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Genital *Candida* Species Detected in Samples from Women in Melbourne, Australia, before and after Treatment with Antibiotics

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Vulvovaginal candidiasis (VVC) remains a common cause of morbidity, with three-quarters of women affected during their lifetimes. Use of antibiotics is an acknowledged trigger for VVC, which adversely affects women's physical and emotional health. Knowledge of patterns of genital *Candida* species-level identification is important for management, as *Candida* species other than *Candida albicans* often fail first-line treatment. A community sample of women with no vaginal symptoms, and who were prescribed antibiotics, was recruited into this study, where the incidence of genital colonization by various *Candida* species was documented, as well as symptoms of VVC plus relevant associations, before and after treatment with antibiotics. Self-collected low vaginal swabs were taken prior to and 8 days after completion of antibiotic treatment, and data on various potential risk factors for VVC were collected simultaneously, with complete data being available for 233 participants. Baseline *Candida* species colonization was present in 21% of women (95% confidence intervals [CI], 17% to 27%), rising to 37% (95% CI, 31% to 44%) after antibiotic treatment. The primary species detected for either period was *C. albicans* (73%), with *Candida glabrata* detected in around 20%. Self-assessed proneness to VVC after antibiotic treatment and baseline colonization with *Candida* spp. were significantly associated with symptomatic VVC after antibiotic treatment. For microbiologically proven candidiasis, VVC symptoms had a sensitivity of 57% and a specificity of 91%. When physicians prescribe antibiotics, the history of risk of VVC is one issue that physicians should discuss with women, particularly those who are self-identified as being prone to VVC. Furthermore, we recommend that definitive microbiological diagnoses be made for women with recurrent symptoms or those failing initial treatment, to guide appropriate therapy.

Epidemiology. Vulvovaginal candidiasis (VVC) is a common problem for women and may affect their physical and emotional health, as well as relationships with their partners (6). Lifetime prevalence figures for VVC are around 70%; this number may be inaccurate, as many studies are based on patient self-reporting, without microbiological confirmation (18).

Prevalence studies of genital tract colonization (asymptomatic) by *Candida* species with microbiological evidence are difficult to compare, as many studies examine tertiary hospital populations and may include or target only those with vaginitis symptoms. Worldwide estimates of rates of genital *Candida* colonization range from 17% in Turkey to up to 30% in a U.S. study of asymptomatic young women followed over 12 months (2, 3, 7, 11, 13, 17, 20).

Knowledge of patterns of genital *Candida* species-level identification for VVC is important for clinicians, as *Candida* species other than *Candida albicans* often fail first-line treatment. Despite fears that the advent of over-the-counter antifungal agents would encourage the emergence of relatively resistant non-*C. albicans Candida* species, the dominant *Candida* species in the United Kingdom remains *Candida albicans*, with non-*C. albicans Candida* species staying at a level of approximately 5% over the past decade (20). The proportion of genital *C. albicans* in symptomatic women ranges from approximately 90% in U.S. and Australian samples (11, 13) to approximately 65% in Belgium (2), Turkey (7), and Saudi Arabia (1).

***Candida* and antibiotics.** Previous work has identified VVC after antibiotic treatment as a concern for many women, which may affect their compliance with prescribed antibiotic treatments (14). However, in a comprehensive literature review, using a combination of "candida," "vagina," "epidemiology," and/or "*Candida* spp." as search terms in December 2005, we found only one prospective community-based study that examined the incidence of VVC after antibiotic treatment. In that study of 74 women who took a variety of antibiotics for nongenital infections, the incidence of vulvovaginitis after antibiotic use was 32% (95% confidence intervals [CI], 22% to 44%) (5).

We previously undertook a randomized placebo-controlled factorial trial, to test whether the popular probiotic *Lactobacillus acidophilus* was effective in the prevention of vulvovaginitis after antibiotic use (16). As the intervention was not effective, and there was no difference in outcome between the oral and vaginal probiotic groups, trial data have been examined post hoc. We describe the incidence of genital *Candida* spp. present before and after antibiotic treatment, and associations with *Candida* colonization as well as VVC after antibiotic treatment, in a community sample of women.

MATERIALS AND METHODS

Participants. The sample was recruited largely through a wide network of family physicians in Melbourne, Australia (15). Women aged 18 to 50 years, with a nongynecological infection requiring short-term oral antibiotic treatment, were recruited just prior to or within 48 h of commencement of antibiotic treatment.

