PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Pediatric critical illness associated with respiratory infection: a single-centre, retrospective, cohort study
AUTHORS	Alfaraidi, Haifa; Luinstra, Kathy; Eshaghi, Alireza; Smieja, Marek; Gubbay, Jonathan; Pernica, Jeffrey

VERSION 1 – REVIEW

REVIEWER	Reviewer name: Anna Zanetti Institution and Country: Italian Society for Rheumatology, Italy Competing interests: None
REVIEW RETURNED	17-Jan-2020

GENERAL COMMENTS	Authors performed a manuscript entitled "Pediatric Clinical respiratory illness associated with Mycoplasma pneumoniae: a single-centre, retrospective, cohort study". The study design is clear and well explained. In the 'Statistical Analysis' paragraph, it is necessary to specify how normality was tested and which alpha was used to define first type error. In table 2, it is not clear how the p-values were obtained. Have anova / kruskal wallis tests been made for the comparison of all categories simultaneously and, subsequently, in pairs of categories? Or are they made directly for pairs of categories? Which p-value was reported? Furthermore, no corrections for multiple tests were made. I suggest also to add the number of missing for each variable.

REVIEWER	Reviewer name: Jesse Papenburg Institution and Country: McGill University, Canada Competing interests: None
REVIEW RETURNED	16-Feb-2020

GENERAL COMMENTS	This is a retrospective cohort study (medical records review) of all children admitted to a single centre's PICU for "respiratory infection". Although the title suggests that the study is primarily about Mycoplasma pneumoniae infections, only 10 of 227 patients tested positive (~4%) for this pathogen, limiting the study's ability to describe these infections. The major finding is that, among the subgroup >5 years of age with pneumonia, M. pneumoniae prevalence was high (12.5%), although the precision of that estimate is low given the small numbers (95% CI 4-27%). The authors conclude that "Consideration should be given to empiric anti-Mycoplasma antimicrobial therapy pending the result of rapid molecular diagnostic testing in this subset of critically ill children." Despite it's limitations (small, single centre, single season, retrospective design), the study highlights an important consideration in the care of critically ill children with pneumonia. MAJOR COMMENTS:

	1. The study population needs to be better defined. More details about the single centre are needed. How large a hospital? How large a PICU? Importantly, how were eligible subjects admitted to PICU identified? At one point, discharge diagnosis is mentioned: was the hospital discharge abstract database queried? If so, what ICD codes were used? The authors state that it is routine practice for patients admitted for respiratory infection to be tested for resp viruses by multiplex PCR: do they have any data to back up their claim? Were nosocomial cases included? If so, why? Would it not be better to focus on community-acquired pneumonia, since MP is not considered a nosocomial pathogen? If the authors want to advocate for testing/treating for MP, they should not do it on the basis of a cohort that includes nosocomial cases. If nosocomial cases are included, then some analysis of prevalence of MP in noso vs. CAP is warranted. 2. Patients are grouped by diagnosis, but is this admitting diagnosis, working diagnosis, PICU transfer diagnosis, hospital discharge diagnosis…? 3. A paragraph on study limitations needs to be added: small overall sample size, small number of MP cases, single centre, single season, retrospective design… 4. Abstract conclusions are much too strong. I would argue that MP infection was indeed rare (4%) in the overall cohort. “Rapid diagnostic testing and targeted treatment should be considered in an effort to avert morbidity and mortality from respiratory infection” is not substantiated from the study data. Simply advocating for testing and treatment in children > 5 years of age with pneumonia (community-acquired pneumonia?) would be a more reasonable conclusion. 5. Table 1: please present age strata (e.g., <1 y/o; 13-23 months; 2-4 y/o; 5-12 y/o; 13-17 y/o; or whatever strata make most sense as per the age distribution). 6. For Table 3, in addition to simply presenting means, please present medians (particularly important given small n of MP+). Also please present by age strata (<= 5y/o; > 5y/o). 7. p. 14. The conclusion should be modulated by stating that detection of MP is rare in critically ill children <= 5y/o. (I’m assuming that it’s rare since half of cases were in children >5 y/o and the 75th percentile of age distribution is 6.15 years). MINOR COMMENTS: 1. Abstract Objectives: remove “systematically”. Statistical analyses compare distributions of variables across groups, and do not assess “systematic” differences. 2. Throughout the text, I would suggest changing “participants” to “subjects” or “patients” as the study subjects were not enrolled and thus did not “participate” in the study. 3. BACKGROUND: what does “minority” mean? Please be more specific. How often does MP infection spontaneously resolve? 4. IDSA pediatric CAP guidelines do advocate for macrolide therapy in some inpatients: “empiric combination therapy with a macrolide (oral or parenteral), in addition to a β-lactam antibiotic, should be prescribed for the hospitalized child for whom M. pneumoniae and C. pneumoniae are significant considerations” “Azithromycin (in addition to β-lactam, if diagnosis of atypical pneumonia is in doubt)”. British Thoracic Guidelines for Pediatric CAP specifically recommend macrolides for very severe disease (presumably, requiring critical care): “Macrolide antibiotics should be used if either mycoplasma or chlamydia pneumoniae is suspected or in very severe disease” The Background should be appropriately modified;

