Author’s response to reviews

Title: MDR1 overexpression combined with ERG11 mutations induce high-level fluconazole resistance in Candida tropicalis clinical isolates

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Version: 2 Date: 07 Mar 2018

Author’s response to reviews:

Dear Editor,

We would like to thank you for the pertinent comments and we have edited the manuscript accordingly. In the following we go through the comments by the reviewers point by point.

Editor Comments:

1. Please provide clarification on whether any (administrative) permission was required, and obtained, in order to review patient records and use the data mentioned in the Methods. Please include this information in the “Ethics approval and consent to participate” section of the Declarations.
Reply: The information about permission to review patient records and use the data has been added in the “Ethics approval and consent to participate” section of the Declarations.

2. As part of our editorial policies we strongly encourage all authors to deposit their sequencing data before publication. Please deposit the sequencing data generated in this study in a suitable public repository such as the NCBI SRA database. Once you have deposited your data, ensure you provide the information on deposition and how to access these data in the ‘Availability of Data and Materials’ section in Declarations, including the permanent link or the unique identifier associated to it.

Reply: We have deposited the sequencing data of ERG11 gene in the NCBI GenBank database. The accession number has been provided in the ‘Availability of Data and Materials’ section in Declarations.

BMC Infectious Diseases operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reply: Thank you.

Reviewer reports:

Francesco Barchiesi (Reviewer 1): The manuscript by Longyang Jin et al., analyzes the molecular mechanisms of fluconazole resistance among clinical isolates of Candida tropicalis. They found that MDR1 overexpression combined with ERG11 mutations induce high-level fluconazole resistance. The content of the manuscript is of interest to readers of BMC Infec. Dis. and the paper merits to be published. Only one comment concerning an issue that the authors probably would briefly talk about in their discussion. It should be very interesting (as more correct from a molecular point of view) to add some experiment of mutagenesis in which single /
double mutations Y132F and S154F would be inserted into wild-type isolate to see the effects on phenotypes.

Reply: We appreciate the reviewer’s comments and suggestions. Y132F and S154F double mutations have been proved to mediate azole resistance by inserting them into wild-type isolate [Ref 14]. This information has been added in the Discussion section in Line 176.

Brunella Posteraro (Reviewer 2): The manuscript by Jin et al. 'MDR1 overexpression combined with ERG11 mutations induce high-level fluconazole' (INFD-D-17-01835_R1) is a molecular investigation study onto high-level fluconazole resistance in Candida tropicalis clinical isolates. To do that, the authors evaluated 12 fluconazole-resistant C. tropicalis isolates, which were isolated from samples of patients at a tertiary care teaching hospital, for efflux-pump gene expression level as well as ERG11 missense mutations. In addition, 10 fluconazole-susceptible isolates were included in the study. The authors show that while Y132F and S154F amino acid substitutions in Erg11p were present in all fluconazole-resistant isolates except one, the MDR1 gene overexpression was detected in all fluconazole-resistant isolates. Among these isolates, seven were high-level fluconazole resistant isolates (minimum inhibitory concentration ≥128 mg/L) and three were pan-azole resistant isolates.

Interestingly, multilocus sequence typing revealed that the 10 fluconazole-susceptible isolates and 12 fluconazole-resistant isolates belonged to nine and seven diploid sequence types, respectively. The authors state that their study is the first to have investigating the relationship between MDR1 gene overexpression and increased fluconazole resistance.

The paper is well written and the results are clearly presented. Below, there are minor comments that need for consideration by the authors to improve the manuscript.

Specific Comments

Line 64. Please add "with" after "or".

Reply: "with" has been added in Line 64.
Line 79. What the rate of resistance observed in this temporal interval?

Reply: In the Peking University People’s Hospital, a high rate of resistance to fluconazole, with 20.30% resistant C. tropicais isolates, was observed between 2013 and 2017. This information has been provided in Line 79.

Line 80. "Identified by CHROMagar Candida and matrix-assisted laser desorption/ionization (MALDI-TOF) mass spectrometry". Please modify the sentence as "presumptively identified by CHROMagar Candida and then confirmed by matrix-assisted laser desorption/ionization".

Reply: This sentence has been written as suggested in Line 81.

Line 83. -susceptible isolates (MIC≤2 mg/L). "MIC, ≤2 mg/L.

Reply: It has been corrected in line 85. This kind of correction has been made throughout the manuscript.

Line 84. "Between 2014 and 2017"? Alternatively, between 2013 and 2017, as said above?

Reply: This typo has been corrected.

Line 131 and so on. It is superfluous indicating isolates with their designation.

Reply: This sentence has been removed.

Line 158. "C. tropicalis-carrying" "C. tropicalis infected patients" should be better.

Reply: This has been revised.

Line 176. "Ct02R: FLU 8" is a jargon designation. Please use "MIC, 8 mg/L".

Reply: Thank you. This has been corrected.
Line 208. As the authors did no provide a genetic proof about the actual involvement of the MDR1 gene in their isolates, I recommend mitigating the strength of this statement by saying the MDR1 gene may have a role in determining a high-level of fluconazole resistance and pan-azole resistance in C. tropicalis.

Reply: We agree with the reviewer’s suggestion and have revised this sentence accordingly in Line 210.

Table 2. Please modify as suggested. First column; "Designation". Third column; "Date of isolation"; "Underlying Disease", "Ward", and "Thirty-day outcome" are really "patient's characteristics". Thus, I suggest to modify the Table title to taking into account all this. A possible modification is "Amino acid substitutions, antifungal agent MICs, and patient's characteristics for 22 Candida tropicalis clinical isolates".

Reply: The column heading and Table title have been modified as suggestion.

Figures. In addition to improve the image quality, I suggest to combine (and homogenize for presentation) Figure 1 with Figure 2. There is a mistake in the Figure 2 legend. "P" should be ">0.05". However, combining the legends, only the significant P value will appear.

Reply: Thank you for your suggestions. Figure 1 and Figure 2 have been combined into the new Figure 1, including the figure legends.

Best wishes,

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