Author's response to reviews

Title: Viral etiologies of lower respiratory tract infections among Egyptian children under five years of age

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Author's response to reviews: see over
Dear Dr Krishnan,

Re: Manuscript reference No. MS: 1835372716666585

Please find attached a revised version of our manuscript “Viral etiologies of lower respiratory tract infections among young children in an Egyptian children’s hospital using three different laboratory methodologies”, which we would like to resubmit for publication as a research article in BMC Infectious Diseases.

Your comments and those of the reviewers were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following pages are our point-by-point responses to each of the comments of the reviewers as well as your own comments.

Revisions in the text are shown using red colour for additions, and strikethrough font for deletions.

In accordance with reviewer 1’s suggestion, we changed the title of the paper to “Viral etiologies of lower respiratory tract infections among Egyptian children under 5 years of age”. We also added to table 1 the description of clinical diagnosis upon admission of patients. We referenced a review at the topic in the background section, and referenced more studies that were conducted in the Middle East region investigating the burden of a number of respiratory viruses. “previous studies” in the discussion section was replaced by “consistent with the results of studies conducted in other countries”.

In accordance with reviewer 2’s suggestion, we have modified the manuscript to mainly focus on the viral etiologies of LRTIs infections among young children. However, the results of the different laboratory methods were highlighted as a minor point in the study.

In accordance with reviewer 3’s suggestion, we added the criteria used for inclusion and exclusion of subjects in the methods section. We also added that the patients’ respiratory rate was counted. We added the mean and the median age of patients in the test. We omitted figure 2 and replaced it by table 3. Figure 3 is changed to bar chart, and now is called figure1. Figure 4 is also changed in a bar chart, named figure 2. Also, the comparative data for each method is given by a two by two tables and is submitted as additional files.

We have also omitted figure 1 and 2 (therefore renumbered the following figures) and added the positive viruses data in numbers and percentages to table 2 as suggested by all three reviewers.
We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in BMC Infectious Diseases.

We shall look forward to hearing from you at your earliest convenience.

Yours sincerely,

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Responses to the comments of Reviewer #1

1-The text throughout the manuscript needs to be edited for clarity and elimination of redundant wording and correct use of idiomatic English.

Response: Extensive effort was made to correct and edit the English language and avoid any redundancy.

2-For clarity and impact, consider revising the title of the paper to read “Viral etiologies of lower respiratory tract infections among Egyptian children under 5 years of age”

Response: We changed the title of the paper as suggested by the reviewer.

3-The Background section requires further elaboration to clearly describe what is known regarding the etiology of pediatric respiratory viral infections in other regions of the world and Egypt in particular. What was the ultimate purpose of the study? To compare diagnostic procedures (this has been extensively done) or to utilize diagnostic procedures to determine the contribution of different viruses to the etiology of pediatric cases of LRTI in the region (this is novel and what readers are interested in)?

Response: We have modified the background section to include studies done in the Middle East region investigating the burden of a number of respiratory viruses in children such as RSV, and human metapneumovirus. However, a comprehensive investigation of the viral etiologies of LRTIs among children was not conducted recently in the Middle East countries. Therefore, the results of similar studies in other parts of the world were cited. The manuscript has been modified to mainly focus on the major topic which is the viral etiology of LRTIs among children.

In the second paragraph of the Background section the authors refer to previous studies. Where were they conducted? In Egypt? If not, it may be better to reference a review on the topic.

Response: Previous studies refer to studies done in Europe, this is now explained in the background and a review has been cited as a reference.

In the last paragraph of the background section the word “role” should be replaced by “performance”.

Response: We replaced the word “role” with “performance” as suggested by reviewer.

4-The paper would benefit from the description of the clinical presentations of the patients sampled. Were all 450 cases pneumonias?

Response: The patients were diagnosed as having LRTIs by assessing the clinical presentation (this has been added to the methods section). The LRTIs could include bronchiolitis, bronchitis and pneumonia. In the study design, the pneumonia could only be confirmed by X-ray. Since x-ray
examination was not available for all patients, we could not state that they were all pneumonia cases though the clinical symptoms matched the IMCI symptoms for pneumonia.

5-Figures 1, 2, 4 and 5 should be replaced by tables to facilitate comprehension of the results.

Response: We omitted Figure 1 and the number of positive samples and percentages were added to table 2. We replaced figure 2 by table 3. Figure 4 (now called figure 2) has been changed to bar chart to reflect the percentages of each viruses detected by each method. This figure is highlighting to the reader that the use of a particular lab method can underestimate the prevalence of one virus or another.