	consider also modifying the tone of several sentences of the manuscript given this context. 5. The use of “astonishing” is a bit of hyperbole (to describe 19% prevalence) 6. Can the authors be more specific than “explore the epidemiology of MP” in the objectives? 7. Can the authors provide a reference for the multiplex resp virus assay and the lab-developed MP/CP assay? Define HRLMP 8. I believe that Chlamydophila is the new genera for CP 9. On p.8 the lengthy description of all the comorbidities does not add value to the text and is redundant with Table 1 10. Only 11% of subjects had not received influenza vaccine? That is surprising. Also, please specify what is meant by that. Do the authors mean that for eligible patients. e.g., patients >5 mos old, 89% had received influenza vaccine for that specific season? 11. DISCUSSION, p. 11. “MP was commonly” detected in critically ill children”. 4% is not common. 12. P. 12 To whom are the authors referring to in “we thought that this…” 13. P. 13. I don’t see the relevance of discussing Legionella treatment 14. Please mention if requirement for informed consent was waived by the research ethics board

VERSION 1 – AUTHOR RESPONSE

In the ‘Statistical analysis’ paragraph, it is necessary to specify how normality was tested and which alpha was used to define first type error.

Normality was assessed visually. Alpha was set at 0.05, with no adjustments for multiple comparisons in this exploratory study. These sentences have been added to the ‘Statistical analysis’ paragraph.

In table 2, it is not clear how p-values were obtained. Have anova/KW tests been made for the comparison of all categories simultaneously and, subsequently, in pairs of categories? Or are they made directly for pairs of categories? Which p-value was reported? No corrections for multiple tests were made. I suggest also to add the number of missing for each variable.

If Kruskal-Wallis testing identified significant differences, nonparametric pairwise multiple comparisons of the groups using Dunn’s test with Bonferroni adjustment were done; this was added to the text. The number of missing values for each variable were added for Table 2.

The study population needs to be better defined. More details about the single centre are needed. How large a hospital? How large a PICU?

MCH is a tertiary care centre serving a population of approximately 2.3 million residents. At the time of the study, the centre had 159 beds (12 PICU beds) and admitted approximately 6500 children yearly, with over 40 000 emergency department visits. This has been added in the paragraph marked ‘Setting’.

Importantly, how were eligible subjects admitted to PICU identified?

MCH Health Records provided a list of all PICU discharges on a biweekly basis. The PI reviewed all diagnoses and flagged any who could potentially have had respiratory infection.

At one point, discharge diagnosis is mentioned: was the hospital discharge abstract database queried?

No. The PI reviewed all discharges from the PICU manually.

The authors state that it is routine practice for patients admitted for respiratory infection to be tested for resp viruses by multiplex PCR: do they have any data to back up their claim?

