Author’s response to reviews

Title: Prevalence and Antifungal Susceptibility of Candida albicans Causing Vulvovaginitis Among Pregnant Women in Lebanon

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Version: 1 Date: 28 Oct 2019

Author’s response to reviews:

Dr Papadimitriou-Olivgeris Matthaios

Editor
BMC Infectious Diseases

Subject: Revision and resubmission of manuscript INFD-D-19-01372

Dear Dr Papadimitriou-Olivgeris Matthaios,
Thank you for your letter and the opportunity to revise our paper on "Prevalence and Antifungal Susceptibility of Candida albicans Causing Vulvovaginitis Among Pregnant Women in Lebanon"

We have included the reviewers comments (In red) immediately after this letter and responded to their questions.

We are happy to consider further revisions if needed, and we thank you for your continued interest in our research.

Sincerely,
Mira El Chaar
Reviewer reports:

Maria Drogari-Apiranthitou, MD, PhD (Reviewer 1):

1- The study design has not been adequately described and there is confusion with regard to the populations included in the study. In the abstract the authors state: "This study determined the presence of Candida species in symptomatic pregnant women", in the Background: "...the prevalence of VVC caused by Candida species in pregnant women", whereas in the Discussion it reads: "The present study was conducted to evaluate the effect of Candida colonization with regard to pregnancy outcomes". This is not made clear in the Methods or Result section of the manuscript, as it should.

All the 258 pregnant women who participated in this study had vaginal discharge. Those 258 women were screened for candida species to get the prevalence of VVC. Candida species were detected in 100 women but the remaining 158 women were symptomatic (with vaginal discharge) but were negative for candida spp. In order to clarify this point in the article, we did the changes as following:
In the Abstract background section: This study determined the prevalence of candida species in symptomatic pregnant women and evaluated the antifungal susceptibility profile of the isolated candida strains.
In the introduction section of the manuscript: The objective of this study was to determine the prevalence of Candida species in symptomatic pregnant women with vaginal discharge at 35 to 37 weeks of gestation and to evaluate the antifungal susceptibility profile of the isolated strains of C. albicans.
In the method section, we added the following modification: “Clinical samples were collected from 258 pregnant women with vaginal discharge” and the following sentence “In addition, the study evaluated the association between the presence of Candida species and gestational complications and outcomes”

In the discussion section we omitted the following: “The present study was conducted to evaluate the effect of Candida colonization with regard to pregnancy outcomes” and amend it to the following: The present study was also conducted to correlate between the presence of candidiasis and pregnancy outcome.

Were all of the 100 Candida-positive women symptomatic for VVC?
All the 100 Candida-positive samples were symptomatic (producing discharge) however the symptoms were not specific for VVC (such as white discharge, itching or soreness). They were all diagnosed with abnormal discharge.

Were they clinically diagnosed having VVC?
Not all women were clinically diagnosed with typical symptoms of VVC

How were they evaluated?
Women were evaluated by both having vaginal discharge and by sending the vaginal discharge to the laboratory for diagnosis

Were all of the remaining 158 women symptom-free or had other types of vaginitis/vaginosis?
The remaining women were symptomatic (abnormal vaginal discharge) however they were negative for candida. They may be positive for other bacterial infections causing vaginal discharge which we did not identify since our aim was to search for candida species.

How many were only colonized?
We did not assess colonization in asymptomatic women. We assessed infection in symptomatic
patients. All the samples that were positive for the candida showed strong growth on the selective media.

How many of the 165 participants who filled out the questionnaire had VVC?
Of 258 participating women, questionnaires were filled by 165 (64%) women to get the socio-demographic data, clinical status and gestational history. Those 165 women who filled the questionnaire were followed up during their delivery (delivery time, delivery type, induced labor, gestational complications). The neonatal outcomes (newborn height, weight and Apgar score) were assessed. In total 48 women had candidiasis and filled the questionnaire.

We added the following sentence in the result section to clarify this point: “The association between the presence of Candida species, isolated from 48 women who filled the questionnaire, was assessed with gestational complications, recurrent UTI infection, induced labor and prevalence of vaginal discharge”

What was the control group of the study? Not the healthy (or not colonized). If the associations have been calculated only between the different species isolated and not compared to a healthy group, objective (iii) as above, cannot be met (as it reads “the presence of Candida species”), comparisons with other studies in the Discussion are not possible and, unless all these questions are answered, the stated conclusions cannot be evaluated.

No control group used as the study is a cross sectional study. We added in the text the following: “In this study, a cross-sectional design was adopted for determining the prevalence of Candida species in Lebanese pregnant women”.

The associations have been calculated only between the species and not compared to healthy group since the study was designed primarily as a cross sectional study. However the group with isolated candida was compared with no isolated candida species in terms of various clinical outcomes to determine any association between presence of candida with any of the outcomes. Future case control studies to compare the effect of presence and absence of any infection (including candida or any other vaginal infection) on the pregnancy clinical outcome can be done.

Comparison with other studies was done based on the percentage prevalence. The studies that we compared to are also cross sectional studies.