Figure 5 was removed from the manuscript.

6- In Table 1, it would be nice to see an added description of clinical diagnosis on admission unless all studied cases were pneumonias.

Response: We added the clinical symptoms of the patients to the text and to table 1. These clinical symptoms are all of the LRTI pediatric patients that were enrolled in the study.

As all the patients were sampled at the same clinic, what does the geographic area refer to? Area of residence of the patient?

Response: Yes the geographic area represents the area of residence of the patient. This has been added to the text for clarification.

The footnote of the table is not clear. It may be best to write a number for each category and describe in the footnote that this n represents the number of patients providing an answer to the questionnaire.

Response: The footnote has been changed as suggested

7- In the discussion at the top of page 6, the use of “previous studies” is confusing as the studies were not performed in Egypt. It may help to write “consistent with the results of studies conducted in other countries” or similar.

Response: We changed the sentence as suggested by reviewer.
Responses to the comments of Reviewer #2

1. This manuscript includes potentially important information on etiology of lower respiratory infections. In conclusion, RSV and adenovirus were found to be common causes. However, this was expected and not a novel finding. What results are considered to be characteristic to children in Egypt, comparing with data from developed countries and developing countries? Such discussions should be added, which may increase significance of this study.

Response: We discussed the agreement and the disagreement of this study with other studies from developed countries and the Middle East region. However, there is no study conducted in the Middle East region that detected several respiratory viruses simultaneously using PCR. We referenced a study conducted in Turkey recently that detected a number of respiratory viruses, and discussed our results in the light of their results, taking into consideration that they used different assay for each virus.

2. In this manuscript, two major research items are included; (1) to identify causes of lower respiratory infections, and (2) to assess the methods for virus identification. However, this reviewer thinks that the inclusions of these two major aims have complicated the context and made focus of this study very unclear. This reviewer feels that the first part of the study, i.e., etiological study, should be the main content of this manuscript. There seems to be no novel finding in methodology to identify virus. It is a matter of course that rt-RT-PCR is the best among the methods authors employed. If any low-cost method was found to be effective and comparable to rt-RT-PCR, this is outstanding finding, and should be reported as a recommendable method, instead of rt-RT-PCR. However, such new methodological development is not described in this manuscript. If authors want to mention that some different methods were tried, this should be written briefly in the method section, and state which method(s) was used for identifying virus, as a final result. Therefore, authors should revise the manuscript considerably, especially structural arrangement of the contents.

Response: We modified the manuscript and considered using different lab methods for viral detection as a minor (not the main) objective of the manuscript. The technical part concerned with methodologies of detection is now used to illustrate how the detection method can help in identification of the true viral burden contributing to the disease.

3. Regarding results of identified viruses: Authors must describe which method was used to obtain the final identification of virus. Did authors used data of rt-RT-PCR only, or rt-RT-PCR and other additional method (when rt-RT-PCR gave no results, but the other method could identify the virus)? This point must be clarified and described in the text.

Response: The real time PCR method was mostly the method to obtain the final results except for 2 samples. The first one is an RSV positive sample that was positive by DFA and negative using real time PCR, and was added to the RSV positive samples. We confirmed the results of the real time PCR, and it was negative. However, we could confirm that it is RSV positive using conventional PCR. The other samples was positive for HPV-3 using the DFA and the SVC and negative by real time PCR. We could not explain this phenomenon, and the samples were depleted. This data is provided in the supplementary files showing the sensitivity and the specificity for each method.
4. Figure 1 should be removed because it has duplicated information with Table 1. If authors want to include total numbers of identified viruses, this information can be added to Table 2, as an additional line at the bottom, as a total number.

Response: Figure 1 is omitted as suggested and the information was added to table 2

5. Figure 4 and 5 should be deleted because methodological evaluation is not the main topic of this manuscript, as described above.

Response: We modified the manuscript to consider using the three methods as a minor point. We also modified Figure 4 (now called figure 2) to highlight that the use of different lab method can affect the relative importance of one virus versus the other. For example, the prevalence of adenovirus is almost the same as HPIV-3 and HPIV-1 according to SVC. However, using a more sensitive method to that particular virus (rt-PCR) revealed its true prevalence, which is much higher than the previously mentioned viruses.

