.

For example, “Our study has limitations, including patients collected over only one year, not including recent data, and only collecting upper respiratory tract samples. No serology was performed and no published evaluation of the accuracy of the diagnostic kit used is available. While good technique was utilized, contamination cannot be 100% ruled out. More than one microorganism was detected for two of the three children, which calls in to question which pathogen was causing disease. However, co-detection of *M. pneumoniae* in 73% of patients with ARI in the study in Lao by Phommasone et al. 2022 and in 93% of cases (hospitalized children with severe pneumonia across Africa and Asia) in the PERCH study. All children improved without *M. pneumoniae* directed therapies, which raises the question of if

M. pneumoniae was causing infection or if it was merely present.

- A period is needed at the end of this sentence “The revised 2020 Lao Pediatric Antimicrobial Prescribing Guidelines recommend oral amoxicillin and intravenous ampicillin as first line therapy for pneumonia not needing and requiring hospital admission, respectively, with option for oral switch to azithromycin, erythromycin or clarithromycin²³” (note: recommend has 2 “m”s)
- The “First”, “Second”, and “Third” transitions are awkward in that the first two occur within a paragraph and the third is the start of a new paragraph. Consider adapting to by making the sentence “Further investigation of the importance of *M. pneumoniae* infection in Laos, and elsewhere where diagnostic services need reinforcing, is important for three key reasons” the start of its own paragraph. Then either include all 3 points in that paragraph or make them each their own bulleted paragraph underneath.
- “Third, macrolide-resistant *M. pneumoniae* has become increasingly prevalent worldwide, probably owing to the widespread use of this antibiotic class.” Is not a completely accurate statement because of the decrease in overall *M. pneumoniae* infections post COVID. Rephrasing to “Third, the percentage *M. pneumoniae* infections with macrolide-resistant *M. pneumoniae* is increasing worldwide, probably owing to the widespread use of this antibiotic class.”
- There should be a comment about how the COVID-19 pandemic affected resistance patterns if possible.
- The last paragraph over states the implications of this work. Please reframe. For example, “This study shows that *M. pneumoniae* existed in Laos in 2013-2014, without clarity on how often it causes infection. Surveillance research into the local prevalence of disease and antibiotic resistance patterns would assist in better understanding the epidemiology of *M. pneumoniae* infection and to inform treatment guidelines. Better understanding of the current burden of *M. pneumoniae* in Laos is important now in light of anticipated resurgence of *M. pneumoniae* infections post COVID-19 and recent data indicating a high incidence of *M. pneumoniae* infection in young children in China in 2023²⁷.”

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 16 Feb 2025

Audrey Dubot-Pérès

Abstract:

- We did not rewrite the abstract, the 2nd paragraph was not in the submitted manuscript, and am not sure where it is coming from. Could the 2nd paragraph be deleted by the journal ?

- Agree, we will keep the 1st paragraph and therefore this sentence will be deleted.
- Thank you, we have added this sentence to the abstract
- The 2nd paragraph must be removed, the 1st paragraph, which is the correct abstract, mentions the need for “enhancement of diagnostic systems for *M. pneumoniae* detection to improve understanding of the epidemiology of *M. pneumoniae* infection in Laos”. We have removed the mention of resistant profile as suggested.

Introduction:

- This is from an outdated paper and we have removed this statement that no longer holds true.
- We were unable to find data supporting this. We would appreciate it if the reviewer could provide the relevant reference.
- This information is based on the experience of individuals from the region. We have revised the statement to: “of which there is little apparent awareness in Laos and adjoining countries”.
- Thank you, we have modified the sentence accordingly: “We describe here three patients positive for *M. pneumoniae*, to highlight the occurrence of this pathogen in Laos in children with ARI.”

Introduction:

- The unpublished manuscript we are mentioning under brackets (Dubot-Pérès *et al.* unpublished) is different from the reference 16.
- The modification has been done.
- The unpublished Dubot-Pérès *et al.* and the reference 16 are 2 different manuscripts.

