Author's response to reviews

Title: Epidemiology, Species Distribution and Outcome of Nosocomial Candida Bloodstream Infection in Shanghai

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Author's response to reviews: see over
Dear Sir,

We are grateful to the reviewers for their comments, which enabled us to markedly improve our manuscript. Enclosed our point by point response to the questions raised:

**Responses to reviewer 1’s comments**

**Reviewer:** Shawn R Lockhart  
**Reviewer's report:**  
In this manuscript the authors document 121 episodes of Candida bloodstream infections in a teaching hospital in Shanghai China. All isolates were identified to species and susceptibility testing was performed. The authors used multivariate analysis to determine risk factors for candidemia and determined that inappropriate antifungal therapy was a risk factor for mortality.  

**Major comments:**  
There are new species specific Candida breakpoints that should be applied to this analysis (M27-S4).

**Reply:** thank you for your advice, the new species specific Candida breakpoints have been used in the full text. Flucytosine, amphoterin B, fluconazole, voriconazole, and itraconazole were performed using the ATB® FUNGUS 3 system (BioMérieux, France). For triazoles, the minimal inhibitory concentration (MIC) was determined by determining the concentration at which a prominent reduction in the yeast cell count was observed after 24 h of treatment. The MIC for amphoterin B and flucytosine were defined as the lowest concentration at which no visible growth was detected. CLSI 24 h resistance breakpoints (CBPs) for fluconazole, itraconazole and voriconazole, recently approved, were used: susceptible (S) ≤2 μg/mL, susceptible dose-dependent (SDD) 4 μg/mL, and resistant (R) ≥8 μg/mL for fluconazole and C. albicans, C. tropicalis, and C. parapsilosis and ≤32 μg/mL (SDD), ≥64 μg/mL (R) for C. glabrata; S ≤0.12 μg/mL, SDD 0.25-0.5μg/mL, R ≥1 μg/mL for voriconazole and C. albicans, C. tropicalis, and C. parapsilosis, and ≤ 0.5 μg/mL (S), 1 μg/mL (SDD), ≥2 μg/mL (R) for C. krusei; S≤0.12μg/m, SDD 0.25-0.5μg/mL, R ≥1 μg/mL for itraconazole and C. albicans. Due to lack of CBPs of amphoterin B and flucytosine for Candida spp., the epidemiological cutoff values (ECVs) were used for them: S ≤2ug/mL, R≥2ug/mL for amphoterin B and Candida spp.; S ≤0.5ug/mL, R>0.5ug/mL for flucytosine and C. albicans, C. tropicalis, C. glabrata and C. parapsilosis; S ≤32 ug/mL, R>32ug/mL for flucytosine and C. krusei, S ≤1ug/mL, R>1ug/mL for flucytosine and C. guilliermondii; S ≤0.5ug/mL, R>0.5ug/mL for itraconazole and C. parapsilosis and C. tropicalis; S ≤1ug/mL, R>1ug/mL for itraconazole and C. krusei and C. guilliermondii; S ≤2ug/mL, R>2ug/mL for
itraconazole and *C. glabrata*. (added in Methods, Paragraph 5) More results of susceptibility tests have now been added in Table 3.

**The susceptibility rate of C. tropicalis to fluconazole is extremely low and probably represents trailing rather than true resistance. Without knowing anything about the reading (not in materials and methods) I will assume that these are 48 hr breakpoints?**

**Reply:** ATB® FUNGUS 3 system (BioMérieux, France) was used for testing susceptibility. For triazoles, the minimal inhibitory concentration (MIC) was determined by determining the concentration at which a prominent reduction in the yeast cell count was observed after 24 h of treatment. The MIC for amphotericin B and flucytosine were defined as the lowest concentration at which no visible growth was detected. This has now been added in methods (Paragraph 5).

Minor comments:

**What is ANTIFUNGUS 3? If this is a commercial kit then it is not broth microdilution by the methods of CLSI. There needs to be a better description of the susceptibility testing.**

**Reply:** Susceptibility test was performed by using the ATB® FUNGUS 3 system (BioMérieux, France). It was a commercially available and automated yeast susceptibility test system, which was widely used in China [Li F et al, Surveillance of the prevalence, antibiotic susceptibility, and genotypic characterization of invasive candidiasis in a teaching hospital in China between 2006 to 2011. BMC Infectious Diseases 2013, 13:353] providing susceptibility to antifungals results markedly concordant with those obtained using CLSI and EUCAST methodologies. (Methods, paragraph 5)

By the new breakpoints, no isolates of *C. glabrata* are susceptible, only SDD or resistance. It would be more meaningful to put the percent resistant in Table 3 rather than the percent susceptible.

**Reply:** thank you for your advice; we put percentage of SDD or intermediate or resistance in the Table 3. Based on the new species-specific clinical breakpoints, [Pfaller MA et al. Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. J Clin Microbiol 2012, 50(9):2846-2856.] no isolates of *C. glabrata* are susceptible, SDD ≤32 µg/mL, R ≥64 µg/mL for *C. glabrata* and Fluconazole, so the errors were corrected in Table 3.

Responses to reviewer 2’s comments

Reviewer: Emilio Bouza

Reviewer’s report:

This article is well written and includes very prestigious and respected authors
but not much new information of usefulness for others is included

**Reply:** Thank you for your critical comments. Indeed, our paper describes the epidemiology of Candidemia at a large teaching hospital in Shanghai. Knowing that during the past 2 years, there were only 5 papers published from China (2 from Beijing and from Shanghai, Nanjing, Chongqing, each) on the topic for candidemia in adult, we still believe that the results are of interest for the readers even outside Asia.

The study is retrospective and the volume of cases of the institution in episodes of Candidemia is relatively low (20-25 cases/year)
The number of blood cultures/1000 admissions obtained yearly in the institution should be included. The low numbers of candidemia episodes may be related to subculturing.
It is remarkable that a very high proportion of episodes of Candidemia occur in ICU’s. Is this because the number of blood cultures obtained outside the ICU is particularly low?

**Reply:** Thank you for your comments. We reviewed the number of blood cultures performed in the last five years in our hospital; the number of blood culture/1000 admissions was 118.2, 116.0, 117.9, 147.6 and 166.1, respectively. In ICUs, the number of blood culture/all blood culture yearly, in studied period, was 11.4%, 11.7%, 11.3%, 11.1% and 12.3%, respectively. These data have now been included in the text (in Results, paragraph 1) and did not reveal that the number of blood cultures obtained outside the ICU was particularly low.

To consider for adequate therapy the limit of 5 days is too broad in my opinion. The data should be provided day by day in the initial 5 days

**Reply:** thank you for your comments. Our data has been provided day by day in the initial 5 days, which showed the mortality rate (28-day) was 19.7%, 25%, 25%, 28.6%, 50% and 43.5% when the appropriate antifungal therapy was administrated to the patient within 48h, 48-72h, 72-96h, 96-120h and >120h or without treatment, respectively. 28-day mortality was significantly lower for those who received appropriate empiric antifungal therapy within 120h (5d) vs. inappropriate or delayed (>5d) empiric antifungal therapy or no antifungal treatment (20.9% vs. 45.7%, p=0.006). The results have been presented as Figure 2 in our revised manuscript.

I hope that our revised manuscript will now be suitable for publication in the Journal.

Sincerely yours,

Pr Olivier Lortholary, M.D.; Ph.D.
Chief, Necker Pasteur Centre for Infectious Diseases, Paris, France.