Author's response to reviews

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

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Author's response to reviews: see over
Dear Tonie,

please find attached the article: MS: 3493394616909122 “Nosocomial outbreak of the pandemic Influenza A (H1N1) 2009 in critical hematologic patients during seasonal influenza 2010-2011: relevance of oseltamivir resistant variant viruses.”

Pollara C P, Piccinelli G, Rossi G, Cattaneo C, Perandon F, Corbellini S, De Tomasi D, Bonfanti C. to be considered for publication in BMC Infectious Diseases.

We have done the required corrections of referees, and hope that the present version of our work will meet the requirements for publication in BMC Infectious Diseases.

Thank you for your attention.

Yours sincerely,

Caterina Pollara

Reviewer: Sallene S Wong

Reviewer's report:

1. The sample size is too small, of the 26 patients was infected by 2009 pandemic H1N1, and only 3 patients were identified to develop the H275Y mutation and one of the three developed the mutation had mixed H275Y.

The infected patients are 23 by 2009 pandemic H1N1

2. It requires some clarification in the manuscript; all 6 patients were listed as oseltamivir resistant in Table1, which is different from the description in the text and Figure 1. Referring to the results in the abstract, it was written as “Three cases (patients) of oseltamivir resistance to 2009 pandemic H1N1 influenza A virus were identified….”

Table 1 corrected: 3 patients have had a oseltamivir resistance
3. There is no mention of the resistance testing result on the second positive Flu A sample collected from deceased Patient 3.

Done is in Fig 1

4. It may not be appropriate to state that mortality rate is 33% (1/3) due to the H275Y mutation because the two survived patients also had the H275Y mutation.

Done, I am agree

5. In the first paragraph of discussion section, “of” is missing from this sentence: “one them died in ICU (33%)”

Done

6. The symbol for “Death FluA pneumoniae” is different in the legend as compared to in Figure 1.

Done: the symbol is Ψ

7. There should be consistence on naming of the products and supplies, for examples, are these two the same: commercial real-time rRT-PCR assay (Flu A/Flu B) and Influenza A/B Q. Alert Kit.

Done

8. There is some redundancy of writing in the method section.

Eliminated

9. The sentence “confirms nosocomial transmission of oseltamivir resistant strain” in the abstract does not seem to support the finding in this study. Further characterization of the virus using whole genome sequencing is warrant to make such statement of nosocomial transmission due to the H275Y resistant virus. It appears to be the resistance mutation was due to the long term use of antiviral rather than nosocomial transmission of the resistant strain.

Changed
Reviewer: THIAGO M SOUZA

Reviewer's report:

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.

Done

2. English revision throughout the MS is necessary

Done

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

Done

   4. Follow WHO recommendation for abbreviation of 2009 pandemic virus

Done

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

Done

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

Done

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

Done

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 emmerged. In the next sentence, which treatment is been mentioned: for cancer?

Done

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.

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

Done

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

Done

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

Done

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 acquired 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.

Done

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.

Done

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?

Done.

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).

Done