Table 1:

The following paragraph has been moved to the discussion in limitations section. “While good laboratory practices were followed, contamination cannot be 100% ruled out. Results are likely to correspond to the co-detection of several micro-organisms (co-infections and/or carriage) in the upper respiratory tract as has been reported in other studies: more than one microorganism was detected in 73% of patients with ARI in the study in Laos by Phommasone *et al.* 2022¹⁸ and in 93% of cases (hospitalized children with severe pneumonia) in multiple countries in the PERCH study”.

Results: The modification has been done.

Discussion:

- I agree that contamination occurs even in laboratories with best practices, however the likelihood for contamination to occur is related to the way the different lab

activities and the workflow are organized. In our lab, microorganism culture is completely physically separated from the molecular lab, with different staff working in the different laboratories and *M. pneumoniae* isolates were not used for molecular testing. Hence, the risk for contamination is decreased compared to laboratories in which isolates are regularly extracted and submitted for molecular testing, with high concentrations of DNA handled in molecular laboratory. Therefore, I think this is important to mention here. As a molecular scientist, I find it reassuring to include this information, reducing the suspicion that this result could come from contamination from isolate DNA if handled in the same lab.

- As mentioned above, there is no citation for this information, it comes from healthcare professional working in Laos and the fact that only one lab in the whole country can and has tested for this pathogen and it has only been described in Lao once before.
- The limitations paragraph has been modified as suggested: “Our study has important limitations, including that patients were recruited over only one year, and do not include recent data, and that we only collected upper respiratory tract samples. No serology was performed and no published independent evaluation of the accuracy of the commonly used diagnostic kit used is available. While good laboratory practices were followed, contamination cannot be 100% ruled out. Results are likely to correspond to the co-detection of several micro-organism (co-infections and/or carriage) in the upper respiratory tract as has been reported in other studies: more than one microorganism was detected in 73% of patients with ARI in the study in Laos by Phommason *et al.* 2022¹⁸ and in 93% of cases (hospitalized children with severe pneumonia) in multiple countries in the PERCH study³. All children improved without *M. pneumoniae* directed therapies, which raises the question of whether *M. pneumoniae* was causing the disease”.
- Thank you, modification has been made.
- Thank you for the suggestion, we have modified all three points in the same paragraph.
- We have modified as suggested.
- A review from Wu *et al.* (2024) reported that resistance remains high after COVID-19 pandemic. We added this to the manuscript. We added the following sentence “The resistance rate continues to be high following the COVID-19 pandemic”.
- We have modified the last paragraph as below: “This study shows that *M. pneumoniae* existed in Laos in 2013-2014, without clarity on how often it causes infection. Surveillance research into the local prevalence of disease and antibiotic resistance patterns would assist in better understanding the epidemiology of *M. pneumoniae* infection and to inform treatment guidelines. More research on the current burden of *M. pneumoniae* in Laos is important in light of resurgence of *M. pneumoniae* infections post COVID-19 and recent data indicating high incidence of *M.*

pneumoniae infection in young children in China in 2023.”

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 27 January 2024

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The authors have investigated 461 children admitted to Mahosot Hospital, Vientiane, Laos, with acute respiratory infection defined by onset of symptoms <14 days, fever (axillary temperature >38.0 °C) or history of fever, and at least one respiratory symptom (dyspnoea, cough, rhinitis) or abnormal pulmonary auscultation on physical examination between December 2013 to December 2014. Pooled nasal and throat swabs were tested using a 33-pathogen multiplex PCR (FTD33). *Mycoplasma pneumoniae* infection was detected in three children 6, 4 years old and 14 years old. In one child, influenza B virus, *Streptococcus pneumoniae* and *Moraxella catarrhalis* were also detected. In another, influenza B virus, *Streptococcus pneumoniae*, CMV, *Staphylococcus aureus* and *Moraxella catarrhalis* were all detected.