Therefore we included the following sentence in our discussion: “Future case control studies should be performed to compare the clinical outcome of pregnant women infected with any microorganism versus non-infected women.”

2- It is well known that species as C. glabrata and C. krusei are less virulent, usually affecting individuals with certain predispositions and peri- or postmenopausal women. If the frequencies of these species were so high (41% and 17%, respectively) the patients having indeed VVC, the authors should present detailed clinical data and discuss these important findings accordingly.

Patients that were confirmed positive for C. glabrata and C. krusei had similar clinical symptoms as the other patients (Vaginal discharge). No other clinical data were specific to these species to report. We mentioned however in the conclusion the following: “In conclusion, increasing rates of NCAC strains among pregnant women in Lebanon should be looked at as both novel and alarming. Extensive surveillance studies should be done on all clinical specimens yielding significant growth of Candida spp. and the effect of resistance pattern on invasive candida infection”.

3- Isolates were identified as C. glabrata and C. krusei using chromogenic agar only. As numerous other species have pink or creamy colored colonies, how confident were the authors that identification was accurate? How did they confirm that? They should comment on that.

The chromatic agar has been well documented in previous studies as for its high sensitivity and
specificity for the identification of the most commonly encountered Candida spp. References were added. We did confirm that the isolates were C. glabrata and C. krusei using the API 20C AUX API kits (Biomerieux). We included in the text.

“The chromatic characteristics of the colonies were the following: green colonies were identified as C. albicans, creamy colored colonies as C. glabrata, and pink with a whitish border colonies as C. krusei. All isolates were confirmed by API 20 C AUX strip (BioMerieux, Marcy l'Etoile, France). Further phenotypic testing was done to differentiate between C. dubliniensis and C. albicans by growing the germ tube positive yeast isolates at 45°C on SDA for up to 10 days. C. albicans isolates were identified by their ability to grow at 45°C”

4- In the Results, the actual numbers with percentages should be presented and not only the interpretation of the statistics; e.g. number of women (%) with x compared to number of women (%) without x, etc.
All the tables were revised

Other points:

1. The demographic and clinical characteristics with patient numbers should be presented in a separate Table.
A table (Table 1) was added to the manuscript that describe the demographic and clinical characteristics of the respondents.

2. The Background is too long. Lines 36-56 in page 3 and 4-19 in page 4, can be omitted. There is also information repeated in the Discussion.
The background was revised and restructured.

3. In the Methods, the description of a commercial method such as the Etest is redundant and should be omitted (lines 46-58, page 6 and lines 4-14, page 7).
The modification was done according to the reviewers comment

4. The Discussion is unnecessarily long. It should be focused on the results of this study and avoid extended, not directly related information, on treatment for example. Information in page 9, lines 43-53 belongs to the Background. The finding that 4 cultures were mixed is trivial and the authors should not start with it. In such cases, C. albicans, if present, is considered the culprit anyway and treatment is primarily focused on that species.
The Discussion was revised and restructured.

5. In Tables and Figures the results of the current study should be presented. Table 2 and Fig 1 are irrelevant to this study. Table 2 would be meaningful if only data from vaginal swabs from pregnant women were presented.
In Table 3, delivery week, delivery type, induced labor and other gestational complications are not specified. Legends to the Tables and Figures are missing. We would like to ask the reviewer to keep Figure 1 since it summarizes the prevalence of candida species isolated from vaginal swabs in various studies published. Such summarized figure was not observed in any other article. This would be interesting for the readers. Table 2 was removed. Table 3 was modified. Legends were added to table 2 and table 4. Table 1, 3 and Figure 1 do not need a legend.

6. The Conclusions, page 13, except for the first sentence and lines 31-33, are too generic and do not derive directly from the results of the current study. The discussion and conclusion were revised.

Some minor errors and suggestions for improvement

1. Page 2, lines 36-38. More accurate to say 'C.albicans was significantly associated only with gestational diabetes'. The correction was done

2. Page 2, line 45. 'Efficient' would be more accurate than 'efficacious'. The correction was done

3. Page 2, line 50. 'antenatal' The correction was done

4. Page 3, line 24 'in' pregnant women The correction was done

5. Page 4, lines 41-46. The resistance profile of circulating C. albicans cannot "predict" appropriate prophylaxis and treatment. It just "should be considered when local prophylaxis and treatment guidelines are established" as stated in ref.26. The correction was done

6. Page 9, 29. "The observed" or "The calculated susceptibility rates were....." in place of "The susceptibility rates … were determined to be…" The correction was done

7. Page 10, lines 7-14. There is no information about vaginal samples in the study in ref 36. This could be a good argument for the authors as to why they undertook the present study. We mentioned this reference in the article to confirm that NCAC have been also shown to increase in other clinical non vaginal samples. We restructured the sentence as following:“The prevalence of candida species causing vaginitis is pregnant women vary from one population to another. In our study, 39% of participating women were infected by candida species. NCAC were more frequently isolated.
(58%) than C. albicans (42%). NCAC were also shown to increase in non vaginal clinical samples isolated from Lebanon; that was observed in a previous retrospective study published where the authors have shown that among all candida strains isolated, C. albicans rates had decreased from 86% in 2005 to around 60% in 2014. However, the NCAC rates increased from 14% in 2005 to around 40% in 2014, comprising mainly of C. tropicalis, C. glabrata, and C. parapsilosis [21]. The objective of the study performed by Araj et al was different than our objective. The authors in their study were reporting the prevalence of candida species from clinical samples that were received to the hospital clinical laboratory department for diagnosis during a duration of 10 years. Vaginal samples were not included in their sample since in Lebanon the gynecologists do not screen for vaginal discharge, they usually treat the patients empirically.

