Author's response to reviews

Title: A Retrospective Analysis of Microbiota-Targeted Therapies in Patients with H7N9 Infection

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Author's response to reviews: see over
Dear Editor:

MS: 1262312262101691 - A Retrospective Analysis of Microbiota-Targeted Therapies in Patients with H7N9 Infection

Our manuscript, referenced above, has been revised according to the reviewers’ comments. Revised portion are underlined in red. And the answers to the questions are listed as follows:

Authors’ Contributions:

Authors’ contributions

All authors made contribution to the manuscript. Haifeng Lu and Guirong Qian helped design the study, collected data, analyzed data and drafted the manuscript. Xinjun Hu and Chunxia Zhang performed the experimental study and acquisition of data. Hua Zhang prepared the figures. Chunlei Chen, Jing Guo, Silan Gu and Hainv Gao had roles in recruitment, field investigation, and were involved in data analysis, interpretation and write-up. Lanjuan Li designed the study and critically revised the manuscript. All authors read and approved the final manuscript. All these have been added in line 415-422.

Competing Interests:

Please be advised that manuscripts must include a Competing interests section. This should be placed after the Conclusions/Abbreviations. If there are none to declare, please include the statement The authors declare that they have no competing interests. The authors declare that they have no competing interests. It has been added in line 413.
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Reviewer's report

Title: A Retrospective Analysis of Microbiota-Targeted Therapies in Patients with H7N9 Infection

Version: 1 Date: 31 August 2013

Reviewer: Liisa K Selin

Reviewer's report:

This is interesting paper showing how antibiotic therapy influences the intestinal microbiota in humans and how it can be restored with pro- and pre-biotics in the vast majority of patients (shown in comparison analysis between all studied groups). The question remains why this does not work in some patients such as group D and E such and why some patients developed secondary infections. Those two groups did include critically ill patients who obviously received more antibiotics to fight different infections so their microbiota may recover differently. It is also possible that the secondary infections were more frequent in older patients (age median for those who had secondary infection was 64.5). If so, it would mean that in general therapy worked but in the case of older people due to impaired immune systems new secondary infections occur even if probiotic therapy was used (maybe this is Group E?). Maybe older people have B/E ratio already changed as compared to younger individuals and the therapy couldn't be successful in general? Therefore, age-dependent comparison may explain those differences. As a matter of fact they were looking at flu infected people which can be more severe in the elderly due to changes in their immune response. The issue becomes even more complicated as the authors found that patients who didn't receive probiotics and were only treated with antibiotics no secondary infections were found (Fig. 5a and 5b). It would be useful if the authors did an age dependent analysis to determine if age influenced outcome.

Thanks for your constructive suggestion. Yes, the medical records showed 11 had secondary bacterial infection in 16 severe cases, and approximately 72% (8/11) of them were older than 60 years (added in lines 113-115, and 217-218).
B/E ratios of most healthy controls were $\geq 1$, except 4 individuals aged $\geq 80$ years (red triangles in Fig. 3), conversely, the ratios of patients were all $<1$ (red dots for individuals aged $\geq 80$ years in Fig. 3), especially for those patients with secondary infections, the values were even far smaller than elder healthy controls. The data revealed most young and elderly adult patients with H7N9 infection had disequilibrium in the balance between *Bifidobacterium* and *Enterobacteriaceae*. The disequilibrium might have many causes such as the aging process, flu virus infection, antibiotics, anorexia, and so on. Previous in vitro and in vivo studies have revealed that bacterial adherence to the surface of cells and respiratory tract is enhanced by influenza virus infection, and contributes to the increased secondary bacterial infection in influenza[1]. A weakening of the immune system in elderly patients might account for the severity of flu and serious complications [2-3]. The infectious complications in the critically ill H7N9 patients were fatal. The mortality of critically ill patients in our hospital was lower than in other parts of China, which largely perhaps contribute to microbiota regulation therapy for compensating the impaired intestinal colonization resistance to reduce bacteremia and sepsis. Our aim was to summarize the effectiveness of these therapies on re-establishing a stable and diverse microbial community, resolving impaired intestinal colonization resistance and reducing the secondary infections. Thus, we classified the patients into five groups according to microbiota-targeted treatment. However, our data showed that CBM 588 failed to prevent respiratory infections in elderly and critically ill patients, and its effect on increasing levels of *Bifidobacterium* in the gut was unsatisfactory. And probiotic prophylaxis of ventilator-associated pneumonia was already reported to be successful[4] (lines 339-340). But Microbiota-targeted prophylaxis strategies were found to be ineffective in these elderly and critically ill patients. And the elderly in particular were still at high risk for
developing secondary bacterial infection due to primary influenza and the complex antibiotic therapies (added in lines 343-348). It is well known that the aging process is contributed for the secondary bacterial infection. It is a challenge for us to find the effective prophylaxis strategies for secondary respiratory infections in these elderly patients (lines 353-355).

Other minor issues in the manuscript that should be addressed are:

1. Information how H7N9 infection was diagnosed
   
   Thanks, it has been added in lines 103-104.

2. Table1: more information (age, gender) about healthy controls
   
   Thanks, the information about healthy controls has been added in Table 1.

3. DGGE analysis (Fig.8): add samples from representative healthy donors to compare with all the analyzed groups of patients infected with H7N9 and it looks like representative patients from group B and E are missing
   
   Thanks, the DGGE profiles of representative subjects from healthy donors, group B and E, has been shown in Fig.5a. And the related results and discussion has been added in lines 269-287 and lines 300-302, 356-366, respectively.

4. It is not clear what is the difference between group A and B since both were treated with antibiotics and had no probiotic therapy applied (Fig.1). Based on Fig. 1 patient B1 was treated with the antibiotic, which is not indicated in legend (pink line).

   Group A, 2 patients without any microbiota-targeted treatment during follow-up; Group B, 1 patient with only one antibiotic; and the results
profiles of B1 showed the imbalance in the commensal gut microbiota due to antibiotic administration most clearly. And the information of antibiotic which used by B was shown in lines 116-118 and Fig.1.

5. Fig. 1-4 descriptions on x and y axis; perhaps all the groups could be shown in one Fig. and divided into subgroups e.g. Fig. 1a for group A etc. Authors would then have one legend with color-coded different antibiotics usage. It would make things clearer if the authors showed in a different way the probiotic usage (different labeling such as open bars or so) to discriminate it from antibiotic treatment.

Thanks for your suggestions. And we have combined Fig.1-4 into Fig 1. The labels of probiotic have been changed according to your suggestion.

We would like to thank the comments of reviewer for the constructive suggestions of the manuscript.

If there are other errors or further requests, please contact me by e-mail.

With best wishes,

Haifeng Lu

Reference:


2. Centers for Disease Control and Prevention: People at High Risk of Developing Flu–Related
