Title: Acute Lower Respiratory Infections in \(\geq 5\) year-old Hospitalized Patients in Cambodia, a Low-Income Tropical Country: Clinical Characteristics and Pathogenic Etiology

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Author’s response to reviews: see over
Dear Editor,

We thank the reviewers for their time on a second revision of our manuscript entitled Acute Lower Respiratory Infections in ≥5 year –old Hospitalized Patients in Cambodia, a Low-Income Tropical Country: Clinical Characteristics and Pathogenic Etiology.

Please find below our responses to the reviewers' comments and attached the revised version of the manuscript. All co-authors agree with these modifications.

Yours sincerely,
Sirenda Vong

Reviewer's report

Referee 1

Discretionary revision
The revisions are great. Only one last comment. I noticed that all the percent positives (i.e., for flu) are among those that tested positive (i.e., for any virus). I prefer to see this as a percent positive for all tested. I think that is more common in the literature and also gives an indication of prevalence. The authors are clear what they present but as a reader I prefer it the other way and would suggest changing it in your paper.

Response: We understand the reviewer's standpoint and we concur; however, to avoid confusing the readers we chose to present viral yield-related percentages using positive results as denominators to mainly be consistent with that of bacterial etiologies. Firstly, obtaining good quality sputum specimens is staff skills-dependent and can be challenging. Secondly, sputum culture may yield different specificity and sensitivity depending on bacteria types, and specimen's collection and transportation. Taking these arguments together, presenting the percent positive of those that tested positive for bacterial etiologies will make it easier to read and more comparable from one setting to another. In contrast, the testing validity is less of an issue when it comes to viruses because throat and pharyngeal swabbing and PCR testing are more sensitive and specific. As a result, viral distribution pattern would be similar using any type of denominators.

Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
None.
Referee 2

General comments
The authors have done a nice job revising the manuscript and addressing most of the suggestions. There were some suggestions that were not fully addressed and have been noted below. The authors are to be congratulated on nice work. It was nice to see inclusion of some vaccine policy considerations in the discussion. This topic could probably be expanded a bit as noted in the comments below. The manuscript would benefit some editorial and content revisions. The comments below detail some suggestions for content revision but do not include detailed editorial suggestions.

Major Compulsory Revisions
The author must respond to these before a decision on publication can be reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

1. Results. Bacterial etiologies. For non-AFB bacteria, especially H. influenzae and pneumococcus, it is important to know how many were diagnosed by positive blood culture (vs. sputum only). The authors stated that this change was made but the change did not fully address the issue.
   - I was not able to determine how many cases attributed to each bacterial etiology were diagnosed based on culture of normally sterile body fluid (eg, blood, pleural fluid) as opposed to sputum. This is an important distinction, because even with good quality sputum, false positives are possible. To this point, the reader will be suspicious of the fact that S. pneumoniae and H. influenzae were more common among ALRI patients without confirmed pneumonia than those with pneumonia (this can be inferred from Table 1).
   - Clear description of cases with positive blood or other sterile site cultures is also very important to allow comparisons with other studies that did not have sputum culture data.
   - The authors added data to table one to show how many bacterial diagnoses overall had positive sputum or blood. However, the reader still does not have the info by pathogen. For example, it would be very important to know the total number of cases attributed to S. pneumoniae, H. influenzae, etc and how many of each were diagnosed due to positive sterile site culture vs. sputum only.

   Response: to address the reviewer’s comments, we inserted data for blood and sputum separately for each bacterium in the bacterial etiologies section of table 1

Minor Essential Revisions

2. Table 1. I have trouble figuring out percentages. For example, 114 cases of H. influenzae is listed as 38.0% of ALRI. The total number of ALRI was 1904. The footnote states that the denominator for this percentage is the total with blood or sputum, which would be 1789 (from higher up in table 1). 114 divided by 1789 would be 6.4%. The authors appear to be using the number with positive blood or sputum culture as the denominator, but this is not what is stated in the footnote. I recommend very careful review of the table to be sure numerators, denominators, and percentages are very clearly shown. This could be very misleading to the reader.