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TABLE 1. Candida cultured at baseline and in post-antibiotic treatment swabs

Type of result for *Candida* for post-antibiotic treatment swab	No. of baseline swabs (% of total swabs) with result for *Candida*		Total
	Positive[a]	Negative	
Positive[b]	42 (18)	44 (19)	86 (37)
Negative	8 (3)	139 (60)	147 (63)
Total	50 (21)	183 (79)	233

[a] Baseline swabs positive for *Candida* included 36 for *C. albicans*, 11 for *C. glabrata*, and 3 for *C. parapsilosis*.
[b] Post-antibiotic treatment swabs positive for *Candida* included 63 for *C. albicans*, 18 for *C. glabrata*, 1 for *C. parapsilosis*, and 4 for other species.

The study received ethics approval from the Royal Australian College of General Practitioners and the Royal Women's Hospital, Melbourne, Australia.

Design. After written and verbal instruction, women provided self-collected low vaginal swabs at baseline and after antibiotic use. Post-antibiotic treatment specimens were generally collected 8 days after the end of antibiotic treatment or at the time of developing symptoms of VVC, if that occurred. Participants also completed questionnaires at baseline and after antibiotic treatment (demographic information, self-assessed likelihood of developing vulvovaginitis after antibiotic treatment, antimicrobial prescribed, infection indication, body mass index, whether sexually active and sexual activity during the trial, other medical conditions, adherence to the antibiotic regimen, any symptoms of VVC, and time to onset of these symptoms).

One laboratory, the Royal Women's and Children's microbiology laboratory based at the Royal Children's Hospital site, managed all microbiological specimens, to ensure consistency of processing and reporting. Vaginal swabs were initially rolled onto a glass slide for microscopic examination following Gram staining. The swab was then rolled onto a half CHROMagar medium (CHROMagar, France) (4) and incubated at 35°C for 2 days. *C. albicans*, *Candida glabrata*, and *Candida krusei* were identified by the specific color of colonies on CHROMagar; other *Candida* spp. were identified by use of the ID32C kit (bioMerieux) (12).

The primary outcome of the trial was symptomatic VVC: this was defined as both participants' report of appropriate symptoms (that is, an answer of "yes" or "maybe" to a question about symptoms of vaginal itch and irritation, with or without a discharge) and a post-antibiotic treatment vaginal specimen positive for *Candida* spp. Participants who did not return a post-antibiotic use questionnaire or a vaginal specimen were followed up by telephone and letter, whereby it was usually possible to attain minimal information about occurrence of VVC symptoms. Women who recorded no symptoms of vulvovaginitis after antibiotic use but had *Candida* spp. in the post-antibiotic treatment swab were contacted at least 1 week after they completed the trial, to exclude development of symptoms of VVC.

Statistical analysis. *Candida* colonization was measured before and after antibiotic treatment for each woman. To account for the pairing of the test results from each woman, McNemar's chi-squared test was used to test for any change in *Candida* colonization before and after antibiotic use. The matched odds ratio (OR) was also calculated using the ratio of discordant pairs, when *Candida* colonization differed before and after antibiotic treatment: that is, the odds of changing from negative to positive for *Candida* compared to changing from positive to negative. The concordant pairs provided no information about the association. The odds ratio is reported with the respective 95% CI.

Risk factors for VVC by culture results were summarized as frequencies and percentages. Associations between symptoms of VVC and culture results were examined using Pearson's chi-squared statistic.

Logistic regression was used to examine association with potential risk factors of the two main binary outcomes, *Candida* colonization at baseline and vulvovaginitis after antibiotic treatment. Vulvovaginitis after antibiotic treatment was defined as women who had *Candida* colonization and reported typical symptoms after antibiotic use. Multivariable logistic regression was used to adjust for possible confounders. Risk factors that showed weak evidence of association ($P < 0.1$) were left in a multivariable model. For the first outcome, *Candida* colonization, none of the risk factors were found to be statistically significant in the multivariable logistic model and hence we have only reported the unadjusted ORs. Associations were reported as OR with respective 95% CI and *P* values. All reported *P* values are two-sided. Participants who withdrew and did not provide outcome data were excluded from the analysis, which was performed using STATA version 8.

