Author's response to reviews

Title: Uniform distribution of three Candida albicans microsatellite markers in two French ICU populations supports a lack of nosocomial cross-contamination

Authors:

Odile O Eloy (oeloy@ch-versailles.fr)
Stephanie S Marque (x.juillet@wanadoo.fr)
Francoise F Botterel (botterel@univ-paris12.fr)
Francois F Stephan (francois.stephan@chu-guadeloupe.fr)
Jean-Marc J-M Costa (jean-marc.costa@ahparis.org)
Virginie V Lasserre (virginie.lasserre@univ-paris5.fr)
Stephane S Bretagne (bretagne@univ-paris12.fr)

Version: 3  Date: 16 September 2006

Author's response to reviews: see over
Madam, Sir
Please find below the answers to the reviewers’ comments and the list of the modifications. We hope that our manuscript has been improved and is now suitable for publication in the Journal.
Sincerely.

Pr Stéphane Bretagne.

**Reviewer 1's report:** General
*The revisions made the manuscript clearer and it reads better.*
We thank Reviewer 1 for his support.

**Reviewer 2's report:**
*English language should be revised.*
English was already checked by a professional translator (invoice: 180 euros) and has been accepted by Reviewer 1.

**Page 3**
*line 5: insert the acronymous (ICU)*
*line 15: replace “intensive care unit (ICU)” with “ICU”*
*done*

**Page 8**
*Line 3: the term “risk factors” is not correct if you consider all colonized patients (at admission and after 72 hours)*
Sentence changed for: Table 1 lists the demographic data and the main characteristics of the patients in the two hospitals who acquired any *Candida* sp colonization after 72 hours of hospitalization.

*Line 19: what does “common” mean?*
Changed for: was a genotype found in other patients

*Line 22: “including” should be changed in “characterized by CDC3…..”*
Done

**Page 9**
*Line 4: what does “also” mean?*
deleted

*Line 6: the genotypes of hospital A are 29, not 37 as you mention and those of hospital B are 24 and not 30.*
No, the comparison have to be done with all the genotypes found as several patients shared a common genotype. 29 and 24 refer only to a unique type of multilocus genotype

*Line 8: Tables 3-5 are non essential as the data are summarized in Table 6.*
Table 3-5 deleted and Table 6 became Table 3. However, if a reader wants to know the allelic frequencies, which is a commonly used data, he/she must recalculate the figures from Table 6.

*Line 9-10: the sentence “Table …” should be clarified*
Changed for: Table 3 reports the multilocus genotypes of the *C. albicans* isolates observed in the two hospitals

Page 10
Line 3-4: patients shared a multilocus genotype (that represents the most frequent genotype in *C. albicans* population) not isolates
Replaced by: eight patients shared a multilocus genotype which is the most common multilocus genotype among independent isolates {Botterel, 2001 #1}.

Line 16-18: sentence should be clarified.
Changed for: Similarities were found regarding the epidemiology of *C. albicans*, i.e. that most fungal infections that arise in an ICU are caused by unrelated strains, although there were differences between the two hospitals which could have led to less similarities..

Line 22-24: the lower percentage of positive cultures in hospital B could be attributed to the use of oral amphotericin B.
Sentence present in the original submission reintroduced here: An explanation could be could be the role of amphotericin B prophylaxis, implemented in Hospital B only, which could have hampered yeast growth.

Page 11
Line 7: *C. albicans* should be added to “population specific…”
Done

Line 20-25 and line 1-4 page 12: the sentences of this paragraph should be shortened focusing the attention on the usefulness of PMM in the screening of a large number of isolates.
The sentences concerning the methods (already reported in the right section) should be omitted.
Paragraph shortened. However, PMM is not designed only for analyzing a large number of isolates. It is well designed for a rapid comparison of two isolates. As people typing microorganisms such as fungi are not yet used to PMM, we think it is interesting to underline the technical issues in our conclusion. It is particularly true for PCR fragments differing in one bp, as perfectly underlined by Reviewer 1 who has already published on PMM.

Page 12
Line 10-13: The absence of nosocomial contamination may be related to the application of good clinical practices, that should not to be disregarded. Therefore the sentence (Line 12-4) “Therefore, attempts …” has to be deleted.
We disagree with this criticism. The application of “good clinical practices” for preventing Candida infection is obviously not the same as preventing Staphylococcus infection. The good practices for preventing Staphylococcus infections must be followed, of course. But for Candida infection, the interest of preemptive or empirical treatments, or another strategy should be investigated. Since colonization is the first step of infection, this is the step we have to control first. It is the meaning of our final sentence.

