Reviewer's report

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

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Reviewer: Brunella Posteraro

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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".

Line 79. What the rate of resistance observed in this temporal interval?

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

Line 84. "Between 2014 and 2017"? Alternatively, between 2013 and 2017, as said above? Line 131 and so on. It is superfluous indicating isolates with their designation.

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

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

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.

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".

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.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable

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