1. No confirmatory serology was performed.
2. No radiology was available (frequently not done routinely in children with ARI).
3. All three children recovered without specific anti-mycoplasma antimicrobial therapy. One child had severe anemia, but cold agglutinins were not measured.
4. Therefore, although mycoplasma infection was likely present, mycoplasma-associated disease was not convincingly proven. Even if these cases do represent mycoplasma disease, they comprise only 0.6% of a cohort recruited 10 years ago.
5. The authors statement that these data support the enhancement of “diagnostic systems for *M. pneumoniae* detection and analysis of its antibiotic resistance profile to raise awareness and improve understanding of the epidemiology of *M. pneumoniae* infection in Laos, enable targeted therapy, and inform treatment guidelines” is not substantiated by their data.
6. Indeed, it would be hard to justify the diversion of public health or clinical research

resources based on these findings. To better understand the burden of *M. pneumoniae*-associated respiratory disease, a more contemporary cohort of children fulfilling the criteria for community acquired pneumonia/ severe acute respiratory infection would have been more informative.

7. The authors have not provided a sufficiently authoritative review of the relevant literature from LMICs (I have provided some examples of studies that could have been included).
8. The authors make no mention of the potential for *M. pneumoniae* to cause epidemics in Asia. The impact of the COVID-19 pandemic on *M. pneumoniae* epidemiology should have been considered more carefully as a limitation to the relevance of this data from 2014.

References

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4. Lee E, Kim CH, Lee YJ, Kim HB, et al.: Annual and seasonal patterns in etiologies of pediatric community-acquired pneumonia due to respiratory viruses and Mycoplasma pneumoniae requiring hospitalization in South Korea. *BMC Infect Dis*. 2020; **20** (1): 132 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My research bridges clinical practice, large-scale field studies, disease prevention and the fundamental understanding of the mechanisms of infectious disease.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 14 Jun 2024

Audrey Dubot-Pérès

Thank you for the comments.

4 : However, very few data on *M. pneumoniae* in Laos are available, with the diagnostic test available in only one hospital in Laos. Even if there is not indisputable evidence that *M. pneumoniae* was the cause of the disease, our results are evidence that the organism is circulating in children in Laos. Therefore, we suggest that it is important to report that fact and draw attention on the need for more investigation to know the true burden in Laos, spread awareness and alertness to this disease and optimize the treatment of children with ARI.

5: Same comment as above. The organism is circulating, and therefore there is a need to investigate more in details, to develop appropriate diagnostic facilities, awareness and optimize the treatment of children with ARI. If subsequent research demonstrate that it is a very rare cause we would be delighted as would simplify treatment of ARI but at the moment such data do not exist, leaving a gap in ARI pathogen surveillance

6: Thank you for the suggestion and indeed this is what we are suggesting: more research investigation to understand the true burden of *M. pneumoniae* in Laos, especially in the view of the recent epidemics in China. We added the following sentence at the end of the discussion: "More research on the burden of *M. pneumoniae* in Laos is all the more crucial in light of recent data indicating high incidence of *M. pneumoniae* infection in young children in China in 2023²²".

7: We added more references to the sentence below in introduction: "Only limited published data on *M. pneumoniae* infection in low and middle income country are available³⁻⁶."

8: The recent *M. pneumoniae* epidemic in China shows the relevance of our paper, the aim of which is to draw attention to the importance of investigating *M. pneumoniae* infections in Laos and elsewhere in SE Asia.

Competing Interests: No competing interests were disclosed.

Reviewer Report 30 November 2023

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Detection of *Mycoplasma pneumoniae* in hospitalized children with pneumonia in Laos [version 1; peer review: awaiting peer review]

Overall: I like this article. I think it is good to know that *Mycoplasma pneumoniae* is a potential pathogen in Laos. I have a few concerns, some of which are easily addressed. First, this article does need a limitations section. In particular there should be some discussion of the accuracy of the results attained and how reliable the PCR testing is in this setting. All tests have a specificity and sensitivity that should be shared. The positive and negative predictive values in this setting were population prevalence is utterly unknown is more difficult. Second, further characterization about what is consistent and what is inconsistent with *Mycoplasma pneumoniae* clinical presentations should be included. Lastly, I am uncertain and defer to the editors about how it appears the disease specific data appears to be presented as separate studies for an overarching acute respiratory infection study.