McMaster Children's Hospital (and Hamilton Health Sciences) has an Acute Respiratory Infection Surveillance Protocol (policy# 080-MED) that clearly states that all patients admitted to hospital must be screened asking about fever and respiratory symptoms (cough or difficulty breathing); those that answer affirmatively have nasopharyngeal specimens taken to identify the aetiology of their illness.

Were nosocomial cases included? If so, why? Would it not be better to focus on community-acquired pneumonia, since MP is not considered a nosocomial pathogen? If the authors want to advocate for testing/treating for MP, they should not do it on the basis of a cohort that includes nosocomial cases. If nosocomial cases are included, then some analysis of prevalence of MP in noso vs. CAP is warranted.

Our institution, and most in North America, would define 'nosocomial infection' as any infection whose symptoms begin >48 hours after admission to a healthcare facility. Unfortunately, we did not record the exact date of onset of all the participants' respiratory symptoms. Furthermore, the incubation period of M. pneumoniae respiratory infection has been estimated to be 1-4 weeks (Waites KB Clin Microbial Rev 2004; 17(4): 697) with a mean incubation time of 23 days (Waites KB Clin Microbial Rev 2017; 30(3) 748-793); as a result, this 48-hour cutoff would incorrectly characterize many community-acquired cases as 'nosocomial.'

In an effort to remove possible nosocomial cases of Mycoplasma pneumoniae, we excluded all study subjects who had NPS samples acquired a week or more after admission to hospital, and re-conducted all analyses. The changes have been made in the manuscript (note that there were no significant differences to the results or conclusions).

Patients are grouped by diagnosis, but is this admitting diagnosis, working diagnosis, PICU transfer diagnosis, hospital discharge diagnosis…?

The groupings were made based on discharge diagnoses from the ICU and from hospital. However, as noted in the methods section, if the clinical team made a diagnosis of 'pneumonia' without consistent radiographic findings, this was reclassified as best as possible (incorporating all of the clinical history present in the chart).

A paragraph on study limitations needs to be added: small overall sample size, small number of MP cases, single centre, single season, retrospective design…

We agree with the reviewer and have added the following paragraph:

There were obvious limitations to our study. As noted previously, this was a retrospective design and included only a single centre over a 13-month period; as outbreaks with this pathogen have been frequently described (19), we cannot be certain that the prevalence of infection documented in this study is an accurate estimate of children hospitalized with critical respiratory illness in our region of Canada. It is also quite possible that hospital clinicians may not have strictly followed hospital infection control policy and failed to sample the nasopharynges of some patients who otherwise would have been eligible. The study cohort only comprised 221 children and there were only 10 found to be positive for M. pneumoniae; consequently, 95% confidence intervals around our point estimates are wide.

Having said that, the prevalence of Mycoplasma infection found in this small study was similar to that found in a much larger study conducted recently in the United States (2).

Abstract conclusions are much too strong. I would argue that MP infection was indeed rare (4%) in the overall cohort. “Rapid diagnostic testing and targeted treatment should be considered in an effort to avert morbidity and mortality from respiratory infection” is not substantiated from the study data. Simply advocating for testing and treatment in children > 5 years of age with pneumonia (community-acquired pneumonia?) would be a more reasonable conclusion.

We would opine that what constitutes ‘rare’ is dependent on the context. Blood cultures are routinely recommended for children admitted to hospital with community-acquired pneumonia, though their positivity rate has been repeatedly demonstrated to be 4% or lower (Kurowski EM Pediatrics 2015, Hickey RW Ann Emerg Med 1996, Bonadio WA Pediatr Emerg Care 1998, Shah SS Pediatr Infect Dis J 2002). Furthermore, the threshold to test critically ill children should be even lower than that to test those solely admitted to the ward, given that their prognosis is almost certainly worse. However, we agree that the reviewer’s opinion will probably be shared with a large proportion of readers and, as such, have revised the manuscript as follows:

M. pneumoniae infection was found more frequently than invasive bacterial infection in a cohort of children admitted to the PICU with severe respiratory infection. Rapid diagnostic testing and targeted treatment in school-aged children should be considered in an effort to avert morbidity and mortality from respiratory infection.