   Response: corrected, thanks
3. Figure 1 and throughout paper. Authors refer to TB cases as ‘AFB’. They define TB disease in the methods as a positive AFB sputum smear. After defining, it would be clearer to simply refer these cases as having TB disease. Authors can mention the minor limitation of not having culture confirmation in the discussion. This point was raised in the first review. The authors said it was addressed, but it was not addressed.

Response: I am not sure what the reviewer meant here. We made the change from AFB to tuberculosis in Figure 2 (formerly Figure 1), as requested.

4. Results. “Of the 300 (16.8%) positive cultures, abscess-prone Gram-negative bacteria (39.6%) (i.e. Klebsiella pneumoniae, Burkholderia pseudomallei, Pseudomonas aeruginosa) and H. influenza (38.0%) were most frequent, followed by S. pneumoniae (17.7%).” What kind of cultures do the 300 refer to? Sputum, blood, other? These details are very important.

Response: we edited the phrase to read: Of the 300 (16.8%) positive sputum or blood cultures, abscess-prone Gram-negative bacteria ....

5. define terms like ‘abscess-associated bacteria’ in tables and figures (eg, figure 2) in a footnote or legend so that the table/figure can stand alone

Response: done, thanks

6. Results. “During hospitalization, 61 (3.2%) patients died, of which the following etiologic agents were identified in 22 (36.1%) patients including 7 (31.8%) with Burkholderia pseudomallei, 3 with AFB, 3 with various bacteria, 7 with viruses (including 5 rhinoviruses) and 2 with mixed infections.” When describing patients with rhinovirus (as in this sentence) or other viruses commonly detected with other viruses and with uncertain etiologic role, it would help the reader to say ‘rhinovirus alone’. It makes it clear than rhinovirus was the only virus detected.

Response: we agree and made the change accordingly

7. Results. “When compared to patients who improved at discharge, the following factors were associated with mortality: B. pseudomallei infection (4.1% vs. 31.8%, p<0.001), diagnosis of pneumonia (45.3% vs. 70.5%, p<0.001) and elevated blood neutrophils count (34.7% vs. 49.2%, p=0.024);...”

In this sentence, it is not clear which percentage goes with which group in the comparisons. Suggest: “When compared to patients who improved at discharge, those who died in hospital were more likely to have B. pseudomallei infection, diagnosis of pneumonia, and elevated blood neutrophils count. Add percentages in parentheses for each comparison with the percentage for the fatal cases listed first.

Response: done, thanks

8. Discussion. “however, detection of some respiratory viruses, especially rhinoviruses and RSV, was frequently found in non-ill or non-respiratory infected control groups when using highly sensitive molecular diagnostic techniques [13, 34-40].” The authors should reconsider
including RSV in the same category as rhinovirus as being frequently found in asymptomatic controls. RSV is certainly detected among controls but at much lower rates than ALRI patients. I think there are several papers to support, including “A Preliminary Study of Pneumonia Etiology Among Hospitalized Children in Kenya. Hammett et al. CID 2012”

Response:  we concur and excluded RSV of this group

Discretionary Revisions

1. Case definitions. From a methodological standpoint, it is not clear why the authors would create a category of patients whose illness was ‘most likely caused by a virus’. This point was raised in the original comments. It also creates some confusion in the results. When the authors refer to “viruses” as in figure 2, does that mean actual viral detection or “likely viral etiology”? Another example of confusion in the results is: “In contrast, a viral etiology was predominant in ALRI patients with a normal CXR (67.5%) including the pleural effusions group (54.5%) and ALRI with normal CXR group (74.3%) (Figure 1)” Further, this definition of ‘mostly likely virus’ could easily include cases caused by certain bacteria, such as Bordatella pertussis, mycoplasma, or chlamydia. The authors imply that this clinical definition will help clarify whether detection of a virus implies causality. It is not clear how this clinical definition helps with inference. The best way to address the question of causality is to test for the same viruses in a control group. I encourage the authors to drop the clinical definition of ‘likely viral etiology’.

