Author's response to reviews

Title: Development of Fluconazole Resistance in a Series of Candida parapsilosis isolates from a Persistent Candidemia Patient with Prolonged Antifungal Therapy

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Dear Editor,


Li Zhang, Meng Xiao, Matthew R. Watts, He Wang, Xin Fan, Fanrong Kong and Ying-Chun Xu.

Thank you for the opportunity to revise our manuscript. We really appreciate your and reviewers’ constructive, detailed comments for improving the manuscript. We are terribly sorry for the delay of resubmission. We are pleased to respond to the journal requirements and reviewers’ comments as below:

Reviewer: Arnaldo Colombo
Reviewer's report:
The authors evaluated mechanisms of fluconazole resistance in *C. parapsilosis* strains isolated from a patient who developed persistent candidemia and was exposed to fluconazole for several months. Microsatellite multilocus analysis indicated that the six isolates selected for the study belonged to a single genotype. Overexpression of MDR1 genes were detected in the two resistant isolates, and this was highly associated with a homozygous mutation in MRR1 genes. The paper is generally well written and the authors used appropriate methodology to investigate mechanisms of resistance.

Major compulsory revisions
1- My main concern with the present paper is that the authors were able to identify only one genetic mechanism putatively related to fluconazole resistance of *C parapsilosis* strains. Different from the present report, it is well established that *C albicans* strains usually requires a combination of 2 different mechanisms to express a fully fluconazole resistant phenotype. I strongly suggest to the authors to repeat experiments checking for overexpression of ERG11 and efflux pumps using the same *C parapsilosis* strains cultured in the presence of sub-inhibitory concentrations of fluconazole, to be sure that only MDR1 genes are overexpressed in this setting of strains.

2- Please, explain why the *C parapsilosis* isolates PU090 e PU131 that are susceptible to fluconazole exhibited higher expression of ERG 11 when compared to the resistant ones. This finding reinforce my suggestion to check for the expression of genes related to resistance after culturing the isolates in media containing sub-inhibitory concentrations of fluconazole. Remember that eukaryotic cells modulate their gene expression in response to environmental challenges.

Thanks for the reviewer’s important suggestions, we have repeated the experiment. The results and its discussion have been added into “results” (Lines 168-181) and “discussion” section (Lines 209-220), respectively.

Minor comments
1- Line 28- Please, replace sis by six

“sis” has been changed to “six” (Line 31).
2- Considering the long period of candidemia experimented by the patients as well as the SNC infection one could suggest that the patient developed an endocarditis. Please check if patient was submitted to an echocardiogram and describe the findings.

During his hospitalization, the patient has complained chest pains several times, especially when undergoing hemodialysis. In the 29th week, he even lost consciousness when he was undergoing hemodialysis. However, the echocardiogram had no obvious changes.

3- Discussion: a) please add a comment on the inappropriateness of using fluconazole for so long time in a patient with persistent candidemia as well as on the use of itraconazole, an antifungal drug that is not approved for the use of invasive candidiasis; b) please check the recent publication from Grossman NT & Lockhart SR, Antimicrob Agents Chemother, 2015 59(2):1030

Thanks for the comment on the inappropriate use of antifungals (Lines 229-233), as well as the important reference (Lines 215-218) has been added in the discussion section.

Reviewer: Diego Rodrigues Falci

Reviewer's report:
The authors present an interesting case report about fluconazole resistance in Candida parapsilosis. It is indeed a rare phenomenon and the resistance mechanism was well investigated and described. However, general structure and clinical presentation must be revised to warrant a publication in BMC Infectious Diseases.

Major Compulsory Revisions
1. Abstract: it is not formatted as requested in BMC Infectious Diseases ‘Instructions for authors’ – Case Reports.

Background is too short. Too much emphasis in laboratory methods, and these tests must be mentioned after clinical presentation, in the case report section. Please re-write the abstract following instructions provided.

