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Vaginal Microbiology of Women with Acute Recurrent Vulvovaginal Candidiasis

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Alterations in the autochthonous vaginal microflora can predispose women to recurring attacks of *Candida* vaginitis. Quantitative aerobic and anaerobic cultures were obtained from 24 premenopausal women with acute recurrent vulvovaginal candidiasis and from 21 healthy asymptomatic premenopausal women. *Lactobacillus* species constituted the predominant flora in both groups, with a mean $\log_{10}$ CFU/ml of 8.7, a total isolation rate of 96%, and a mean of 1.6 *Lactobacillus* species isolated per patient compared with a mean $\log_{10}$ CFU/ml of 8.9, a total isolation rate of 90%, and a mean of 1.2 *Lactobacillus* species isolated per patient in the vaginitis and control groups, respectively. The results of this small study failed to provide evidence of an altered or abnormal vaginal bacterial flora in women with non-antibiotic-induced recurrent vulvovaginal candidiasis suffering from acute *Candida* vaginitis.

Vulvovaginal candidiasis (VVC) is the second-most-common form of vaginitis in the United States (4). VVC is associated with antibiotic therapy, pregnancy, use of oral contraceptives containing high levels of estrogen, estrogen therapy, and uncontrolled diabetes (9, 25). In most patients with acute symptomatic VVC, a precipitating cause is not found. A subgroup of women with VVC develop recurrent symptomatic episodes of vaginitis or even chronic VVC (25).

It has been postulated that the normal vaginal bacterial flora and specifically *Lactobacillus* species play a critical role in the prevention of vaginal infections and the transmission of pathogens responsible for sexually transmitted diseases (8, 9, 16). The production of hydrogen peroxide and the maintenance of a normal pH by *Lactobacillus* species are thought to reduce the overgrowth of microorganisms associated with bacterial vaginosis (7, 11). Similar information in relation to VVC is surprisingly lacking. There also is a lack of information on the role of vaginal flora associated with recurrent and chronic VVC, and few studies have addressed this issue (1, 22, 26).

We studied the vaginal flora of women with histories of recurrent VVC during episodes of acute *Candida* vaginitis, with particular reference to lactobacillus quantitation and speciation.

MATERIALS AND METHODS

Women with acute VVC who were otherwise healthy were included in the study. Exclusion criteria were pregnancy, age of <18 or >50 years, and the presence of mixed infections (including, e.g., bacterial vaginosis, genital herpes, and trichomoniasis). Acute VVC was diagnosed by the following criteria: (i) acute onset of pruritus or vulvovaginal irritation and/or soreness, (ii) vulvovaginal erythema and/or edema, (iii) vaginal pH from 4 to 4.5, (iv) negative amine test, (v) presence of budding yeast cells or hyphae as shown by wet mount–10% potassium hydroxide (KOH) microscopy, and (vi) confirmation by culture. A control group of healthy asymptomatic women was also studied. Each patient and control subject signed an informed consent form. All the patients met the study case definition of recurrent VVC, that is, each had a history of three or more documented episodes of acute VVC in the previous year.

During speculum examination, swab specimens were obtained from the middle third of the vagina and placed on Sabouraud glucose agar and used for pH determination and wet mount-KOH microscopy, and an amine test was performed. With 3 ml of 0.85% prereduced saline, the vagina was gently lavaged. The solution was immediately aspirated and placed in an anaerobic collection tube and introduced in an anaerobic chamber. Serial dilutions were performed for microbiologic quantitation. Standard methods were used for the isolation and identification of aerobic and anaerobic bacteria. Lactobacilli were identified according to the method of Holdeman et al. (14), which is based on carbohydrate fermentation, demonstration of lactic acid production by gas-liquid chromatography, and determination of the amount of L-lactic acid production by spectrophotometry. Yeasts were identified as *Candida albicans* if they were able to produce germ tubes in human serum and by chlamydospore formation. Yeasts that were germ tube negative were identified and speciated by using the API-C system (Sherwood Medical, Plainview, N.Y.).

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RESULTS

The study group comprised 24 women with acute recurrent VVC. The mean age was 32 years (range, 21 to 44 years). Three patients had a history of recent administration of systemic antibiotics, including cephalexin, metronidazole, and an unknown antibiotic for bronchitis.

The control group consisted of 21 healthy, asymptomatic female volunteers with a mean age of 26 years (range, 21 to 43 years). A vaginal pH of >4.5 (4.6) was found in only one woman. Wet mount microscopy showed coccobacillary flora for this woman, and she was excluded from this study. Four asymptomatic control women had positive KOH microscopy and cultures but had no evidence of vaginitis and were included in this study.