Table 1
Title: table refers to patients who were colonized (56 and 43), not to patients who acquired colonization after 72 hours (45 and 36)
No. The title was specified:
Main characteristics of the patients who acquired any *Candida* sp colonization after 72 hours of hospitalization in Hospital A (45/56, i.e. 80.3%) and in Hospital B (36/43, i.e; 83.7%).

**Table 2**
*Title: source instead of body location*
Done

**Table 6.**
*Title: the sentence “The most ....” must be deleted as comment;*
Done
*Heading of the last two columns: the number is of patients, not of isolates*
No, it is well isolates for which we have kept the genotype. In Hospital A we have kept two isolates for one patient since two different multilocus genotypes were found.

**Reviewer 3's report: General**

The authors have, I believe, misunderstood the main motivation behind my comments. I have no serious doubt that the science is sound, but thought that modifying the manuscript based on my suggestions would strengthen it and make it more accessible. The authors have provided some of the additional information I requested in their letter of response, but if I were to see the revised manuscript for the first time, my suggestions for improvement would be quite similar to those I made when reviewing the original manuscript.

I also note that some typos have to be introduced in the revision, and that the nearest neighbor method is not a phylogenetic method which requires knowledge of the number of events that bring about changes in the microsatellite length.

These are the comments that were not addressed:

**Background.** Stating discriminatory power alone is not sufficient given the results the authors have obtained. If microsatellite pattern were highly irreproducible, nosocomial transmission could not be identified, because the same strain would give different patterns when analysed on different occasions. I believe that there is evidence from earlier work that the microsatellite patterns produced by the authors' method are very reproducible. The authors should state that and/or provide a reproducibility-corrected discriminatory power. I.e. they should state how likely it is that the genotypes of two unrelated isolates are less similar to each other than the genotypes obtained in two repeat analyses of the same isolate (or, if they do not use distances, calculate the probability that two unrelated isolates have a different genotype, corrected for the probability that two analyses of the same isolates will result in a different genotype).

**Results.** Difference in equipment. The authors describe controls they have undertaken, but a
clear statement as to the impact of the two different machines would be useful, i.e. what was the probability that two analyses of the same isolate, each carried out on a different machine, would not result in the same genotype (and/or they could give the reliability-corrected discriminatory power for comparisons involving pairs of genotypes determined on the two different machines.

Methods. I believe that many readers would benefit from a more extensive explanation of the principle underlying multiple correspondence analysis.

Results. Description of diversity of isolates on individual patients. It might be worth pointing out that only one colony per anatomical site was analysed and that therefore possible diversity at a given site was undetectable, as would be the presence of low levels of additional strains - possible some sort of a detection threshold could be calculated and shown.

Results. Statistical analyses. I think a more thorough explanation of the multiple correspondence analysis would be useful. Because I do not understand what is involved in this analysis, I am also not sure if it would detect hospital-specific clusters of genetically similar but nonidentical isolates, which would be a sign of nosocomial transmission (if the test will detect such clusters, the authors should state that). In any event, a neighbour-joining tree would do much to convince readers with limited statistical knowledge that the authors’ conclusions are warranted. Also the underlying genetic distances could be used to confirm lack of nosocomial separation between hospitals by nearest-neighbour analysis (Edelmann et al. 2006, Journal of Clinical Microbiology 43: 6164-6166): If there is no nosocomial transmission, isolates from a given hospital should not have isolates from the same hospitals as their closest related counterpart more often than expected by chance.

We had identified two main criticisms we tried to answer in our revised version of our manuscript. The first point is the reproducibility of the methods. This reproducibility is 100%. In contrast with RFLP, there is no adjustment to bring to the raw data to compare different lines. The PCR fragments length is automatically calculated. The point we wanted to underline for people interested in using PMM is that the calculated length depends on the electrophoresis (capillary versus gel). But for a given electrophoresis mean, the length is always the same. The resulting genotype is unambiguous and always the same (that is why we can use a
reference strain). Therefore, we are really sorry not to see what is a reproducibility-corrected discriminatory power for our data.

The second point is our choice to use a nonhierarchic test (i.e. multiple correspondences analysis) to illustrate our results. Multiple correspondences analysis is described in numerous web sites and text books, was previously used by us in other published papers without a full description which is not, we believe, the main scope of the Journal. We have added some explanations but we do not know where we have to stop.

We agree that nearest-neighbor joining is more commonly used. However, it is a hierarchic method and the dendrogram (phenogram would be more correct) generated implies, de facto, a phylogenetic relationship by a lot of readers not aware of the limits of the methods (PAUP, the software used by Reviewer3 stands for Phylogenetic Analysis Using Parsimony). We wanted to decrease this “false” feeling by using a different test. However, to present our data differently, we have added a neighbour analysis of our data (see Fig 1 B) which corroborates Fig 1A.