Author's response to reviews

Title: Nosocomial outbreak of the pandemic Influenza A (H1N1) 2009 in critical hematologic patients during seasonal influenza 2010-2011: detection 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: detection of oseltamivir resistant variant viruses.” Pollara C P, Piccinelli G, Rossi G, Cattaneo C, Perandin 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 the manuscript was been corrected by a native English speaker. I 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

All revisions are yellow colour on the manuscript.
The corrections of native English speaker are in green colour.

**Reviewer:** Sallene S Wong

**Reviewer's report 28 November 2012-12-18**

**Minimal Essential revisions**

1. Should be consistency with the terminologies, and differentiate H1N1 and
pandemic influenza A (H1N1) 2009, because H1N1 can be interpreted a seasonal H1N1 (pre-2009 seasons).

Done

2. H1N1pdm09 is the correct name; please check spelling on (H1N1dpm09) throughout the manuscript

Done

3. Referring to Background, first paragraph; correct the spelling of neuramindase (NA) gene.

Done

4. Referring to METHODS>Study population, last paragraph describing clinical samples was collected as nasopharyngeal swab. It is inconsistent stated that in Table1; diagnosis of Flu in lower respiratory tract infection was confirmed in patient 1 and 4, but not mentioned in the METHODS

Done: the analyzed samples were: nasopharyngeal swab, bronchoalveolar lavage fluids or respiratory secretions

5. Referring to METHODS>RNA extraction, real-time RT-PCR, multiplex and one step RT-PCR; first paragraph, “DNA was automatically extracted” should be written as total nucleic acid was automatically extracted. Third paragraph; the first sentence is required clarification on “subtype H1N1” and “viral RNA was carried tested by Fast set H1N1v”.

Done

6. Table1 is still very confusing; symbol is not clearly identified in the legend for “Inpatient ward without outbreak”. Under Clinical events, what is the double line arrow for “Other”? I cannot find the description in the RESULT section. The color and symbol for Flu A PCR- are not quite the same as in the legend.

Done: is Fig 1.

7. Referring to RESULTS, there is no mention of viral clearance in patients 3, 5 and 6, but the symbol for Flu A PCR- was added to 3 and 5 in this new Figure1.

Explained in RESULTS

8. Referring to RESULTS, fourth paragraph; Patients 2,3,5,6 were subjected to oseltamivir therapy at various times; and patients 2, 4, 5 were infected by oseltamivir resistant H1N1dpm09. I suggest this sentence should be rewritten as: Patients 2,3,5,6 were subjected to oseltamivir therapy at various times; and oseltamivir resistant H1N1pdm09 with NA gene H275Y mutation was emerged in patients 2, 4 and 5.

Done
9. Referring to RESULTS, the last paragraph indicated that 12 presented ILI symptoms of lower respiratory tract (LRT), was any LRT specimens collected and performed viral testing? Usually more different source of specimens will be collected for testing in ICU patients.

Explained in RESULTS

10. Spelling error in the first paragraph of DISCUSSION, “whether” should be “whether”

Done

11. In the DISCUSSION, referring to this sentence: the 91% of nosocomial patients with Influenza A virus was H1N1pdm09 infected and 9% was infected by influenza A (H3N2). Was any resistance testing done on these 91% H1N1pdm09 samples? These samples can be used as control case study.

Yes! All 91% H1N1pdm09 were subjected to resistance testing and only 5 were resulted resistant.

12. Second paragraph in the DISCUSSION: “The mortality rate in the patients subjected to H1N1pdm09 test in our Hospital was to 15%, while in Haematology ward was to 13.1%.” I do not understand the word “test” used in this sentence and the relevant to the objective of this study?

It is true! I have cancelled the word test; all patients have been subjected to H1N1pdm09 RT PCR but the mortality rate in Hematology ward is very high respect to all units of hospital.

13. Clarify “H1N1” in the third paragraph of DISCUSSION.

Done

**Discretionary Revisions:**

1. Referring to articles such as Baldanti et.al 2010, Chutinimitkul et.al 2010 JVI and Drews et.al 2010 on the subject of the relevance of D222N and D222G to influenza pathogenesis and transmissibility in H1N1pdm09; and expand on the discussion related to fatality caused by H1N1pdm09 infection. Was any sequence performed to identify the D222 mutation in Hemagglutinin gene of H1N1pdm09 virus found in this group of patients?

No : we have performed only H275Y mutation.

2. There is alternative anti-viral therapy available for oseltamivir resistance should be included in the discussion.

No : only oseltamivir therapy used
3. Infection prevention and control in hospital setting is important to reduce nosocomial transmission in ILI patients, especially in hematologic/oncologic wards, and this subject should be included in the discussion.

Done

Reviewer: THIAGO M SOUZA

Reviewer's report 28 november 2012-12-18

Minor Essential Revisions

1 – Professional English revision is still required. There are a number of mistakes throughout the manuscript, such as sentences lacking subject (“…thus increases the chances…”), sentences and predicates separated by commas (“…the reduction of host defenses in these hospitalized patients, has favoured…”), wrong verbal agreement (“…clinical samples (nasopharyngeal swab) was collected…”; “…all these patients had were admitted…”), wrong spelling (“neuraminidase”; “Haematoly”), non-sense phrases (“…also if only few cases were reported…”), wrong use of prepositions (“…respiratory status died for fatal influenza…”), use of contract forms (“…the outbreak disease spread: it wasn’t possible…”). Notice that these are just examples, major English revision is necessary in the manuscript.

Done

2. Although the most used abbreviation for the 2009 pandemic Influenza A/H1N1 virus is H1N1pdm09, the authors have chosen to abbreviate it as H1N1dpm09. Please, correct it throughout the manuscript.

Done

3 – Authors mentioned in the Results section, third paragraph from the bottom, that 13.1% with respect to H275Y mutation. Is that the frequency of mutant quasi-species in the viral population? I am confused, because it is mentioned that only in patient 4 mixtures of mutant and WT virus was detected. Please clarify.

In the our viral population studied, the frequency of mutant quasi-species resulted very low: only 1 patient (patient 4) resulted with mixed viral population (wild type and H275Y). We have considered all the three patients who showed the H275Y mutation, included the patient 4 (13.1%).
The number of patients with oseltamivir resistance was in all the wards of the hospital 5 (9%).

4 – In the discussion section, when the authors list the factors that could account for nosocomial transmission, geographic proximity of the patients is mentioned. Please clarify or remove it, because this would mean that virus could have been acquired outside the hospital environment, un-characterizing nosocomial transmission.
5 – I would recommend to the authors to mention in their point-by-point responses where the changes were made in manuscript text and to mark it in the manuscript. This improves the reviewers analysis and speed up the manuscript processing.

Done: the corrections are in yellow colour.