Premenstrual vaginal colonization of *Candida* and symptoms of vaginitis

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Although premenstrual exacerbation of vulvovaginal symptoms attributed to *Candida* spp. is well documented, the causation of these symptoms is not well understood. This study describes the daily vaginal colonization of *Candida* in three women. A single pilot study was designed to test the methodology of the proposed randomized controlled trial, Garlic and *Candida*. This study reports the colonization of *Candida* spp. in three women. Ten women aged 18–50 who reported at least one episode of vulvovaginal candidiasis were recruited by the University of Melbourne. Each participant took daily vaginal swabs for 2 weeks during the luteal phase of their menstrual cycle, which were analysed for quantitative colony counts of *Candida* spp. Of these, three women were colonized with *Candida* spp. For the first time, to our knowledge, daily colonization of *Candida* during the luteal phase of the menstrual cycle is described in three women, demonstrating an increase in the colony count preceding symptom development. This small study demonstrated the colonization of *Candida* spp. during the luteal phase of the menstrual cycle in three women. *Candida* colonization is poorly understood, yet investigating the relevance of the link between symptom exacerbation and the menstrual cycle in those women who experience recurrent episodes of vulvovaginal candidiasis may influence the management of this condition.

**INTRODUCTION**

Recurrent vulvovaginal candidiasis (VVC), defined as four or more symptomatic episodes in a 12-month period, is notoriously difficult to manage, causes women considerable suffering and is poorly understood (Sobel, 2007). Understanding the mechanism of how vaginal *Candida* change from commensal to pathogenic organisms may be a step further in improving the management of candidiasis.

Some studies suggest that colonization rises in the luteal phase of the menstrual cycle (Engberts *et al.*, 2007; Eschenbach *et al.*, 2000; Odds *et al.*, 1988); consequently, it is often thought to be associated with hormonal changes in the menstrual cycle. Several studies have documented point prevalence of vaginal colonization of *Candida* spp. (Beigi *et al.*, 2004; Goldacre *et al.*, 1979) and others have shown that colony counts of *Candida* spp. vary from month to month and from day to day within individuals (Sautter and Brown, 1980). Despite previous validation of consistency between multiple swabs from the same individual (Odds *et al.*, 1987), no published reports have been found that document the daily colonization pattern during the luteal phase of the menstrual cycle using quantitative methods.

We undertook a pilot study to refine the methods and establish the feasibility for a randomized controlled trial investigating the use of oral garlic to prevent VVC, as garlic has been shown *in vitro* to have antifungal effects against *Candida* spp. (Lemar *et al.*, 2002). The self-collection of swabs by participants is a method used in previous studies (Schwebke *et al.*, 1997; Passos *et al.*, 2007; Pirotta *et al.*, 2004). Self-collection of swabs has been validated, demonstrating a similar sensitivity and specificity for detecting
Candida spp. as the clinician collection of swabs (van de Wijgert et al., 2006). In this paper, we report, using self-collected vaginal swabs, the daily candida counts in three participants who were colonized during the latter half of their menstrual cycle.

METHODS

Participants. Ten non-pregnant healthy asymptomatic women aged between 18 and 50 years, with a self-reported history of at least one episode of VVC, were recruited by the University of Melbourne (Victoria, Australia). These numbers were chosen by convention for pilot studies and considered sufficient to test the study methodology, scientific technique and acceptability by participants. Ethics approval was gained through the University of Melbourne Human Research Ethics Committee (ID 0933026.1).

Self-collection of swabs. Participants who required the use of antifungal medication were asked to withdraw from the study. The study was undertaken during the luteal phase of the menstrual cycle due to the greater likelihood of positive culture for Candida spp., as well as to reduce the burden on participants and to optimize compliance and retention in the study. During the last 2 weeks of their menstrual cycle, participants self-collected a daily vaginal swab to quantitatively determine Candida colonization. All participants followed pilot-tested, comprehensively written instructions for the self-collection of vaginal swabs. No participants withdrew from the study.

Colony counts. Swabs were placed in Amies transport medium and posted to the laboratory in a sealed biohazard bag in a marked envelope, complying with local postal regulations. Quantitative colony counts of Candida spp. were performed based on methods detailed by González et al. (2009), in which five serial dilutions of vaginal swabs were performed. Initially, each swab was vortexed for 5 s in 0.9 ml sterile physiological strength saline in a capped sterile Wasserman tube. One hundred microlitres of the saline suspension was spread on the surface of a Brilliance Candida agar (BCA) plate (Oxoid) that had added chloramphenicol (0.4 g l⁻¹). Each plate was incubated at 36 °C for 24 and 48 h. The remaining suspension was capped and stored at 4 °C for further dilutions if growth of yeast occurred after 24 h of incubation. Further dilutions were performed (from 10⁻³ to 10⁻⁸) using sterile saline and 100 μl was spread on the surface of Sabouraud dextrose agar (SDA; Oxoid) containing 0.4 g chloramphenicol l⁻¹. If no colonies were present on the BCA plate, 500 μl initial saline suspension was added to the surface of an SDA plate and incubated for a further 48 h. The colony count was recorded as c.f.u. (100 μl)⁻¹ and (ml saline suspension)⁻¹ at 48 h of plate incubation. Yeast was identified as Candida albicans if green colonies were present on BCA as well as being germ tube-positive.