We omitted figure 5.
Responses to the comments of Reviewer #3

1. What definition for LRTI was used? Were the IMCI criteria used for classification of lower respiratory tract infection, fever is not a criteria for defining LRTI, the criteria described for LRTI in the paper i.e. difficulty in breathing, chest in-drawing, and inability to feed are all criteria of severe disease and these children are usually admitted to the hospital, further was respiratory rate measured for these children? Please provide definition of LRTI used in the study.

Response: The exact case definition of LRTIs that was used to enroll the children in this study is as follows:

The presentation of a child of age less than or equal 60 months to the emergency room, or the outpatient clinic with any combination of cough, difficulty breathing, fever, chest indrawing, and fast breathing of more than 50 respiratory rate/minute for children under 12 months and >40 respiratory rate/minute for children from 12 months to 5 years.

Results of chest X-ray (if available) was obtained at the time of admission. The episode would be designated as a radiologically confirmed pneumonia case if an area of consolidation and/or pleural effusion were determined on the chest x-ray.

Our criteria matched the IMCI criteria for pneumonia, however, we preferred radiological confirmation (if available) to differentiate pneumonia from bronchiolitis. Of the 450 patients 339 (75%) had chest indrawing, 438 (97%) presented with cough, 415 (92%) had difficulty in breathing, and 325 (72%) were feverish. Sixty-four patients (14%) were hospitalized.

This information was not mentioned in the previously submitted version of the manuscript, but was added to the revised submitted version.

2. For each virus before the % the number positives should be given. Figure 1 can be deleted as this can be described in text figure does not provide any additional information. In fact if a total row is added in table 2 at the end, the same information can be compiled in table 2 and figure 1 can be omitted.

Response: We omitted Figure 1 is as suggested and the number of positive samples and their percentages was added to table 2.

3. Figure 2 is not well representing the facts, this figure is showing how many cases of each virus infection were co-infections, the % is from the total positivity for that virus, thus in a graph it gives a wrong impression about the % prevalence for each virus. This data can also be presented in a table giving the % prevalence for the virus and then how many of these were co-infections

Response: We omitted figure 2 and replaced it by table 3. Table 3 is showing the exact viral etiology of patients, which shows reader the co-infections as well as the frequent co-infections detected. We hope that the table is now reflecting the exact situation.
4. Figures 3 is all jumbled up this should be a bar graph for each virus and for each month further if the numbers are very few then data should be represented in numbers rather than %. In the line graph also some of the colours of the lines are very similar and thus not easy to decipher

Response: Figure 3 (now called figure 1) is changed to bar chart (Figure 1) for ease of comprehension.

5. Figure 4 is totally wrong this not a trend that it should be represented in a line graph. The comparative data for each method should have been given in a two by two table with sensitivity and specificity.

Response: Figure 4 (now called figure 2) has been changed to bar chart to reflect the percentages of each viruses detected by each method. This figure is highlighting to the reader that the use of a particular lab method can under estimate the prevalence of one virus or another. We also prepared the two by two table for the sensitivity and the specificity of the methods. We are submitting these tables as additional files.

Minor revisions

1. I am surprised that all children with LRTI were enrolled from the out patients department that would exclude all children with severe LRTI as they are usually admitted to the hospital

Response: In this hospital the children initially present to the outpatient clinic or the emergency room for initial diagnosis. The severe cases are usually hospitalized. Sixty four of the 450 patients (14.2%) enrolled in the study were hospitalized. Also, some of the outpatients needed supplementary oxygen. These data has been added to the text in the results section.

2. Were all children seen in the OPD with LRTI enrolled in the study if not what was the criteria for enrollment?

Response: No, not all the children seen in the OPD were enrolled. Patients were excluded, if they have been hospitalized in the week prior to presentation, they have already entered the study for the same episode of illness, or if their parents were unwilling to participate.

3. The mean and median age of the children should have been given along with M: F ratio

Response: The mean and median age have been added to the text

4. Page 4 prevalence of respiratory viruses among patients : Respiratory viruses were detected in how many children in total by all methods ? only % is given

Response: At least one respiratory virus was detected in 269 (59.9%) of the patients. The number 269 was added to text.
5. It is strange to note that the sensitivity of each method (fig. 5) increased on day 21-28 or was similar to day 0-3. The authors have tried to give some explanation for this in discussion but the figure gives a wrong impression to the reader.

Response: We omitted figure 5.

6. The legends to the figures are not explanatory and are very brief.

Response: We modified the legends to be more clear.
Editorial Board Comments
Editorial Requirements:
**Request for copy editing.

After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further.

Response: The manuscript has been copyedited by the recommended Edanz group. (www.edanzediting.com/bmc1).