Author Response 1

REVIEWER 1.

Major remarks:

1. "Statistics is partially doubtful. e.g. lines 202-203. SD is higher than mean values. How to interpret 9±24 days? Is the difference of 1 day significant in such case? Wrong statistical test was used. Line 202-303. Simple calculation with T-test shows no significance in both cases. This way I cannot be certain about other p-values, and the conclusions of the study."

We thank the reviewer for their feedback. We used non-parametric tests for the hospital and ICU length of stay due to the skewed distribution of the data. We acknowledge the reported mean and standard deviations was led to apparent contradictions and difficulty in interpretation. In our revision, we have replaced these values with the median and interquartile range in the text and Table 4 to more accurately represent the findings. We have chosen to evaluate length of stay using non-parametric rank based tests including the Wilcoxon Rank Sum and Kruskall-Wallis, which have shown to result in lower error rates (see new reference #28: Chazard, Ficheur, Beuscart, and Preda, 2017). As t-tests rely on assumptions of normally distributed errors and/or large sample size for the central limit theorem to apply, we believe that the non-parametric tests are more appropriate in this case.

Minor comments.

1. "Line 136. Unit is not clear."

Thank you very much for catching this error. We have changed the units to the correct kg/m2 units.

2. "Figure 3 is difficult to interpret."

Thank you for pointing this out. To aide in interpretation of the distribution, we have created violin plots of the hospital and ICU length of stays for the VI and NI groups. We have further included reference lines indicating the median value for each group.

REVIEWER 2.

"I thank the authors for this clear and informative manuscript. In this retrospective observational cohort study Melamed et al. compared two groups of patients: a group with influenza infection (including more than 50% of H1N1) and a group with other viral infections. This manscript aimed to describe secondary pulmonary infections and outcome of these patients in Non-Influenza Viral Respiratory Infection. They observed that co-infections with bacteria are also frequent in this context."

Thank you; we are glad that the reviewer found our manuscript informative, and we welcome the comments below. We hope that we have satisfactorily addressed them.

Major comments:

-"Inclusion criteria: "positive results from viral studies within the first 10 days of admission"; exclusion criteria: "Patients with onset of secondary pneumonia late into the admission (defined as more than two weeks) were excluded". If I understand correctly: patients were included if they had bacterial co-infection at admission and up to 15 days post admission. There is a mix of community

acquired pneumonia (i.e. bacterial infection acquired before/during/after viral infection outside the hospital) and health associated pneumonia (nosocomial pneumonia). These two diseases are strictly distinct regarding physiopathology, pathogens, etc…It is not possible to perform a global analyze. It is a critical point."

We acknowledge the reviewer's concern, and wanted to express that the primary intent of this study is to analyze whether the epidemiology (e.g., incidence and etiology) of secondary bacterial pneumonias that complicate a primary viral infection differs based upon the type of viral infection, regardless of whether the infection was initially acquired in the outpatient or inpatient setting. We agree that the pathogenesis of pneumonia in a generally healthy community dweller is different from a nosocomial pneumonia acquired in a chronically institutionalized host. However, the primary aim was to test the hypothesis whether influenza is uniquely associated with secondary bacterial pneumonias, as is often suggested. We found that indeed, influenza does not uniquely predispose to secondary infections. In addition, we wished to investigate the clinical impact of secondary pneumonias following primary viral infections, and whether these secondary infections contribute to increased death or hospital length of stay, or are simply an "epiphenomenon" as some have recently argued. Our data suggest that secondary infections do contribute somewhat to mortality with almost twice the mortality rate compared to viral-infected patients without pneumonia. Altogether, these findings form a basis for mechanistic studies that can help us understand why different patterns of co-infections seem to emerge, which we (and other groups) are trying to model in experimental animals with co-infections.

- "There is an important difference between the two groups regarding the number of lung-transplant and immunocompromised patients (higher in the NI group). The immunocompromised status is known to favor bacterial or fungal infections and may bias the final significant difference of secondary pneumonia in the NI group (44%) compared to the VI group (23%). Furthermore, the presence of CMV or HSV pulmonary infections may sign a severe immunosuppression in some patients of the study increasing the bias between the 2 groups. Finally, these immunocompromised patients are more likely to be infected by specific types of microorganisms because of their more frequent contact with the healthcare system. It seems complicate to extrapolate these results to the general population."

We completely agree with this statement. That was one of the interesting findings of this study, because frequently, in large population-based studies, patients with acute respiratory viral infections and pneumonia are often all grouped together, but our study design enabled us to demonstrate the considerable heterogeneity of patients with viral infections and secondary pneumonias. Furthermore, our findings underscore the importance of recognizing the host immune state in assessing risk of secondary infections. We hope that our work will provide the reader with an improved understanding of this group of patients, allowing for different empiric antimicrobial treatment on admission. We have expanded our discussion to reflect these points. In addition, with increasing use of immunosuppressive medications for a variety of different conditions, it would be helpful for clinicians to understand that the standard treatment approaches for a general population may not cover the appropriate organisms found in immunocompromised populations. However, comparing the epidemiology of post-viral pneumonias in immunocompetent and immunocompromised was not the primary focus of this study.

