Vaginal Microbiota of Women with Frequent Vulvovaginal Candidiasis

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Vulvovaginal candidiasis (VVC) is an insidious infection that affects a large proportion of women of all ages, and 5 to 8% of affected women experience recurrent VVC (RVVC). The aim of this study was to explore the possible importance of vaginal bacterial communities in reducing the risk of RVVC. The species composition and diversity of microbial communities were evaluated for 42 women with and without frequent VVC based on profiles of terminal restriction fragment polymorphisms of 16S rRNA genes and phylogenetic analysis of cloned 16S rRNA gene sequences from the numerically dominant microbial populations. The data showed that there were no significant differences between the vaginal microbial communities of women in the two groups (likelihood score, 5.948; bootstrap P value, 0.26). Moreover, no novel bacteria were found in the communities of women with frequent VVC. The vaginal communities of most women in both groups (38/42; 90%) were dominated by species of Lactobacillus. The results of this study failed to provide evidence for the existence of altered or unusual vaginal bacterial communities in women who have frequent VVC compared to women who do not have frequent VVC. The findings suggest that commensal vaginal bacterial species may not be able to prevent VVC.

Vulvovaginal candidiasis (VVC) is a widespread and common disease affecting a large proportion of women of all ages. Classically, VVC is described as consisting of a white “cottage cheese” discharge with associated vulval and vaginal inflammation (31). In typical cases, the symptoms include vulva erythema, edema, excoriations, and fissures (33). Alternatively, women can test positive for Candida species during routine exams with no clinical evidence of infection (31). Several factors predispose women to VVC, including genetic factors (3), pregnancy (5), uncontrolled diabetes mellitus (6), and the use of high-estrogen contraceptives (11), steroids, and antibiotics (28). Approximately 75% of women experience at least one episode of VVC during their lives, most commonly when they are childbearing age (33). About 40 to 50% of these women experience a recurrence (17), and 5 to 8% of these women have recurrent VVC (RVVC), which is defined as four or more proven episodes of VVC in a 12-month period (35). The quality of life is greatly diminished for women who experience RVVC. In addition, the diagnosis and treatment of VVC cost an estimated $1 billion per year in the United States (12). The high incidence and healthcare costs associated with treatment of VVC highlight the need for understanding the pathogenesis of the infections and host defense mechanisms so that effective strategies can be developed to control and prevent this disease.

Relatively little is known about possible associations between the bacterial species found in the vagina and colonization by Candida species. While it is commonly thought that the normal vaginal microbiota plays an important role in the prevention of vaginal infections and transmission of pathogens responsible for sexually transmitted disease (20, 29), there is no consensus about the impact of vaginal bacteria on control of VVC and RVVC. Siegler (32) first suggested that vaginitis caused by Candida was associated with intermediate flora patterns (a Nugent score between 4 and 6 [23]), and then Hillier et al. (15) reported a similar association between VVC and intermediate or normal flora patterns. In contrast, Sobel and Chaim (34) compared the microbial flora of vaginal secretions in healthy, nonpregnant women of reproductive age with the microbial flora of vaginal secretions in similar women with acute RVVC and found that women with VVC did not have reduced numbers of Lactobacillus species. Further, there is no consensus on whether H2O2-producing lactobacilli in the vaginal microbial communities protect their hosts from VVC (16, 19, 39). It has also been observed that the use of antibiotics can lead to yeast infections (28), perhaps by killing or inhibiting bacterial populations that have antymycotic properties. This suggests that normal vaginal microbiota may have an important role in restricting yeast infections. Trials to evaluate the use of oral or vaginal probiotics containing Lactobacillus spp. to prevent postantibiotic VVC have been limited and have yielded inconsistent results (7, 27).

Studies done previously to examine the importance of vaginal bacterial communities in determining susceptibility to RVVC have used cultivation-dependent methods. These traditional, “cultivation-dependent” methods have historically been used in clinical microbiology laboratories and in research to characterize the microbial populations associated with the human body. They are tedious and labor-intensive, and so their use for the analysis of large numbers of samples is impractical and costly. They are further limited by their reliance on selective media, and many bacterial populations are refractory to...
The color of the pH paper was compared with the pH paper chart and recorded.

side wall with a cotton-tipped applicator after placement of the vaginal speculum.

NY). The practitioner obtained a sample of vaginal secretions from the vaginal

from pH 3.0 to 5.5 (pHydrion pH papers; MicroEssential Laboratory, Brooklyn,

10°C. One of these swabs was shipped to the University of Idaho, while the other

scopically for the presence of

clinical signs of infection. After this, a speculum (lubricated with sterile saline if

were not asked whether they had used vaginal probiotics in the past. The study

completed questionnaires on habits and practices, medical history, and vaginitis

symptoms. Individuals who were currently participating in another clinical study,

bathing or showering for at least 2 h prior to the scheduled visit. Each participant

Subjects enrolled in the study were required to refrain from douching, vaginal

medications (e.g., Vagisil), and sexual intercourse for 48 h and to refrain from

were pregnant, or were menstruating at the time of a scheduled visit were

symptoms. Individuals who were currently participating in another clinical study,

were pregnant, or were menstruating at the time of a scheduled visit were

Subjects in the control group were required to self-report that they had had no yeast

infections in the past 2 years.

were excluded from the study, as were individuals diagnosed with vaginal infections

such as trichomoniasis, chlamydial infection, vulval skin disease, or bacterial

vaginosis at the time of a scheduled visit. Likewise, individuals currently using

any of the following forms of birth control were excluded: an intrauterine device,

spermicides, Depo-Provera, NuvaRing, and Seasonale. Individuals with preex-

isting systemic diseases or chronic conditions, such as diabetes or an immuno-

logical disease (human immunodeficiency virus or systemic lupus erythematos-

us), were excluded, as were individuals currently using immunosuppressive

drugs, undergoing chemotherapy, or taking prescription medication of the fol-

owing kinds: systemic antimicrobial or antifungal drugs or antifungals or anti-

microbials to treat a vaginal infection within the previous 30 days. The subjects

were not asked whether they had used vaginal probiotics in the past. The study

protocol and informed consent document were reviewed and approved by the

University of Iowa Institutional Review Board. Documented informed consent

was obtained from all subjects prior to participation in this study.

At each scheduled visit the practitioner did a pelvic exam to assess possible

clinical signs of infection. After this, a specimen (lubricated with sterile saline if

necessary) was used to collect three vaginal samples from the left side of the

upper half of the vaginal canal. One cotton swab was placed in a vial containing

~1 ml of sterile saline and sent to a clinical laboratory to be evaluated micro-

scopically for the presence of Candida sp., trichomoniass, and “clue” cells. The

second and third cotton swabs were placed in separate vials and stored at 70 ±

10°C. One of these swabs was shipped to the University of Idaho, while the other

was retained as an archived specimen. A standardized protocol was utilized to
determine the vaginal pH at each visit of a subject using pH paper with range

from pH 3.0 to 5.5 (pHydrion pH papers; MicroEssential Laboratory, Brooklyn,

NY). The practitioner obtained a sample of vaginal secretions from the vaginal

side wall with a cotton-tipped applicator after placement of the vaginal specular.

The color of the pH paper was compared with the pH paper chart and recorded.

Quality control was performed weekly to ensure the accuracy of the pH deter-

minations within 0.5 pH unit.

The study was done in a blind fashion; the histories of frequent VVC in the

women sampled were unknown to the investigators who analyzed the microbial

communities. Once these analyses were completed, the blind was broken.

Genomic DNA isolation. The swab samples were thawed on ice and mixed

vigorously by vortexing to dislodge cells from the swab. Prokaryotic genomic

DNA was isolated from a 0.5-ml aliquot of each cell suspension using a two-step

cell lysis procedure (42). First, bacterial cell walls were disrupted enzymatically

by addition of mutanolysin (50 µg) and lysosome (500 µg), followed by incuba-

tion for 1 h at 37°C. Second, the cells were mechanically disrupted by six

freeze-thaw cycles. Each cycle consisted of 2 min of incubation at 100°C that was

immediately followed by 2 min in a dry ice-ethanol bath. Between freeze-thaw

cycles, the cell suspensions were incubated for 1 min in an ultrasonic cleaning

bath (FS60; Fisher Scientific, Pittsburgh, PA). Proteins in the disrupted cell

suspension were digested with proteinase K (Qiagen, Hilden, Germany) during

a 1-h incubation at 55°C. Further isolation and purification of the total DNA

extract were performed using a Wizard DNA purification kit (Promega, Madi-

son, WI).

T-RFLP analysis of 16S rRNA genes. The species composition and diversity

of the numerically dominant populations in vaginal microbial communities were

not related to the species composition of vaginal bacterial communities.

MATERIALS AND METHODS

Study design and subjects. In this cross-sectional case-control study vaginal

swabs were collected from 42 subjects who were between 18 and 40 years old in

a clinical study conducted at the University of Iowa (Iowa City, IA). One half the

samples were collected from patients with frequent VVC, while the other half

were obtained from a control group. Subjects who had had at least four vaginal

yeast infections during the past 2 years, at least one of which was diagnosed by a

health care practitioner, were classified as having frequent VVC. Subjects in the

control group were required to self-report that they had had no yeast infections

in the past 2 years.

Comparison of women with and without frequent VVC.