Abstract: No comments

Intro:

- Is *Mycoplasma pneumoniae* smaller than *Mycoplasma genitalium*? I think that *Mycoplasma* as a whole are considered the smallest, but I think *genitalium* is smaller than *pneumoniae*. I would defer to others here.
- Several statements from the conclusion should be included in the intro to show to readers the importance of this article. Specifically the following:
 - "*M. pneumoniae* has been described from adjoining Cambodia, Vietnam and Thailand, but not, as far as we are aware, from Myanmar/Burma^{14,15}. Further investigation of the importance of *M. pneumoniae* infection in Laos, and elsewhere where diagnostic services need reinforcing, is important for three key reasons. First, only one hospital in Laos has diagnostic facilities for *M. pneumoniae*, and these are not generally available for routine diagnosis in children with respiratory infection. Second, lacking a cell wall, *M. pneumoniae* is not susceptible to β -lactam antibiotics that would most commonly be used for respiratory tract infections¹¹. Macrolides, tetracyclines and fluoroquinolones are most commonly used to treat *M. pneumoniae* infection but with a poor evidence-base for efficacy^{3,4}. More information is needed to understand which patients should receive empirical macrolide therapy in addition to β -lactam antibiotics. The situation is analogous to the treatment of central nervous system

infections in Laos and elsewhere in rural Asia, to cover not just the 'conventional' bacteria but also the common causes that are regarded wrongly as 'atypical', such as scrub typhus and murine typhus, that require non- β -lactam antibiotics for effective therapy^{16,17}. The revised 2020 Lao Pediatric Antimicrobial Prescribing Guidelines recommend oral amoxicillin and intravenous ampicillin as first line therapy for pneumonia not needing and requiring hospital admission, respectively, with option for oral switch to azithromycin, erythromycin or clarithromycin¹⁸ When to add these anti-*Mycoplasma* antibiotics is difficult to judge given the paucity of local data "

Methods:

- It appears that this is a subset of the data for from an overarching unpublished study in reference # 10. It looks like the methods, etc. were similar but the previous published data is for RSV, while this is only looking at Mycoplasma. Ideally the complete study would have been published together, perhaps with editorials or perspective articles providing additional perspective on specific illnesses.

Results:

- It is unclear why the sex is shared in relation to the abnormal pulmonary auscultation and hypoxia. Either share the sex when it is relevant to the findings only, or don't share it at all or share it all the time. If sharing only when it is relevant, then please explain why it is relevant.
- It is unclear why the 3.9-year-old female had so many positive organisms (similar for the 5.7-year-old). Are these false positives? If she did have all of these I would expect her to be very sick and immunocompromised, or at least to have an elevated WBC. It seems likely that there was an error in the test (contamination?) or the WBC was done after significant treatment?

Discussion:

- The primary argument of this paper – that *Mycoplasma pneumoniae* is an entity that should be considered in Laos is important. That there are positive tests at all is suggestive of this. Several factors should be further discussed to flush out this argument.
 - What are the reasons for false positive results or other reasons to question these results? I.e. lab contamination, or testing that has lower specificity?
 - How does the presentation of infection compare to that other places? I appreciate the comment that the anemia could be cold agglutination. I also appreciate the comment about all 3 improving without the recommended treatment. How often does that occur in other settings? Also, the ages... in many recommendations azithromycin is recommended after a given age (ex. Above 8-years-old). How does this compare to ages of hospitalized infection from *Mycoplasma pneumoniae* in other places.
- *Mycoplasma pneumoniae* is thought to be an outpatient presentation. What percentage of infections are thought to cause hospitalizations? If there are 3 in this sample, what would the expected number of cases be that would present to outpatient care? Or be asymptomatic?
- What other factors should be included in a discussion of whether or not to change recommendations? Or is it simply enough for now to know that this is a possibility.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I study medical education and medical communication, including having taught clinical practice in Laos. I can comfortably identify that the information in this article is important to clinicians in Laos. When it comes to specifics laboratory research and pathogen identification, that is beyond my scope of practice.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 10 Jun 2024