- "The screening Methology used ICD-9 codes. While this method is known acknowledged as a powerful epidemiologic tool, the codes for microbial etiology have poor performance (especially ten years ago) and it is not clear how it is used here. Were the 2824 admission files reviewed or screened by ICD-9 codes? Can you: (i) clarify this point and (ii) refer to articles that validate this strategy."

We have clarified this point in the text (under "Methods, lines 106-109 in revised version) to address any ambiguity. ICD-9 codes for all viral pneumonias or pneumonias caused by "other" or "not specified" organisms were used to select the 3,316 charts from all admissions during the study time period. From there, manual review of each chart took place to arrive at final 134 charts use for final analysis. Since ICD-9 code was used only to obtain our first sample from which to perform manual chart review, we did not find an additional reference to validate this screening method, as we did not use ICD-9 codes to determine microbial etiology. We agree, this would have been a poor method to accurately identify true viral respiratory infections. Instead, ICD-9 codes were used only to narrow the patient list from all admissions over this 3 year period to admissions that might be relevant to our inquiry. Actual viral infections and presence/cause of secondary infections were ascertained by manual review of the 3,316 charts by the investigative team.

Minor comments:

-*" The correct nomenclature for the influenza virus responsible for the 2009 pandemic is A(H1N1)pdm09. Please edit accordingly."*

Thank you; we have made these changes throughout the text.

- *"The paper deserves to be clearer about the ICU stay of patients and ideally proportion of mechanically ventilated patients. The number of patients admitted to ICU is described in the figure 3, but must be also indicated in the text or even in the table 2, ideally with a score to compare the patients' gravity. The proportion of ICU patients seems higher in the NI group."*

This is an excellent point. We have added the statistic regarding percentage of patients admitted to the ICU in both the VI and NI groups (39% vs. 62%, p = 0.011). As you suggested, the NI group was admitted to the ICU more often, indicating that this was a sicker group overall. Unfortunately, we do not have data on mechanical ventilation, but we hope that the ICU admission data will serve a similar purpose to indicate the degree to which each group was critically ill.

- *"The paper deserves to be also clearer about the "respiratory symptoms" having justified a hospitalization and if possible, objective vital parameters such as respiratory rate. Criteria based on scores validated in pulmonary infections would bring even more informations (e.g. CRB65, CURB65)."*

Thank you. We have clarified what we meant by respiratory symptoms (cough, rhinorrhea, shortness of breath, or hypoxia) in the revised methods (lines 109-110 in revision). Since this was a retrospective review, clinicians did not uniformly use a scoring system, such as CURB65, to determine patient disposition, as this information was therefore not readily available. We acknowledge that this may led to some heterogeneity among the patients who were admitted to the hospital rather than discharged home. We had addressed this in the limitations section as well.

- *"In the VI group, it would be preferable if the authors provide more details about the proportion of secondary infected patients specifically in the A(H1N1)pdm09-infected patients."*

This is a valid point, and we have described these infections further. We have now added this information to the results section. Nearly half of all secondary infections in viral influenza were caused by S. aureus. The remainder of the infections are detailed in the results section.

- *"H1N1 pandemic in 2009 was a specific time in the hospitals. In the VI group, the study mixes H1N1 patients (> 50%) and with "most classic" influenza infection. Can we so easily mix these categories of patients?"*

While there are differences between these 2 viral infections, our intent was primarily to draw attention to non-influenza respiratory viral infections. There have been many descriptions of secondary pneumonia in influenza as well as influenza A(H1N1)pdm09 specifically. For purposes of this discussion, we chose to discuss these together. Other work has examined secondary infections after influenza A(H1N1)pdm09 infection (references 13-19).

- *"The predominance of the S. aureus as the first found pathogen in the VI group is, as you say in your paper, quite astonishing and very interesting. In ICU, the study by Yap et al. have found an increase in Methicillin-Resistant Staphylococcus aureus (MRSA) implicated in ventilator-acquired pneumonia (VAP) after the SARS-CoV1 pandemic in 2002-2003. It is important to note that approximately half of the VI group was infected by H1N1, also during an epidemic time: the secondary S. aureus infections in your study may have been caused by MRSA which may emerge in epidemic situations, because of nonscrupulous hygiene precautions (e.g. increased use of gloves, less frequent hand hygiene) or heavy use of antimicrobials active against gram-negative organisms. What was the proportion of MRSA infections? The high proportion of S. aureus infections can be eventually explained by a bacterial cross-transmission because of the epidemic situation, and we probably need a clarification about this eventuality."*

This is an interesting point. We have re-examined our data, and found that 3 of the 6 *S. aureus* infections were confirmed MRSA. This new information has been added to our manuscript.