Reviewer's report

Title: Nosocomial outbreak of the 2009 pandemic H1N1 Influenza A in critical hematologic patients during seasonal influenza 2010-2011: relevance of oseltamivir resistant variant viruses

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Reviewer: THIAGO M SOUZA

Reviewer's report:

The manuscript (MS) entitled “Nosocomial outbreak of the 2009 pandemic H1N1 Influenza A in critical hematologic patients during seasonal influenza 2010-2011: relevance of oseltamivir resistant variant viruses” by Pollara et al., is presented by the authors as a retrospective observational study on hematological patients that had nosocomial infection by the influenza A H1N1 2009 pandemic virus (H1N1pdm09). The magnitude of the nosocomial infection in these patients varied from mild to severe/fatal. The authors also argue that some of their patients “acquired” oseltamivir-resistant (OST-r) viruses.

The topic this MS regards is very important, emergence of OST-r H1N1pdm09 virus in immunocompromised individuals, and merits publication after major revision. Although it has been described that: i) circulation of OST-r H1N1pdm09 virus in Italy occurred (Puzeeli et al., 2011, Campanin et al., 2010 and Esposito et al., 2010), ii) immunocompromised hospitalized patients with H1N1pdm09 are expect to shed influenza for longer periods of time (Souza et al., 2010, among several other reports), and iii) immunocompromised individuals are more likely to have worse clinical outcomes for influenza then the general population – it is still important to registered the original data from this work in the literature. This is because influenza studies on immunocompromised patients came in general from single centers with very limited number of patients included and only accumulation of these data in the literature may lead to further meta-analysis on the topics mentioned above, in order to produce more statistical sound information.

1. Is the question posed by the authors well defined? yes
2. Are the methods appropriate and well described? Fair, although additional experiments (such as virus isolation in cell culture and pyrosequencing assays would enhance the quality of the work) – the methods used by the authors are enough to address the questions posed by the authors.
3. Are the data sound? yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? No, the authors should have been more careful in presenting the Table 1 and Figure 1. For both, data are missing.
5. Are the discussion and conclusions well balanced and adequately supported
by the data? Fair, the authors should make a more detailed comparison with other cases of OST-r in Italy and with other works in prolonged H1N1pdm09 shedding in immunocompromised patients. They are also encouraged to weak their conclusions on nosocomial transmission.

6. Are limitations of the work clearly stated? Although it is, in my option the limitation presented precluded further conclusions on nosocomial transmission of the virus.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Not extensively, please see the answer to question number 5.

8. Do the title and abstract accurately convey what has been found? The authors should weaken their conclusions on nosocomial transmission.

9. Is the writing acceptable? Major English revision should be made.

Minor Essential Revisions
1. Do not mention % for very small number of patients. For example, just state 3/23 patients instead of 33%; or, in results, it is not necessary to refer to 100% deaths in the ICU when the authors mentioned that all three patients admitted to the ICU died.

2. English revision throughout the MS is necessary

3. Standardization of terms: influenza like, influenza-like or flu A likeness; immunocompromised or immune-compromised; rRT PCR or RT-PCR.

4. Follow WHO recommendation for abbreviation of 2009 pandemic virus

5. Background: The pandemics started in Mexico and USA.

6. Punctuation revision throughout the MS, specially next to the references

7. Methods: Remove the ethical aspects of the MS from the Sanger sequencing of NA sub-section. An new section on ethical aspects should be created

Major Compulsory Revisions
1. Semantic revision is necessary throughout the MS, see an example below:
   a. Abstract/results: “patients who developed the H275Y mutation”; in fact, the viruses carrying this mutation emerged. In the next sentence, which treatment is been mentioned: for cancer?

2. Sanger sequencing is mentioned to be the “gold standard” method for sequencing – it is hard to say that in the ultra-deep sequencing era.

3. Methods/Study population and setting: Last sentence should come previously in the text

4. Methods/RNA extraction…: Which nucleic acid did the authors extract RNA or DNA?

5. What were the criteria to define mixed bases at position 823? Did the authors considered any peak as relevant?

6. Results and Discussion: The authors stated: “patients who developed the
H275Y mutation”, “…patients 2, 4, 5 acquired oseltamivir resistant 2009 pandemic influenza…”, “… patient 5 became infected by a drug resistant virus after prolonged therapy…” and “We found a relatively high incidence of drug resistant viruses…”. In these passages there are some misconceptions. according to the data presented by the authors drug resistant virus emerged during treatment. Patients did not acquire resistant virus or develop the H275Y mutation. Besides, no isolation of virus in cell culture was performed to confirm that viruses detected in the patients are infectious.

7. Results: More attention should be paid in regard to the presentation of Table 1 and figure 1.

a. Table 1 is cut at patient 6. Besides, the reason for admission for all patients seems to be basically the same, fever, making the 4th row unnecessary.

b. Figure 1 is very confusing. Symbols for PCR diagnosis are positive a day prior to the onset of illness and, only with a new sample in the subsequent day, PCR for H275Y was performed. I believe that the charts do not translate what the authors did. In the list of labels, the symbols for Private room droplet and contact isolation and Therapy with Tamiflu are missing (presumably one is the blue line and the other is the gray box). For the patient 6 there is a black thick line, not mentioned in the list of labels. The symbol for death due to Flu A pneumonia presented in the list of labels was not used; instead, two other different symbols were used to assign the patients that deceased. Which are the other symptoms appeared in patients 3, 5 and 6 (double-lined arrow)? Controversially, although the authors state that there was a nosocomial outbreak, onset of illness for patient 6 did not occur during hospitalization – he/she was an outpatient.

8. Criticism on case definition and conclusion on nosocomial transmission: The study was performed during the 2011 winter in the northern hemisphere, was the circulation of H1N1pdm09 virus significant in the community? Phylogenetically, does the virus detected in the hospital cluster separated from other sequences form Italy in 2011? Why was patient 1 considered the index case? The authors should explain, in the context of nosocomial transmission, how patients 4 and 5 onset of illness occurred while patients 1, 2 and 3 were in contact isolation. Immunocompromised patients have atypical manifestation of influenza, since flu-like illness could be masked by a variety of symptoms. Did the authors systematically search for respiratory virus outbreaks in this hospital? How is it performed?

9. The authors should compare their results on OST-r H1N1pdm09 virus with other studies on drug resistance in Italy (Puzeeli et al., 2011, Campanin et al., 2010 and Esposito et al., 2010) and with other studies H1N1pdm09 shedding (Souza et al., 2010).

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