8. Page 10, lines 48-56. "However…delivery.", citation is needed for this sentence.
The reference was added as recommended by the reviewer.

9. Page 11, lines 7-9. In a more recent study also using a molecular typing method (pulsed-field gel electrophoresis), it has been shown that vertical transmission has the principal role in the neonatal colonisation by C. albicans in the very first days of life [Filippidi A, Galanakis E, Maraki S, Galani I, Drogari-Apiranthitou M, Kalmanti M, Mantadakis E, Samonis G. The effect of maternal flora on Candida colonisation in the neonate. Mycoses. 2013 Jun 12. doi: 10.1111/myc.12100.] The authors may add this information here.
The reference was added as recommended by the reviewer. The paragraph was revised and was rewritten in the introduction.

10. Page 11, line 21. Only colonization? Please see Major point 1, above. We mean presence of candida, not colonization.
We corrected the sentence as following: “In the current study, we aimed to correlate between the presence of candidiasis and pregnancy outcome”

11. Page 11, line 31. 'in' Iran
The correction was done

12. Page 11, line 36. 'in' agreement
The correction was done

13. 'C. krusei' should be corrected throughout the text and tables.
The correction was done
14. 'Candida' should be in italics. The correction was done

Terezinha Svidzinski (Reviewer 2):
The contribution seems interesting, but there are many points to be reviewed before acceptance for publication.
There are important concerns regarding to microbiological procedure:
The word "Candida" is written in different ways and some of them totally wrong, please correct it.
Only the spelling "Candida" is correct in any situation
The correction was done

There are various yeasts names written without italic. Please, check it into the entire manuscript
The correction was done

Page 3, line 48 the expression "non-C. albicans species (NCAS) should be replaced by "Non-C. albicans Candida (NCAC). In addition, every time the "NCAS" appears it should be replaced by "NCAC".
The correction was done

Page 6, lines: 6-9: The sentence "The second swab was used to prepare a smear for gram staining and wet mount with KOH." Swab with culture media, cannot be used for microscopic analysis, as well as wet mount with KOH, it is useful just for culture and yeasts identification, please rewrite completely. Besides the Gram stain neither KOH was not mentioned in results or discussion, thus I suggest delete this information
The information was deleted as requested

Page 6, line 17: add the word "human" before the …. pooled serum
The correction was done

Page 6, lines: 9-39: The methods used for yeasts identification was too rudimentary, it is not wrong, but I suggest to authors mention this information in discussion session as a limitation of this study.
We did use the conventional method for identification for candida species. These conventional methods are still being used by most of the diagnostic laboratories in the country since they are reliable and cost effective. We couldn’t use MALDI-TOF or VITECK for a rapid identification of the isolates due to budget limitation. We did not add this section as a limitation in the manuscript since conventional methods are still reliable for identification and are currently used in most of the hospital laboratory medicine departments.

Page 8, line 38 and other places: What does "Candida kreusi" mean? Did the authors wanted to write Candida krusei? It appears in other sentences, please get it right
The correction was done

Figure 1: is interesting, but authors need add the respective reference for each country, for instance as foot note.
We added the references in the text: Recent studies from countries such as China, Brazil, Tunis, Kuwait, India and Turkey have reported that C. albicans remains the most commonly isolated yeast (60%-80%) in women diagnosed with VVC [24-29]. On the other hand, an increasing trend in the
occurrence of NCAC (58%-60%) over time has also been observed in Pakistan and Burkina Faso [30, 31] (Figure 1).

Table 1 and 2: Please, pay attention to format: remove all vertical lines and internal horizontal ones. All tables were fixed.

Minor points:
Please check the total time of this study, authors mention 12 months (abstract) and 13 months (manuscript's body), but the period from June 2015 to July 2016 corresponds to 14 months, right? The modification was done. Apologies for the error.

Page 2, line 10: Replace the word "prevalent" by common. The modification was done.

Page 5, line 41: Please delete the sentence: "while 36% (N=93) were not filled by the gynecologists"; The modification was done.

Page 7, line 53: delete the coma. The modification was done.

Page 8, line 19: add "the cultures from" after the word "Among". The modification was done.

Page 9, line 51: Add the word "cultures" after "vaginal swabs". The modification was done.

Page 9, line 53: The sentence "More often than not" is weird, please rewrite it. The sentence was changed.

Page 10, line 19: The reference number 26, is no related to VVC, therefore should be excluded from here. The reference was omitted.