Table 1: please present age strata (e.g., <1 y/o; 13-23 months; 2-4 y/o; 5-12 y/o; 13-17 y/o; or whatever strata make most sense as per the age distribution).

This has been added to Table 1.

For Table 3, in addition to simply presenting means, please present medians (particularly important given small n of MP+). Also please present by age strata (<= 5y/o; > 5y/o).

We presume that the reviewer is referring to the age variable. This data has been added.

The conclusion should be modulated by stating that detection of MP is rare in critically ill children <= 5y/o. (I’m assuming that it’s rare since half of cases were in children >5 y/o and the 75th percentile of age distribution is 6.15 years).

We have changed the manuscript to the following:

The fact that Mycoplasma was commonly detected in critically ill children would argue that routine surveillance for this pathogen should be considered, as others have suggested (13), although infection was more rare in infants or preschool-aged children.

Abstract Objectives: remove “systematically”. Statistical analyses compare distributions of variables across groups, and do not assess “systematic” differences.

This has been done.

Throughout the text, I would suggest changing “participants” to “subjects” or “patients” as the study subjects were not enrolled and thus did not “participate” in the study.

The word ‘participants’ has been changed to ‘subjects’.

BACKGROUND: what does “minority” mean? Please be more specific.

By ‘minority’, we mean that the number of children admitted to intensive care is less than the number of children admitted to the paediatric ward for acute respiratory illness – this is almost certainly true for all hospitals in North America. This proportion will vary as the characteristics of the hospital and the health care system of the region vary, and so precise estimates may not greatly aid the average reader’s comprehension of the study results and conclusions – but we have added the value noted in the large EPIC study (~20%).

How often does MP infection spontaneously resolve?

Presumably, it self-resolves very frequently, given how commonly it has been isolated from school-aged children with non-severe CAP, who are typically treated with antimicrobials not active against this pathogen…and yet have an excellent prognosis. However, the specific proportion of MP nonsevere CAP that self-resolve has never been well defined. In a cohort of children with macrolide-resistant MP respiratory infection in Japan, fever lasted for a mean of 1 day in those treated with minocycline (to which the isolates were sensitive) as compared to a mean of 4.6 days and 5.5 days in those treated with azithromycin or clarithromycin, respectively (Ishiguro N PLoS ONE 2017 12(3): e0173635). In a systematic review of prospective cohort studies and small RCTs, many at higher risk of bias, a statistically significant benefit of antimicrobial therapy was not found; this was probably due to a high rate of self-resolution, especially for non-severe illness, rather than ineffectiveness of antimicrobials per se.

IDSA pediatric CAP guidelines do advocate for macrolide therapy in some inpatients: “empiric combination therapy with a macrolide (oral or parenteral), in addition to a β-lactam antibiotic, should be prescribed for the hospitalized child for whom M. pneumoniae and C. pneumoniae are significant considerations” “Azithromycin (in addition to β-lactam, if diagnosis of atypical pneumonia is in doubt)”.

We agree with the reviewer that the IDSA guidelines do include that text. However, our statement ‘Neither the American, Canadian, nor British guidelines recommend antimicrobials with activity against M. pneumoniae as first-line empiric treatment for pediatric CAP’ is also correct, given that the IDSA guidelines also state very explicitly ‘Amoxicillin should be used as first-line therapy for previously healthy appropriately immunized school-aged children and adolescents with mild to moderate CAP…’ and ‘Ampicillin or penicillin G should be administered to the fully immunized infant or school-aged child admitted to a hospital ward with CAP when local epidemiologic data document lack of substantial high-level penicillin resistance for S. pneumoniae.’