Response: we agree and deleted the group from the methods section. In the results section, we added more clinical and radiographic information regarding these patients whose ALRI’s etiology is compatible with viral infection alone. We hope we conveyed the point more clearly

Interestingly, of the 796 patients whose blood and sputum specimens were negative for bacteria, 121 (15.2%) patients presented with an elevated blood lymphocyte count or normal neutrophil count on admission. The patients had no radiographic images indicative of TB, no necrotizing pneumonia, and no use of antibiotics preceding hospitalization.

2. Results. Factors associated with identification of microorganisms. The association between age and viral vs. bacterial etiology is heavily influenced by the lack of sputum culture in younger children. This is in addition to the known challenges of getting high volume blood specimens for culture in children. I don’t think this comparison adds much to the paper and could be deleted.

Response: We respectfully disagree with the reviewer’s suggestion. We agree with the reviewer that bacterial infection is difficult to detect in children, which makes comparison of results between age groups challenging. However, we also want to mention that positivity rate for viral infection is inversely proportional to age. This result is less disputable as viral detection from throat and pharyngeal swabs and PCR testing are more specific and sensitive methods.

3. Figure 3. For the reasons stated above, this figure does not add much to the paper and could be deleted. Further, these results could be easily summarized in the text and does not need a figure.
Response: thanks, we removed the figure

4. Results. “Report of antibiotic intake prior to hospital admission was not associated with a negative sputum (p=0.854) or blood (p=0.275) culture.” The reader cannot interpret this comparison without the percentages in each group, or just give the overall percentage of pre-culture antibiotic use for blood and sputum cultures and state that positivity rates did not differ by pre-culture antibiotic use.

Response: we added a sentence to reflect the reviewer’s comment

5. Discussion. Paragraph 1. ‘Poor’ is non-specific. Suggest using ‘low income’ as more standard term.

Response: ok

6. Discussion. “On the other hand, there is emerging evidence, particularly shown in African studies, that M. tuberculosis is commonly present in acute community-acquired pneumonia in children [15,19] and adults...” Reference 15 does not seem appropriate for this sentence. Please confirm.

Response: Ref # 15, which refers to studies conducted in Asian settings, also mentions TB as possible cause of acute pneumonia. We edited the phrase to reflect appropriately the references

7. Discussion. “However, of a random sample of 24 ALRI cases (median 58 years of age, range 18 – 78) for which we also collected urine samples, three (13%, 95% confidence interval 3% - 32%) patients tested positive for S. pneumoniae antigen by Binax NOWÒ rapid immunochromatographic assay (Scarborough, ME). (IPC, unpublished data).” The point of including these data is not completely clear. Are the authors using the data to support that pneumococcus is likely causing more or less pneumonia than they documented by sputum and blood culture? The paragraph may just need editing to make the point clear.

Response: we hope our edits have made the phrase clearer

8. Discussion. “…which could have been prevented with childhood anti-pneumococcal and H. influenzae b vaccines in Cambodia.” It is good that the authors raise this point. I suggest using the precise name of the vaccine. For instance, for pneumococcus, presumably the authors are talking about pneumococcal conjugate vaccine and not polysaccharide vaccine. It would be nice if they mentioned the current status of considering PCV and Hib conjugate vaccines in Cambodia, a country that is eligible for GAVI supported vaccine implementation.

Response: we added a sentence to reflect the reviewer's comment

9. Discussion. “Moreover, sputum tests, which are commonly used to diagnose pneumonia, have poor sensitivity and are frequently contaminated by saliva or upper respiratory tract colonization.” The authors should also acknowledge the lack of specificity of sputum culture.

Response: this information has been added
Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests