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Prevalence of Non-*Albicans Candida* Infections in Women with Recurrent Vulvovaginal Symptomatology

Jason D. Mintz[1], Mark G. Martens[2]

[1]Rutgers-Robert Wood Johnson Medical School, Piscataway, USA; [2]Department of Obstetrics and Gynecology, Jersey Shore University Medical Center, Neptune, USA.
Email: mmartens@meridianhealth.com

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ABSTRACT

Background: *Candida* vulvovaginitis is one of the most frequently diagnosed conditions in women's care practices. Historically, 90% of cultured yeast species were *C. albicans*. However, due to a variety of interventions, the proportion of non-*albicans Candida* (NAC) infections appears to be increasing. We sought to estimate the current prevalence of *Candida* vulvovaginitis and the species-specific distribution of such infections in recurrent cases. **Methods:** Women with recurrent vulvovaginal symptomatology referred to an Obstetrics and Gynecology practice were tested by genital fungus culture, *Candida*-specific polymerase chain reaction (PCR), or both between July 2010 and February 2013. **Results:** A total of 103 women were tested. Mean age was 45.6 years. Including only their most recent positive test result, 29.1% (30/103) of women tested positive for *Candida* by any of the above testing measures. Of those, 50% (15/30) tested positive for *C. albicans* and 50% (15/30) tested positive for a NAC species. Across all visits, 60% (18/30) tested positive for *C. albicans*, 56.7% (17/30) tested positive for NAC, and 16.7% (5/30) tested positive for both a *C. albicans* and a NAC species. Among all isolated NAC species, 28.6% (6/21) were determined to be *C. glabrata*, 23.8% (5/21) *C. krusei*, 23.8% (5/21) *C. parapsilosis*, and 23.8% (5/21) other *Candida* species. **Conclusion:** Approximately 30% of women with recurrent vulvovaginal symptomatology have detectable *Candida* strains and it appears that NAC species may cause half of all these infections. This is imperative because NAC infections are usually more difficult to diagnose and are resistant to most treatments.

Keywords: Recurrent Vulvovaginal Candidiasis; Non-*Albicans Candida*; Fluconazole; Yeast Infections; Candida Vaginitis

1. Introduction

Candida vulvovaginitis is a frequently diagnosed condition in women's care practices [1]. It has been reported that approximately 75% of women will experience at least one yeast infection in their lifetime and that 5% - 8% of women will meet the criteria for recurrent vulvovaginal candidiasis (RVVC), having more than four episodes in a given year [2,3]. The cost associated with diagnosis and treatment of vulvovaginal candidiasis (VVC) is proposed to be in excess of $3 billion by 2014 [1]. This figure is likely a gross underestimate, as the number of *Candida* infections resistant to conventional therapeutics appears to be increasing [4].

The reported *Candida* prevalence in patients with vulvovaginal complaints is approximately 30% [5]. Historically, 85% - 95% of cultured yeast species were *C. albicans* [6-8]. However, due to a variety of interventions including single dose treatment, low-dosage azole maintenance regimens, and the use of over-the-counter antimycotics, the proportion of NAC species appears to be increasing [2,9-11]. Reports published in the most recent decade suggest a NAC prevalence of 10% - 30% in patients with VVC [4,12-16]. Among a study involving RVVC patients, 20% were found to be infected by a NAC species, but this study cultured only a limited number of NAC species [17]. Discrepancies in NAC prevalency figures may be reflective of differences among the respective patient cohorts sampled with regard to climates, cultures, geographic conditions, and prescribing patterns [2].

Within the past few years, there has been some evi-

dence put forth questioning the pathogenicity and overall significance of NAC species [17,18]. These authors propose that symptomatic patients testing positive for NAC species may not need to be treated, as their symptoms may result from an alternative diagnosis [17,18]. A major tenet of one such claim is that 27% of patients treated for a NAC infection had a persistence of symptoms despite negative culture [17]. Though a case-specific determination of NAC significance may be warranted in women with other potential diagnoses, it is worth noting that *Candida* infection can exist despite negative cultures [19]. With the advent of PCR, an additional cohort of women with vulvovaginal symptoms, yet negative cultures can be detected.

Other evidence also suggests that NAC infections warrant significant concern. Several reports note that NAC species appear to be associated with severe or recurrent cases of VVC [10,13]. Zeng *et al.* notes that a greater percentage of NAC infections than *C. albicans* infections is associated with more severe symptoms [13]. Similarly, Girgoriou *et al.* reports that NAC caused more frequent vaginal soreness and dyspareunia than *C. albicans* [10]. The predominant NAC species cited in these studies, *C. glabrata*, as well as other strains including *C. krusei*, do not reliably respond to azoles [4,16,20,21]. Therefore, identification is needed to better direct therapy.

Due to the significance of NAC species in clinical practice, we sought to determine the current prevalence of *Candida* vulvovaginitis and the species-specific distribution of such infections in recurrent cases.

2. Methods

2.1. Study Design

From July 2010 to February 2013 a retrospective analysis of all patients (103 in total) referred to an obstetrics and gynecology practice for recurrent vulvovaginal symptomatology was conducted to determine the prevalence of *C. albicans* and NAC infection. All women involved in the study presented after repeated courses of oral and topical antifungals for their recurrent symptoms. They were tested for *Candida* by genital fungus culture, *Candida*-specific PCR, or both. Since the compiled database included results from multiple visits, only the most recent positive *C. albicans* or NAC culture and/or PCR result was used to determine prevalence rates. However, positive *C. albicans* and NAC results from multiple visits were also collected. NAC species frequency was calculated using the positive NAC culture and/or PCR results from all visits.

2.2. Specimen Collection

Specimens for each patient were taken by direct vaginal wall collection during a speculum-assisted vaginal exam using sterile swabs (BBL CultureSwab, Becton, Dickinson and Company, Sparks, Maryland).

2.3. Culture

Primary genital fungal culture and yeast identification from the primary culture was performed according to the methodology of Hazen and Isenberg [22].

2.4. PCR

Yeast was detected for the following six species: *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. dublinensis* utilizing the amplification of *Candida* species target DNA by PCR based on dual priming oligonucleotide technology per the method of Luo and Mitchell [23].

3. Results

From July 2010 to February 2013, 103 women were examined and tested by culture and/or PCR for the etiology of their recurrent vulvovaginal symptoms. In total, 29.1% (30/103) of the women tested positive for any *Candida* species (**Figure 1**). An analysis of the diagnoses in these women suggested that *Candida* was the sole pathogen in 60% (18/30) of the patients with the remaining 40% (12/30) having a mixed infection of *Candida* plus a bacterial pathogen. Of the thirty woman who tested positive for *Candida*, 50% (15/30) tested positive for *C. albicans*, while 50% (15/30) tested positive for a NAC species (**Figure 1**). These numbers reflect the most recent positive culture and/or PCR result in each woman.

Across all visits and inclusive of any positive culture and/or PCR result in the thirty patients testing positive for *Candida*, 60% (18/30) had a *C. albicans* infection and 56.7% (17/30) had a NAC infection. Among these women, 16.7% (5/30) were infected by both *C. albicans* and a NAC species at some point in the study period with one woman having a simultaneous *C. albicans* and NAC infection.

Throughout the duration of the study, a positive NAC result was obtained in 21 samples in 17 different women. Of the four recurrent positive results, three women had different NAC species from an earlier visit and one woman had two different NAC species detected on the same visit. Among the NAC species identified, 28.6% (6/21) were determined to be *C. glabrata*, 23.8% (5/21) *C. krusei*, 23.8% (5/21) *C. parapsilosis*, 9.5% (2/21) *C. lusitaniae*, and 4.8% each *C. famata*, *C. tropicalis*, and *C. dublinensis* (**Figure 2**).

4. Discussion

Vulvovaginal symptomatology is a frequent complaint prompting a physician's office visit, with yeast infections

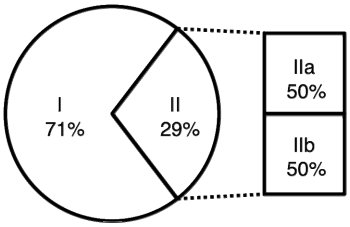

Figure 1. Prevalence of *Candida* in patients with recurrent symptoms, *n* = 103. I, percent prevalence in which *Candida* was not detected; II, percent prevalence in which *Candida* was detected; IIa, percent of II in which *Candida albicans* was detected; IIb, percent of II in which a non-*albicans Candida* species was detected.