RESULTS

Sample characteristics and participant flow have been described previously (16). Complete data are presented for 272 participants at baseline and 233 women after antibiotic treatment. In addition, post-antibiotic treatment symptom data are available for 257 participants. Most women (96%) self-reported good compliance ("took most or all") with prescribed antibiotics. Moreover, only six women (2%) were recruited more than 24 h after commencing their antibiotic treatments. All vaginal specimens were processed in the study laboratory, except for three specimens sent to external laboratories.

***Candida* species.** At baseline, 21% (59/275; 95% CI, 17% to 27%) of the asymptomatic women were colonized with *Candida* spp. Of these, *C. albicans* was the primary species in 43 (73%), 12 (20%) had *C. glabrata* (one of whose specimens also grew *C. albicans*), three (5%) had *Candida parapsilosis* (from one of whose specimens *Rhodotorula mucilaginosa* was also cultured), and *Candida fomata* was cultured from one woman's sample.

Table 1 shows results of *Candida* growth from baseline and post-antibiotic treatment specimens for women who supplied both ($n = 233$). Approximately 2 weeks after baseline and 8 days after completing a course of antibiotics, 37% (86/233; 95% CI, 31% to 44%) of women had specimens culture positive for a *Candida* species. In 63 women (73%), the main growth was *C. albicans*, while in 18 women (21%) *C. glabrata* was predominant. The odds ratio of a positive result for *Candida* after antibiotic treatment, if the baseline test was negative, was 5.5 (CI, 2.6 to 13.5; $P < 0.001$), compared to having an initial positive result followed by a negative one.

TABLE 2. Risk factors for vulvovaginal candidiasis in women colonized and not colonized with *Candida* species at baseline ($n = 272$)

Risk factor	No. (%) colonized with *Candida* spp. ($n = 58$[a])	No. (%) not colonized with *Candida* spp. ($n = 214$[a])	Unadjusted odds ratio (95% CI)	*P* value
Current smoker	10 (20)	39 (80)	0.93 (0.43–2.0)	0.85
Using estrogen-based contraception	19 (23)	62 (77)	1.19 (0.64–2.23)	0.58
Current sexual relationship	47 (24)	152 (76)	1.74 (0.85–3.58)	0.13
BMI[b] of >30	4 (12)	29 (88)	0.50 (0.17–1.50)	0.41
Self-assessed proneness[c]	34 (28)	86 (72)	2.17 (1.19–3.98)	0.01
Regular yogurt consumption	37 (21)	136 (79)	1.01 (0.55–1.85)	0.97

[a] Some missing data.
[b] BMI, body mass index.
[c] Answered "half the time," "often," or "always" to the question "How often would you estimate you get thrush when taking antibiotics?"

TABLE 3. Risk factors for vulvovaginal candidiasis by postantibiotic vulvovaginitis cases and noncases ($n = 257$)

Risk factor	No. (%) of postantibiotic vulvovaginitis cases ($n = 55$)	No. (%) of postantibiotic vulvovaginitis noncases ($n = 202$[a])	Unadjusted odds ratios (95% CI)	Adjusted odds ratios (95% CI)[b]
Estrogen-based contraception	18 (33)	56 (28)	1.27 (0.67–2.41)	
Current sexual relationship	46 (84)	143 (71)	2.10 (0.97–4.58)	
Intercourse during trial	26 (47)	93 (53)	0.81 (0.44–1.48)	
BMI[c] of >30	7 (13)	25 (13)	1.05 (0.42–2.59)	
Candida spp. cultured at baseline	27 (49)	29 (14)	5.79 (2.99–11.18)	4.64 (2.34–9.18)
Broad-spectrum antibiotic use	20 (36)	57 (28)	1.46 (0.78–2.74)	
Self-assessed proneness[d]	21 (38)	46 (23)	3.11 (1.63–5.94)	2.61 (1.33–5.11)
Regular yogurt consumption	33 (60)	132 (65)	0.80 (0.43–1.45)	

[a] Omits participants who provided outcome data inadequate for ascertaining case status.
[b] Calculated using multivariable logistic regression.
[c] BMI, body mass index.
[d] Answered "half the time," "often," or "always" to the question "How often would you estimate you get thrush when taking antibiotics?"

Association of possible risk factors for VVC and *Candida* colonization at baseline. Table 2 shows associations between possible risk factors for VVC and *Candida* species colonization at baseline. Only self-reported proneness to developing VVC after antibiotic use was associated with baseline *Candida* colonization in univariate analysis.

Association of possible risk factors for VVC and post-antibiotic use symptoms of vaginitis. A final report of vaginitis symptoms was established in 257 women. Participants reporting no symptoms of VVC were classified as noncases. Table 3 shows characteristics of women who developed VVC after antibiotic treatment compared with those who did not. *Candida* spp. in a preintervention swab (OR, 5.79; CI, 2.99 to 11.18) and self-assessed proneness to vulvovaginitis after antibiotic use (OR, 3.11; CI, 1.63 to 5.94) were the only characteristics to be significantly associated with the development of vulvovaginitis after antibiotic use in univariate analysis. Moreover, after adjustment for possible confounders, these two factors remained strong predictors of vulvovaginitis after antibiotic use. There was no evidence of an interaction between baseline *Candida* colonization and self-assessed proneness to vulvovaginitis after antibiotic use.

The antibiotics taken by study participants were grouped into narrow-, moderate-, or broad-spectrum drugs, according to Australian antibiotic guidelines (19). There were no statistically significant differences in development of VVC after antibiotic treatment by antibiotic group in univariate analysis. Of women taking the most frequently prescribed antibiotic, amoxicillin ($n = 80$), 18% (14 women) developed VVC compared to 21% of those taking any other antibiotic (OR, 0.81; 95% CI, 0.38 to 1.65). Fewer participants used other antibiotics: the second most common group was cephalexin ($n = 28$). Compared to narrow-spectrum antimicrobials, women taking broad-spectrum antibiotics had an OR of 1.24 (95% CI, 0.55 to 2.78) for VVC; compared to those taking moderate-spectrum antimicrobials, the OR was 0.79 (95% CI, 0.36 to 1.7) using logistic regression.

Table 4 shows women's report of vulvovaginitis symptoms and results for post-antibiotic treatment swabs. Overall, 23% (55/235; 95% CI, 18% to 29%) of women developed vulvovaginitis with a post-antibiotic treatment swab culture positive for a *Candida* species. Women with symptoms of vaginitis were three to four times as likely to be culture positive as negative. *C. albicans* was detected in 49 (89%) cases of VVC, with *C. glabrata* accounting for a further four cases. The final two cases' specimens were processed at outside laboratories: one was reported to be a *Candida* species other than *C. albicans* and the other was not identified to species level.

Of 51 women with a baseline specimen positive for *Candida* spp., 42 (82%) also had a positive swab after antibiotic treatment. Of these 42 participants, 27 (53%) also had symptoms and were therefore defined as cases of post-antibiotic treatment vulvovaginitis. Of women reporting definite post-antibiotic treatment vulvovaginitis symptoms, 79% (48/61) had a post-antibiotic treatment swab positive for *Candida* spp., whereas 28% (7/25) of women reporting possible symptoms had a *Candida* species in such specimens.

Reporting of definite symptoms of vaginitis after antibiotic use had a sensitivity of 57% and specificity of 91% for a post-antibiotic treatment swab with *Candida* spp. The positive and negative predictive values of symptoms of VVC after antibiotic use for culture of *Candida* spp. in this study were 79% and 78%, respectively.

Further observations (to be interpreted with caution due to small numbers) were those of women with *Candida* spp. cultured from baseline swabs: 56% (24/43) with *C. albicans* and 25% (3/12) with *C. glabrata* developed vulvovaginitis after antibiotic use (OR, 5.1; 95% CI, 1.1 to 31.0). Of those with *Candida* spp. cultured from post-antibiotic treatment swabs, 78% (49/63) of those with *C. albicans* and 22% (4/18) of those with *C. glabrata* had VVC symptoms (OR, 12.3; CI, 3.1 to

TABLE 4. Symptoms of vulvovaginal candidiasis and postantibiotic culture results ($n = 228$)[a]

Symptoms of vaginitis	No. (%) of postantibiotic swabs culture negative for *Candida* spp.	No. (%) of postantibiotic swab cultures positive for *Candida* spp.	*P* value[c]
No	113 (78)	29 (35)[b]	
Maybe	18 (13)	7 (8)	
Yes	13 (9)	48 (57)	<0.001

[a] Missing data: three women reported definite symptoms but used treatment before obtaining a specimen; four did not provide post-antibiotic treatment specimens but reported symptoms of VVC on follow-up.
[b] Three in this group reported symptoms after the conclusion of the trial.
[c] Using Pearson's chi-squared statistic.

