Author’s response to reviews

Title: A survey of fungal microbiota in airways of healthy volunteer subjects from Puglia(Apulia), Italy

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Title: “A survey of fungal microbiota in airways of healthy volunteer subjects from Puglia(Apulia), Italy.”

Dear Dr. Julian Wei Tze Tang

Editor, BMC Infectious Diseases,

Thank you for the review of our paper: “A survey of fungal microbiota in airways of healthy volunteer subjects from Puglia(Apulia), Italy.”

We are very grateful for all your helpful comments.
We have revised our manuscript according to the reviewers’ comments and highlighted changes in colored text. A native English speaker (working for our University as Collaboratore ed Esperto Linguistico) revised the manuscript as confirmed by the certificate attached.

Our replies to the specific comments of the reviewers are as follows:

Reviewer reports:

Reviewer 1

C1: Peter Wilson (Reviewer 1): The term 'photograph' is not appropriate - suggest survey or description. The sample of volunteers was small and it is not clear if it is representative. Geographical variation is not known. The results are likely to reflect spores common in the air of the region and be changed by weather and season. Therefore a longitudinal study would be useful to determine the colonization duration, as mentioned in the discussion.

R1: Yes, we agree about the usefulness of a longitudinal study, but the people was occasionally recruited in an exhibition center to evaluate the presence of fungi supposed or self-declared healthy individuals. The purpose of the study was just a first step towards understanding the possible presence of fungi in airways, with particular attention to toxigenic species which could represent a further risk for human health. We believe that the presence of fungi in airway’s for germination and toxins production is an interesting finding that let us suppose the importance of future research programs on this field.

C2: Selective media were used - as this was a descriptive study looking for any fungi were there any not likely culturable on these media. Could PCR be performed on the original samples?

R2: Prediction power of a diagnostic success lies in the ability of method in detection of unknown and new potential pathogens. PCR approach is based on consensus (genus/family level) primers (in this case we used consensus primers for fungi), a powerful tool that produces specific, high-resolution data that allows for quicker detection of known and very closely taxa. Alternatively, metagenomic sequencing allows researchers to comprehensively sample all organisms present in a given complex sample. In this case, case-studies should be planned, due to costs and time needed for bioninformatic data analyses.

C3: The number of patients is too small to correlate effectively with respiratory diseases. A larger number of patients should be tested and over a longer period.

R3: The enrolment of a big number of healthy not-hospitalized voluntaries and related follow-up are very difficult to achieve.
Reviewer 2

Nandini Shetty (Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors.

C1: The authors should not use the term colonised unless the fungi were seen to have germinated and therefore are growing in the respiratory tract which is unlikely. The term contaminated would be more accurate to reflect the presence of fungal spores in the lungs. As we all inhale as many as a thousand spores per day these results would not be surprising. The authors should take care with using colonisation and contamination interchangeably.

R1: Thank you for your comment. We agree that it is appropriate to replace colonization with "contamination" throughout the manuscript.

C2: How closely do these results reflect the normal air flora present in the ambient air at the time of sampling? All of the species documented would be part of normal air flora and may just have been inhaled on the way to the clinic.

R2: Thank you for this important observation. We agree that probably the presence of some spora reflect the normal air microbial flora present in the ambient at the time of sampling and that maybe it would have been interesting also sampling the ambient air to support this hypothesis. However, this was a not expected finding that opens now new interesting research studies that absolutely will include a careful analysis of the breathing air. However the presence of fungi in airways let us to support the necessity of modify our previous knowledges on the normal flora, including now fungi whether our findings would be confirmed. Furthermore, it is important to start thinking that whether these fungi find good conditions in airways for germination and subsequent production of mycotoxin, often dangerous for human health, also to look at the complete airways flora of healthy subjects, could become very important for prevention programs. We modified the text and conclusion adding this important concept and explanation.

C3: In the three patients with concomitant lung disease (COPD and asthma) were respiratory samples examined for the presence of fungal hyphae?

R3: Unfortunately we don’t have this information because subjects were enrolled during the annual public regional meetings “Fiera del Levante”, when they didn’t perform lung function or gave us their clinical data as they stopped themselves just for analysis of exhaled air.

C4: Overall this study is of limited interest and the authors have already described the longitudinal studies that would make their findings more interesting.
R4: Yes, we agree about the usefulness of a longitudinal study, but the people was occasionally recruited in an exhibition center to evaluate the presence of fungi in supposed or self-declared healthy individuals. The purpose of the study was just a first step towards understanding the possible presence of fungi in high airways, with particular attention to toxigenic species which could represent a further risk for human health. If fungi find in airways conditions for germination and toxins production is an interesting question which could be the basis for a subsequent and very interesting research program.

Hoping the revised version of our manuscript meets the selection criteria of your Journal,

Sincerely,

Giovanna Elisiana Carpagnano.