Audrey Dubot-Pérès

- 1) We have added a paragraph in discussion to mention the limitations of our study: "Our study has limitations, including patients collected over only one year, not including recent data, and only collecting upper respiratory tract samples. No serology was performed and no published evaluation of the accuracy of FTD33 kit for the diagnosis of *M. pneumoniae* infection is available."
- 2) FTD33 is a commercial kit, in the validation document provided by the manufacturer only information on analytical sensitivity and specificity is provided - we have included this in the methods section: "validation by the manufacturer showed a detection limit of 100 copies/mL of plasmid and no reaction with *M. pneumoniae*-negative samples by real-time PCR reference methods (communication from Fast-track Diagnostics)."
- 3) We did not find any published articles on the evaluation of sensitivity and specificity of FTD33 for *Mycoplasma pneumoniae* detection.
- 4) We added in the discussion that with no published data on the accuracy of FTD33 for the

diagnosis of *M. pneumoniae* infection we could have underestimated the number of cases.
5) All symptoms for the three patients (rigors, sputum, runny nose, sore throat, vomiting, nausea) are consistent with *M. pneumoniae* infection, as it is mentioned in discussion section.

6) I added one sentence in footnote of the Table 1. *M. pneumoniae* infection can have protean clinical manifestations and therefore we would prefer not to label clinical manifestations as inconsistent.

7) If the reviewer is pointing out that these data are part of the larger data set of ARI in Laos, we confirm this but argue that a more detailed report on *M. pneumoniae* infection is important on public health grounds to highlight this disease that is neglected in Laos and elsewhere in SE Asia.

- Author response to "Intro" section in Review Report:

1) Thank you for the comment. We replaced *Mycoplasma pneumoniae* by *Mycoplasma* spp. We have moved the statement "*M. pneumoniae* has been described from adjoining Cambodia, Vietnam and Thailand, but not, as far as we are aware, from Myanmar/Burma » to the introduction, and we have mentioned in the introduction the fact that only one hospital has the facilities for the diagnosis of *M. pneumoniae* infection and that there is a need for more information on the burden of *M. pneumoniae* infection in Laos.

- Author response to "Methods" section in Review Report:

1) The writing up of the complete study over several years is in progress. However, all single microorganisms will be not discussed individually, therefore we felt important to publish a separate paper to highlight the importance of *M. pneumoniae* and to raise clinical and public health awareness.

- Author response to "Results" section in Review Report:

1) We removed the mention of patient gender in that sentence. Although we cannot 100% rule out contamination, the test were performed following good laboratory practice using quality controls. Those results are likely to correspond to co-detection of several micro-organism (co-infections and/or carriage) in upper respiratory tract as it is reported in other studies. We added this information in the footnote of the Table 1: "Contamination cannot be 100% ruled out, however the test were performed following good laboratory practice using quality controls. Those results are likely to correspond to co-detection of several micro-organism (co-infections and/or carriage) in upper respiratory tract as it is reported in other studies: more than one microorganism was detected in 73% of patients with ARI in the study in Lao by Phommasone *et al.* 2022²² and in 93% of cases (hospitalized children with severe pneumonia) in PERCH study²³."

- Author response to "Discussion" section in Review Report:

1) The detection test used was PCR which is highly specific and sensitive. In addition as *Mycoplasma pneumoniae* isolates from patient samples were not handled in the laboratory, it is very unlikely that those results could be false positives due to contamination. We added this sentence in the discussion. Thank you for the suggestion. Discussion of these important issues would greatly increase the size of the paper and make it more of a review than a case description from Laos. We would be grateful for the editors advice as to whether expanding the paper is felt appropriate or not. We would prefer to keep it as a case report from Laos. We think we already have addressed this in the discussion by the following sentence: « Hence, enhanced awareness and routine diagnostic systems for *M. pneumoniae* detection in Laos, and research into the local prevalence of antibiotic resistance

in this pathogen, are needed, both for individual patient care and surveillance to understand the epidemiology of *M. pneumoniae* infection and to inform treatment guidelines. »

Competing Interests: No competing interests were disclosed.