After those first-line recommendations, the IDSA does include a proviso to give macrolides to children ‘for whom M. pneumoniae and C. pneumoniae are significant considerations’; unfortunately, this is not defined extensively nor accurately. On page e15, it is noted that ‘Atypical pneumonia is characteristically slowly progressing, with malaise, sore throat, low-grade fever, and cough developing over 3-5 days’ – a description that has been shown to be both insensitive and nonspecific (in addition to the references in the text, see Wang K Cochrane Database Systematic Rev 2012; CD009175) – and on page e23-25, it is simply stated that testing for Mycoplasma is recommended for those ‘when the pretest probability is intermediate or high’, without specifying how that is to be determined. As a result – since the IDSA guidelines only recommend macrolide therapy as an afterthought for those at increased risk, without giving a clue as to how to determine that, the guidelines effectively do not advocate for macrolide use.

British Thoracic Guidelines for Pediatric CAP specifically recommend macrolides for very severe disease (presumably, requiring critical care): “Macrolide antibiotics should be used if either mycoplasma or chlamydia pneumoniae is suspected or in very severe disease” The Background should be appropriately modified; consider also modifying the tone of several sentences of the manuscript given this context.

We agree with the reviewer that the BTS guidelines do include that text. However, our statement 'Neither the American, Canadian, nor British guidelines recommend antimicrobials with activity against M. pneumoniae as first-line empiric treatment for pediatric CAP' is also correct, given that the BTS guidelines also state very explicitly 'Amoxicillin is recommended as first choice for oral antibiotic therapy in all children…' They also go on later to state 'IV antibiotics should be used in the treatment of pneumonia in children when the child is unable to tolerate oral fluids or absorb oral antibiotics or presents with signs of septicaemia or complicated pneumonia; recommended IV antibiotics for severe pneumonia include amoxicillin, coamoxiclav, cefuroxime, cefotaxime, or ceftriaxone.'

After this first-line recommendation, the BTS guidelines (like the IDSA guidelines) give a 'classical' description of clinical signs/symptoms of atypical pneumonia, which is not helpful, as defined above. We would also argue that a blanket recommendation to give all children with severe CAP macrolide treatment is also not helpful, given that a majority of these will not have Mycoplasma infection.

The use of "astonishing" is a bit of hyperbole (to describe 19% prevalence).

That word has been removed.

Can the authors be more specific than "explore the epidemiology of MP" in the objectives?

We have changed 'explore the epidemiology' to 'determine the prevalence.'

Can the authors provide a reference for the multiplex resp virus assay and the lab-developed MP/CP assay? Define HRLMP.

The HRLMP acronym has been expanded in the text (Hamilton Regional Laboratory Medicine Program). The multiplex respiratory virus assay has been used extensively by the Hamilton Regional Laboratory Medicine Program (HRLMP) for 10 years and with >100,000 clinical and research specimens. It was validated against culture and the Luminex RVP, and ongoing external quality control. It has been presented in abstract form and used in clinical studies and trials (see references below). The laboratory-developed MP/CP assay was validated against sequencing and external quality control materials, and has been used for clinical specimens for the past 3 years.

Ali M, Han S, Gunst CJ, Lim S, Luinstra K, Smieja M. Throat and nasal swabs for molecular detection of respiratory viruses in acute pharyngitis. Virol J.

2015 Oct 29;12:178. doi: 10.1186/s12985-015-0408-z. PubMed PMID: 26511714; PubMed Central PMCID: PMC4625558.

Loeb M, Dang AD, Thiem VD, Thanabalan V, Wang B, Nguyen NB, Tran HTM, Luong TM, Singh P, Smieja M, Maguire J, Pullenayegum E. Effect of Vitamin D supplementation to reduce respiratory infections in children and adolescents in Vietnam: A randomized controlled trial. Influenza Other Respir Viruses. 2019 Mar;13(2):176-183. doi: 10.1111/irv.12615. Epub 2019 Jan 4. PubMed PMID: 30328294; PubMed Central PMCID: PMC6379634.

