Reviewer’s report

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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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 gen expression in response to environmental challenges.

Minor comments

1- Line 28- Please, replace sis by six

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 a echocardiogram and describe the findings.

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

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare I have no competing interests