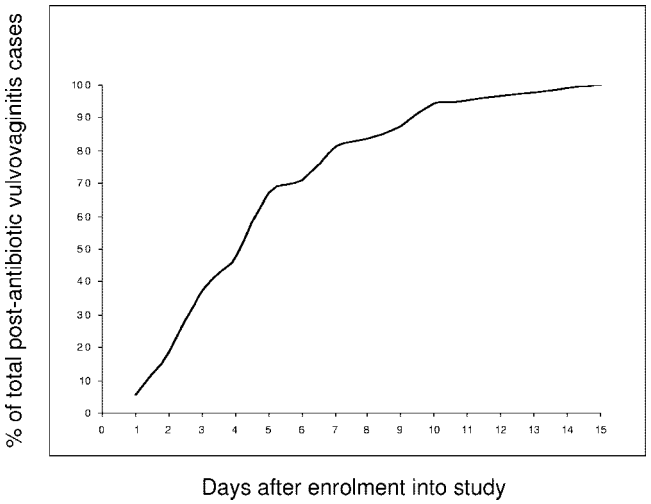

FIG. 1. Cumulative percentage of cases of post-antibiotic treatment vulvovaginitis symptoms by days from enrollment into the study ($n = 55$).

57.2). No other detected *Candida* spp. were implicated in vulvovaginitis after antibiotic treatment.

Figure 1 demonstrates the time to onset of post-antibiotic treatment vulvovaginitis symptoms, which was a median of 5.3 days (interquartile range, 3.0 to 7.0 days) from enrollment into the study for those with proven vulvovaginitis after antibiotic use and a median of 5.1 days for those with suggestive symptoms but no candidal growth.

DISCUSSION

We report the results for *Candida* colonization and VVC after antibiotic treatment of a prospective community sample taking antibiotics for nongenital infections. At baseline, 21% of asymptomatic women aged 18 to 50 years had *Candida* spp. cultured from a self-collected vaginal swab; this figure reached 37% after antibiotic treatment, with 20% of women symptomatic for VVC. *C. albicans* was the dominant species (nearly three-quarters of both pre- and post-antibiotic treatment swabs) as described in other studies. Adjusted self-reported proneness to vulvovaginitis after antibiotic use and *Candida* colonization at baseline predicted later symptomatic vulvovaginal candidiasis after antibiotic use, whereas use of estrogen-based contraception and recent sexual activity did not.

Our results should be generalizable to other primary care populations, as recruitment was across a large city, covered a wide range of sociodemographic areas, and was done through primary care clinics.

It is difficult to compare our findings of asymptomatic colonization by *Candida* spp., as most other studies have been in selected populations, often those with gynecological problems. Our findings of 21% *Candida* colonization (95% CI, 17% to 27%) in otherwise asymptomatic women at baseline lie within the range of incidences reported from other countries.

The incidence of vulvovaginitis after antibiotic treatment in our series of 23% (55/235; 95% CI, 18% to 29%) is in the lower range of the value from the only comparable prospective community study, 32.4% (95% CI, 22% to 44%) (5). This sample was also drawn from a primary care setting and had the advantage of women being examined at the time of recruitment but was a much smaller sample size ($n = 74$) and the average length of antibiotic courses was longer, 9 days. In that study, *Candida* colonization at baseline was not predictive of vulvovaginitis after antibiotic treatment (risk ratio, 0.58; CI, 0.25 to 1.37). However, a further study in a pregnant population found a lower rate of VVC after antibiotic treatment at 6% (7/115; 95% CI, 2% to 12%), but with a risk ratio of 3.3 (CI, 1.68 to 6.49) if an initial swab was positive for *Candida* spp. (10).

Another interesting finding of our study was that *C. albicans* at baseline was more likely to be associated with VVC after antibiotic treatment than other species were, suggesting greater potential for clinical disease. However, *C. glabrata* accounted for at least 7% of VVC cases. Therefore, it is important that clinicians make a definitive diagnosis for candidiasis and that microbiology laboratories culturing *Candida* identify organisms to species level, for those women with recurrent symptoms or those failing initial treatment, so that appropriate antifungal medications are prescribed. The results also confirm previous work reporting that symptoms of VVC are not accurate predictors of *Candida* presence (3).

The implications for practice of this study are that women's self-reporting of proneness to vulvovaginitis after antibiotic treatment is a good predictor of the condition. In the absence of evidence to guide management of these women, one approach may be to consider antifungal chemoprophylaxis when antibiotics are to be prescribed for them.

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