A summary of the vaginal bacterial flora of the control women is shown in Table 1. *Lactobacillus* species constituted the predominant flora, with a mean $\log_{10}$ CFU/ml of washing of 8.9, and lactobacilli were recovered from 18 of 20 control women (90%). The predominant *Lactobacillus* species was *Lactobacillus salivarius* subsp. *salivarius*, with an isolation rate of 35%. Overall, a mean of 1.2 *Lactobacillus* species per subject were isolated. *Gardnerella vaginalis* was isolated in 3 of 20 women (15%). Strictly anaerobic bacteria were isolated from 10% of control women. *C. albicans* was isolated from five women (25%).

A summary of the bacterial flora of the 24 women with acute VVC is shown in Table 2. *Lactobacillus* species were again the predominant flora, with a mean $\log_{10}$ CFU/ml of washing of 8.7 and an isolation rate of 96%. The mean number of *Lactobacillus* species isolated per patient was 1.6, and the predominant species was *Lactobacillus catenaforme*, with an isolation rate of 42%, and this was closely followed by *L. salivarius* subsp. *salivarius* and *L. salivarius* subsp. *salicinius*, both with an isolation

TABLE 1. Summary of vaginal flora of 20 healthy adult premenopausal women[a]

Organism	Mean (range) $\log_{10}$ (CFU/ml of washing)	Rate of isolation (%)
Candida albicans	2.1 (2.0–2.3)	25
Aerobes or facultative anaerobes		
Lactobacillus species	8.5 (4.0–9.0)	15
Lactobacillus salivarius subsp. *salivarius*	8.3 (4.7–9.2)	35
Lactobacillus salivarius subsp. *salicinius*	8.3 (4.1–9.8)	15
Lactobacillus catenaforme	9.2 (5.7–9.7)	15
Lactobacillus casei subsp. *alactosus*	8.6 (4.3–9.2)	25
Lactobacillus plantarum	6.1 (4.6–6.4)	10
Lactobacillus acidophilus	6.3	5
Total facultative lactobacilli	8.9 (4.0–9.8)	90
Streptococcus species	8.5 (2.0–9.6)	15
Staphylococcus species	7.1 (2.3–8.0)	35
Corynebacterium species	8.8 (2.0–9.5)	25
Micrococcus varians	2.0	5
Gardnerella vaginalis	9.3 (3.0–9.8)	15
Escherichia coli	4.6	5
Anaerobes		
Peptococcus magnus	8.0 (2.3–8.4)	10
Peptococcus morbillorum	6.1	5

[a] Per patient, the mean numbers of *Lactobacillus* and bacterial species isolated were 1.2 and 3, respectively (ranges, 0 to 3 and 1 to 6, respectively), which correspond to rates of isolation of 90 and 100%, respectively.

rate of 37.5%. *C. albicans* was isolated from 22 of 24 patients (92%) and *Candida parapsilosis* was isolated from 2 patients.

Non-*Lactobacillus* species were isolated with low frequency in both groups, with a trend towards a higher number of *Corynebacterium* species in the control group; however, the low overall frequency of isolation of members of those genera fails to indicate any significant difference in the two groups.

DISCUSSION

Since Doderlein's description in 1892, the presence of lactobacilli in vaginal secretions has been associated with a normal status (3, 17). It has been suggested that lactobacilli in the vagina have a protective role, preventing the development of bacterial vaginosis and the transmission of pathogens responsible for sexually transmitted diseases (9, 16, 21, 26). In 1921, Schroder, in stressing the protective role of lactobacilli, described three vaginal flora patterns in relation to lactobacillus presence and pathogenicity, viz., (i) lactobacillus dominant, the least pathogenic; (ii) intermediate; and (iii) lactobacillus free, the most pathogenic (23). Siegler first suggested that *Candida* vaginitis is associated with an intermediate flora pattern (24). Hillier et al., reviewing Gram-stained preparations of vaginal flora from 7,918 pregnant women, similarly reported an association between VVC and intermediate or normal flora patterns (12). Hillier et al. further reported an association between antibiotic use and the intermediate pattern of vaginal flora (12). Several defense mechanisms for lactobacilli have been proposed, including vaginal acidity, but the exact glycogen breakdown mechanism for lactic acid production is still unknown. Other protective mechanisms of bacterial flora against colonization and invasion by *C. albicans* involve the production of bacterial toxins, stearic interference with adherence, and a decrease in the available energy source (16). Lactobacilli have been shown to inhibit germ tube formation by *C. albicans* (20) and in vitro growth of microorganisms, including *C. albicans* (5). *Lactobacillus acidophilus* has been found to produce acidolin (10) and lactacin B (2), an antibiotic and a bacteriocin, respectively, which are in part responsible for their protective activity. More recently, it has been demonstrated that peroxidases combined with H_2O_2 and a halide have properties toxic against a variety of microorganisms. Lactic acid-producing bacteria, e.g., *L. acidophilus*, release H_2O_2 required for this peroxidase-mediated antimicrobial system (16, 18). *Lactobacillus* species capable of releasing H_2O_2 have been observed in the vaginae of 98% of normal women (7).