I believe that Chlamydophila is the new genera for CP

There are two distinct lineages within the family Chlamydiaceae, and so it was suggested to split the genus Chlamydia into two (Chlamydia and Chlamydophila). However, this was a controversial decision, and more recently it was established that the family would only contain a single genus, Chlaymdia (Greub G Int J Syst Evol Microbiol 2010; 60: 2694).

On p.8 the lengthy description of all the comorbidities does not add value to the text and is redundant with Table 1.

This description has been removed.

Only 11% of subjects had not received influenza vaccine? That is surprising. Also, please specify what is meant by that. Do the authors mean that for eligible patients. e.g., patients >5 mos old, 89% had received influenza vaccine for that specific season?

We agree with the reviewer that this would be an unusually high rate of influenza vaccination. This was a retrospective review, not a prospective study – so if it was written that 'immunizations were up to date' in the chart, then it would be recorded as such. However, it is entirely possible that many study subjects were not actually up to date with influenza vaccination but that their caregivers stated that they were 'up to date' when interviewed by the clinical team. As a result, we have removed that datum, since it does not seem reliable.

DISCUSSION, p. 11. "MP was commonly" detected in critically ill children". 4% is not common.

This adverb has been removed.

P. 12 To whom are the authors referring to in "we thought that this…"

We have removed the word 'we' in that statement.

P. 13. I don't see the relevance of discussing Legionella treatment.

That sentence has been removed.

Please mention if requirement for informed consent was waived by the research ethics board

This has been clarified (the REB did waive the requirement for informed consent).

There are some limitations that need to be more clearly reflected in the title and text of the manuscript (please see reviewers specific comments that will need to be fully responded to). A major one being the actual relatively small number of positive Mycoplasma cases in the series. The most appropriate language/terms to use hinge on what may be regarded as rare vs. common, this is obviously debatable, but 4% in the overall cohort most would argue is not common but the important finding of 12.5% in the over 5's is relevant.

Reviewer-specific comments have been responded to, as suggested. The title has been modified (removing mention of Mycoplasma pneumoniae).

More discussion re. ?clinical relevance of 'low level positive' PCR results for Mycoplasma and some critique of the evidence for treatment directed against Mycoplasma and actual efficacy would be relevant in the discussion. The issue of macrolide resistance is hinted at but could be discussed more.

We have modified the following paragraph in the discussion to address these points:

One obvious issue is that we cannot be certain of the therapeutic benefit of antibacterials (such as macrolides or doxycycline) for pediatric CAP presumed to be caused at least in part by M. pneumoniae; one systematic review found no clear difference in outcomes between children treated with Mycoplasma-active agents and those without (20). Furthermore, the detection of Mycoplasma in the respiratory tract does not prove causation, as coinfections have been shown to be common (10) and some investigators have documented high rates of PCR-positivity in control persons (21) (although others have not (10,22)); some investigators have identified novel serologic tests that can confirm active infection (23).

Title delete "associated with Mycoplasma pneumoniae". Your findings are of interest in relation to all your patients NOT just the 10 with Mycoplasma.You can mention mycoplasma but it should not be your main message.

This has been done.

Amend your abstract and what this study adds accordingly.

This has been done.

Add a table describing all the organisms detected including the 8 bacterial organisms.

This information has been added to a new Table 3.

Table 1 - Highest level of resp support and antibiotics given in PICU should be in a separate table alongside duration of stay in PICU

This information has been added to a new Table 2.

VERSION 2 – REVIEW

REVIEWER	Reviewer name: Jesse Papenburg Institution and Country: McGill, Canada Competing interests: None
REVIEW RETURNED	19-Mar-2020

GENERAL COMMENTS	Thank you for the thoughtful responses to the initial comments; all concerns have been adequately adddressed

VERSION 2 – AUTHOR RESPONSE

We would like to again thank both reviewers and both editors. We very much appreciate your help with our research and for having given us this opportunity to submit our manuscript to your journal.