The “abstract” has been rewritten following instructions. (Lines 20-39)

2. Manuscript, General Structure:
Please suppress ‘Methods’ and ‘Results’ sections to adequate the manuscript to the Case Report type of article. Investigation of resistance mechanism is a very important merit of this work, however it should be shortened, with focus on its results. Put it into the ‘Case Report’ section, after the clinical presentation.

The methods and results section has been shortened and put into the ‘Case Report’ section as required (Lines 110-181).

3. Manuscript, Case Report: The clinical description has some uncommon aspects:
What is the hospital protocol to initiate antifungal therapy? Patient had not received antifungal after a positive blood culture, and had received antifungal after a sputum smear with yeast forms.
Did the sputum culture yield any fungi? Did the patient have any pulmonary radiographic abnormalities, or an abnormal neutrophil count? Please clarify antifungal indication in the 66th week.
Thanks and we would agree that the clinical had some uncommon aspects and so we have added the description in detail to the case presentation section (Lines 87-91).

In the 49th week, he had a fever of 39°C, and blood culture was done immediately. In the meantime, he was given meropenem as initial empirical therapy. His temperature recovered after 3 days before blood culture reporting positive for *C. parapsilosis* after 7 days, so *C. parapsilosis* isolate was not supposed to cause infection, and he was not given any antifungal therapy.

In the 66th week, he had a fever of 38°C, a sputum smear showed a large amount of yeast, and the sputum culture showed *Candida glabrata* (++). Enhancement CT scan on chest showed nodules in the upper lobe of the right lung, bilateral pleural effusion. The patient was considered as pulmonary fungal infection, so he was commenced on fluconazole. Although *Candida glabrata* has ever been isolated from sputum culture, *Candida glabrata* was not isolated from the following blood culture. While still on the antifungal therapy, *C. parapsilosis* susceptible to fluconazole was isolated from blood cultured in the 71st week following admission.

I have added the description in detail to the case presentation section. (Lines 87-91)

Are there any restrictions on polyene (any formulation) use at the hospital? An amphotericin (preferably a lipid formulation) would be the choice for treating a breakthrough infection, or for savage therapy.

In our hospital, amphotericin B (lipid formulation) is uncommonly used because of its high cost. In addition, the amphotericin B (lipid formulation) made in China is still with relatively higher hepatotoxicity and toxicity. The patient in this case suffered chronic renal failure and undergo hemodialysis regularly. Therefore, the physician in charge of this patient did not choose amphotericin B to treat his systemic fungal infection.

Please describe how central nervous system infection was diagnosed – how could it be distinguished from sepsis-associated encephalopathy. Did a lumbar puncture was performed? Did cerebrospinal fluid culture yield any fungi?

In the 81th week of hospitalization, the patient showed symptoms of convulsion of the limbs, combined with disturbance of consciousness, and then he was in deep coma. Physical examination indicated that the patient was with muscular hypertonia, Babinski sign positive, and neck rigidity. The Glasgow score of the patient was E1V1M3. MRI or lumbar puncture was not performed because he was in critical condition. After the consultation with a neurologist, the patient’s signs and symptoms might be caused by central nervous system infection, but metabolic encephalopathy could not be excluded. The description in detail has been added in the case presentation section (Lines 103-109).

4. Manuscript, References: Reference #6 is repeated in #28. Please correct it.

Thanks and the repeated reference has been deleted.

Minor Essential Revisions
1. Figure 1. The timeline is a good way to understand temporal clinical aspects, but I recommend to reconsider to use “Drug doses” in the ordinate axis. Comparison of dosing of different drugs (fluconazole, itraconazole and caspofungin) is somewhat inappropriate. 

We have deleted “Drug doses” in the ordinate axis, and drew a new figure.

Discretionary Revisions
1. Ethics statement: a mention to ethical aspects is appropriate, but detailing on that could be suppressed.

Thanks and the ethics statement has been suppressed. (Lines 111-113)