Few published studies on vaginal flora have specifically addressed the qualitative and quantitative aspects of the flora of women with VVC, and fewer reports have included quantitative anaerobic cultures (1, 22, 26). Auger and Joly investigated the vaginal microbial flora associated with *Candida* vulvovaginitis and found a decrease in the associated aerobic bacterial flora of the vagina in the presence of *C. albicans* vaginitis (1). This study failed to utilize anaerobic microbiologic techniques. Auger and Joly hypothesized that gram-negative bacteria are more antagonistic to *C. albicans* than are gram-positive species, supporting the observation that broad-spectrum antibiotics induce yeast infections. Salvaggi et al. (22) reported the frequent association of vaginal candidiasis with the presence of gram-positive cocci and gram-negative bacilli, with no reference at all to *Lactobacillus* species. In 1979, Levison et al. reported similar titers of both aerobic and anaerobic lactobacilli in asymptomatic women and those with various types of vaginitis, excluding bacterial vaginosis (19). Recently, Hillier et al., in a large cross-sectional study of predominantly asymptomatic pregnant women, observed that the frequency of *Candida* colonization was higher among women with H_2O_2-nega-

TABLE 2. Summary of vaginal flora of 24 patients with acute VVC

Organism	Mean (range) $\log_{10}$ (CFU/ml of washing)	Rate of isolation (%)
Candida albicans	4.4 (2.3–5.0)	92
Candida parapsilosis	5.7 (4.3–6.0)	8
Aerobes or facultative anaerobes		
Lactobacillus species	7.7 (4.6–8.6)	29
Lactobacillus salivarius subsp. *salivarius*	6.1 (4.6–6.9)	37.5
Lactobacillus salivarius subsp. *salicinius*	8.8 (3.6–9.8)	37.5
Lactobacillus catenaforme	8.5 (4.6–8.8)	42
Lactobacillus casei subsp. *alactosus*	9.2 (5.4–9.8)	17
Lactobacillus jenseni	8.9	4
Lactobacillus plantarum	6.7 (4.0–7.1)	8
Total facultative lactobacilli	8.7 (4.6–9.8)	96
Streptococcus species	5.7 (4.5–5.9)	8
Staphylococcus species	3.0 (2.0–3.6)	37.5
Corynebacterium species	4.6 (2.6–5.3)	5
Unidentified gram and rod bacilli	6.4	4
Gardnerella vaginalis	9.8	4
Moraxella species	2.0	4
Anaerobes		
Peptococcus magnus	3.0	4
Lactobacillus salivarium subsp. *salivarius*	6.5	4

[a] Per patient, the mean numbers of *Lactobacillus* and bacterial species isolated were 1.6 and 2.6, respectively (ranges, 0 to 3 and 1 to 6, respectively), which correspond to rates of isolation of 96 and 100%, respectively.

tive lactobacilli (11). Similarly, asymptomatic *Candida* infection was more frequent in women having only H_2O_2-negative lactobacilli. These observations suggest that H_2O_2-producing lactobacilli might play a greater role than H_2O_2-negative lactobacilli in inhibiting *C. albicans* growth in vivo (11). *C. albicans* both produces and tolerates low concentrations of H_2O_2 (24); however, in vitro higher concentrations of H_2O_2 may be candidicidal (6). Paradoxically, in the same study Hillier et al. found no evidence of increased colonization or symptomatic candidiasis in pregnant women completely lacking lactobacilli; hence, the importance of H_2O_2 requires clarification (11).

The present study compared the microbiologies of vaginal secretions in healthy, nonpregnant women of reproductive age with those of similar women with acute recurrent VVC. Results for a series of predominantly non-antibiotic-induced cases of VVC failed to demonstrate a reduction in colonization with *Lactobacillus* species, both with regard to population size and the number of species of lactobacilli isolated. Unfortunately, the level of H_2O_2 production by the lactobacilli isolated was not measured; nevertheless, we failed to identify a single patient with reduced lactobacillus population numbers. It is apparent that additional studies that focus on functional aspects of vaginal lactobacilli such as H_2O_2 production are needed, since the present study corroborates the observation that women with VVC do not lack lactobacilli and are not colonized with unusual species of lactobacilli. Minor changes in the population number of some members of non-lactobacillus genera were seen in a minority of women only. The significance of the latter observation is unclear and could represent the results of acute inflammation rather than indicate a causal role.

In conclusion, the results of our study show that acute exacerbations of symptomatic *Candida* vaginitis in women with recurrent acute VVC are associated with normal *Lactobacillus* species populations. These results cast doubt on to the potential benefit of therapeutic measures such as the oral ingestion of yogurt or douching as a means of preventing recurrent VVC (13, 15).

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