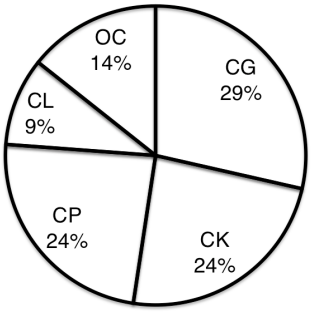

Figure 2. Non-*albicans Candida* species frequency, *n* = 21. CG, *Candida glabrata*; CK, *Candida krusei*; CP, *Candida parapsilosis*; CL, *Candida lusitaniae*; OC, other non-*albicans Candida* species.

representing one of the most common etiologies [1]. Approximately 5% - 8% of women experience RVVC, resulting in a significant cost to the healthcare system [1-3]. In this study, it was determined that 29.1% of women with recurrent vulvovaginal symptoms tested positive for *Candida* by culture and/or PCR. Among these women, 50% were determined to be infected by a NAC species.

Historically, limited data has been published on the prevalence of NAC infections in RVVC populations, most likely due to the focus on the preponderance of *C. albicans* infections in non-recurrent cases. However, NAC infections may cause more severe symptoms [10,13] and are often more difficult to treat [2]. In 2010, Kennedy *et al.* estimated the prevalence of NAC infection in RVVC patients to be 20% [17]. Although a large study, this prevalence was limited by the culture identification of a small subset of NAC species including *C. glabrata*, *C. parapsilosis*, and *C. lusitaniae* [17]. As evidenced by our data, other species such as *C. krusei* may also significantly contribute to the prevalence of clinically relevant NAC infections.

This greater NAC prevalence likely reflects an increasing trend. In a large retrospective study of a vaginitis clinic population, Spinillo *et al.* reported an increase in NAC prevalence from 9.9% to 17.2% between 1988 and 1995, a trend which was upheld even after a subgroup analysis of self-referred patients without a history of RVVC [9]. Similarly, Martens *et al.* reported a NAC prevalence of 18.9% in a cohort of women with yeast infections from 1994-1996, two years following over-the-counter approval for topical antifungals, but prior to the popularization of oral fluconazole [24]. In a gynecology infectious disease practice, Nyirjesey *et al.* found a 25% NAC prevalence among a population of chronic vaginitis patients between 1991 and 1993 [25]. Most recently in 2011, Guzel *et al.* reported a 50% NAC prevalence in their mixed acute and chronic vaginitis patient population [11].

With regard to patients diagnosed with RVVC, the recent, sharp increase in NAC infection possibly stems from the increased and repeated usage of fluconazole. Fluconazole was Food and Drug Administration approved for the treatment of vulvovaginal candidiasis in 1994 and since then its prescription by physicians has risen [26]. Unlike *C. albicans*, which has demonstrated uniform susceptibility to fluconazole without a significant increase in the MIC50 or MIC90 between 1986 and 2008, NAC species including *C. glabrata*, *C. lusitaniae*, and *C. krusei* do not reliably respond to fluconazole [4,16,20,21].

Fluconazole, rather than topical azoles, is likely the major etiologic factor for increased NAC prevalence because it is absorbed through the gastrointestinal tract. Previous studies indicate that the gut serves as the initial reservoir for vulvovaginal colonization[2] and that there is a high degree of homology between yeast found in both the intestines and vagina [2,27,28]. We suspect that repeated trials of fluconazole select for more resistant NAC species in the gut, particularly in RVVC patients who have received multiple courses over several years. These species then colonize and infect the vulvovagina relatively unopposed from competing yeast species. Consequently, it is expected that an analysis of primary referral data may reveal, a preponderance of patients initially infected by *C. albicans* who developed NAC infections over the course of repeated fluconazole treatment.

Along with the increased prevalence of NAC infections due to increased fluconazole use, the advent of PCR has allowed us to detect a greater proportion of such infections. Culture is only able to detect infection in patients harboring 10^3 organisms per milliliter; yet, as few as 10^2 organisms are sufficient to cause symptoms [19]. PCR can identify an additional group of patients in which definitive diagnostic evidence would not otherwise be available. However, despite the heightened sensitivity of PCR, concordance between both culture and PCR may be lacking [22]. In their study, Mardh *et al.* reported a 43% concordance rate between culture and PCR with 21% of vulvovaginal *Candida* samples positive only by culture and 18% positive only by PCR [22]. Reduced concordance rates may be partially attributable to detection thresholds

set by laboratories. Standardization among laboratories should be adopted.

5. Conclusion

As a result of the increased prevalence of NAC species in patients with recurrent symptoms, the identification of offending *Candida* strains is of high priority to better direct therapy and eradicate infection early. Further investigation into the most reliable and cost effective means of identification should be performed.

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