RESULTS

Seven of the ten women recruited for the study were culture-negative. Of these seven, two experienced symptoms. These were participant number 7, who reported abnormal discharge on day 19 and itching on days 21, 22 and 24, and participant number 8, who reported itching on days 22, 24 and 25, while having no vaginal colonization with Candida. Participant number 7 reported recurrent VVC, unlike participant number 8.

Three of the ten women were found to be colonized with C. albicans, participant numbers 2, 9 and 10. In all three women, Candida levels peaked from day 19 to 25 of the menstrual cycle. One woman (participant number 2) experienced no symptoms, with levels reaching 5.5 x 10⁴ c.f.u. ml⁻¹ on day 22. Participant numbers 9 and 10 both experienced itching and/or discharge following a rise in colonization, starting on days 23 and 16, respectively. Only participant number 10, who had more days of colonization and symptoms than the other two women, reported four or more episodes of VVC during the previous 12 months. The colony counts of the three women colonized with Candida spp. and their reported symptoms are presented in Table 1.

DISCUSSION

This study was set up as a pilot study to test the methodology of a proposed randomized controlled trial. It was not powered for statistical analysis and is the first study, to our knowledge, to investigate the daily candidal colonization patterns observed during the luteal phase of the menstrual cycle in three women. Despite finding no previous studies that recorded the daily quantitative counts of Candida spp., one study concluded that colonization levels may fluctuate from day to day and persist for several days before resolving spontaneously (Priestley et al., 1997). This study supports this finding.

Several studies claim that colonization generally rises in the second half of the menstrual cycle (Larsen and Galask, 1982; Odds et al., 1987; Priestley et al., 1997). The development and rise of colonization levels before menstruation were noted in these case studies. Interestingly, both participant numbers 9 and 10 reported symptoms during days 25–27, when their colonization levels were lower than those previously associated with no symptoms. Participant number 10, who reported more than four episodes of VVC in the previous 12 months, had higher levels of colonization and more days of reported symptoms than the other two culture-positive participants.

Some studies challenge the notion that higher levels of colonization equate with symptomatic episodes (Linhares et al., 2001; Odds et al., 1987). Comparing one woman with another could indeed lead to this conclusion, as evidenced by these case studies. In each of these three cases, there was no single trigger point of the Candida burden beyond which symptoms occurred. Symptoms were experienced by one woman (participant number 10) with a colonization level of 5.0 x 10⁵ c.f.u. ml⁻¹, while another (participant number 9) was asymptomatic with levels of 3.7 x 10⁴ c.f.u. ml⁻¹. The likelihood of experiencing vaginal symptoms in this study appeared to increase as individual colonization levels rose. Two women experienced initial symptoms after a rise in colonization levels. At their individual colonization peak levels, both symptomatic women reported discharge and itching. This small study may add evidence to the debate about whether the level of Candida colonization correlates with the development of symptoms. A limitation of this study is the inherent difficulty of standardization of the...
amount of secretions in self-collected daily vaginal secretions within an individual.

It is uncertain why the development of symptoms has been observed in the luteal phase of the menstrual cycle. Previous studies investigating the changing levels of cytokines and other vaginal immunoregulatory mechanisms during different phases of the menstrual cycle found that although changes with immunoregulatory markers were present in the luteal phase of the menstrual cycle, no conclusions could be drawn about protective immunity in acute VVC (Fidel et al., 2003). It is possible that the changes in immunoregulation could predispose a woman to symptomatic episodes in the luteal phase of the cycle. It is also possible that a consistently high level of colonization could cause mucosal irritation so that symptoms persist when colonization levels begin to fall. Another hypothesis is that tolerance of vaginal colonization of candida may be lower in the latter part of the luteal phase of the menstrual cycle.

### Concluding remarks

By carefully documenting daily quantitative counts of Candida and recording the symptoms experienced during the luteal phase of the menstrual cycle, this study has contributed to information from previous studies. The case studies in this study support observations that colonization levels appear to rise during the luteal phase of the menstrual cycle, with colonization levels rising approximately 6 days before menstruation in the three cases observed. These case studies also contribute new data, demonstrating rises in colonization preceding the onset of symptoms.

Future studies with a larger sample size may confirm whether variability both within and between subjects is as large as noted in this study. They would also confirm whether a sharp rise in colony counts does precede symptoms, determine clinical significance of colonization patterns, as well as identify host or other factors contributing to a rise in colonization.

### ACKNOWLEDGEMENTS

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### REFERENCES


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