The species composition and diversity of

microbial communities can be discerned

(13, 14, 18, 21, 25, 43). In this study, we used these methods to

characterize the composition and structure of the vaginal microbiota of healthy women and women with frequent VVC to
test the null hypothesis that the risk of frequent VVC is related to the species composition of vaginal bacterial communities.

The sam-

ple papers were not used to determine the phylogenetic diversity within each microbial community.

The presence of frequent VVC based on profiles generated by T-RFLP analysis. Student’s t

test was used
to determine differences in microbial community structures for women with frequent VVC and the microbial community structures for women without frequent VVC based on profiles generated by T-RFLP analysis. Student’s r test was used to assess the difference in vaginal pH between women with frequent VVC and women without frequent VVC. A difference was considered significant when the

P value was <0.05.
RESULTS AND DISCUSSION

Classification of vaginal microbial communities from the women sampled based on T-RFLP data. The vaginal microbial communities of the 42 women sampled were characterized on the basis of the T-RFLP of 16S rRNA genes derived from the numerically dominant populations present in each community. The data were subjected to cluster analysis to identify similar communities, and the number of clusters was evaluated. The communities were placed into clusters on the basis of differences in the sizes and abundances of terminal restriction fragments derived from 16S rRNA, providing a means for distinguish the communities in terms of the species (phylotypes) present and their abundance. In the 42 women, there were five different clusters of bacterial communities (Fig. 1), four of which were found in three or more women (clusters I, II, III, and IV) and one of which was found in a single individual (cluster V).

Differences between the women with and without frequent VVC. The frequencies of vaginal microbial community types in women with and without frequent VVC are shown in Table 1. A likelihood ratio test and bootstrap analysis (43) showed that there were no significant differences in the distribution of women with and without frequent VVC among the various community types (likelihood score, 5.948; bootstrap P value, 0.26). This suggests that women who have frequent VVC do not have altered or unusual vaginal bacterial communities and that the risk of recurrent yeast infections is not correlated with the composition of vaginal bacterial communities. This finding is consistent with the of findings of Sobel and Chaim (34), who also showed, using culture-dependent methods, that the development of RVVC was not correlated with an abnormal vaginal microbiota. However, the possibility that rare bacterial populations are correlated with risk of frequent VVC cannot be excluded. It should also be pointed out that only one of the women with frequent VVC had acute VVC at the time of sampling, and thus nothing can be concluded about shifts in community composition that may accompany an acute infection.

Compositions of the vaginal microbiota in women with and without frequent VVC. The species compositions of vaginal microbial communities in women who had frequent VVC and women who did not have frequent VVC were determined by

<table>
<thead>
<tr>
<th>Cluster</th>
<th>No. of women with frequent VVC</th>
<th>No. of women without frequent VVC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>12</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>21</td>
<td>42</td>
</tr>
</tbody>
</table>
TABLE 2. Species compositions of vaginal communities in women with and without frequent VVC

<table>
<thead>
<tr>
<th>Phylotypea</th>
<th>Cluster I (n = 96)</th>
<th>Cluster II (n = 96)</th>
<th>Cluster III (n = 96)</th>
<th>Cluster IV (n = 96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y12 (n = 96)</td>
<td>Y14 (n = 96)</td>
<td>Y19 (n = 96)</td>
<td>Y30 (n = 96)</td>
</tr>
<tr>
<td>(L.) crispatus</td>
<td>86.5</td>
<td>89.5</td>
<td>94.6</td>
<td>99.0</td>
</tr>
<tr>
<td>(L.) iners</td>
<td>0</td>
<td>7.4</td>
<td>2.2</td>
<td>0</td>
</tr>
<tr>
<td>(L.) gasseri</td>
<td>13.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(L.) jensenii</td>
<td>0</td>
<td>1.1</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>(L.) gilainarum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(L.) kitasatosi</td>
<td>0</td>
<td>0</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>(L.) vaginals</td>
<td>0</td>
<td>0</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>(Lactobacillus) sp. strain KC38(b)</td>
<td>2.1</td>
<td>0</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>(A.) vaginae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(Diphtherobacter) sp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(Prevotella) bivia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(a\) Clones were classified by comparing their 16S rRNA gene sequences to those of known organisms. Genus and species names were used if the sequence similarity to a type species was \(\geq 97\%\); only the genus only was used if the sequence similarity was \(< 97\% \& 90\%\). Community clusters are based on T-RFLP analysis of 16S rRNA genes. See Fig. 1. The numbers of women with the clusters were as follows: cluster I, 24; cluster II, 10; cluster III, 4; and cluster IV, 3. (b) Woman with frequent VVC. \(n\) is the number of clones in the library that were sequenced. (c) \(Lactobacillus\) sp. strain KC38 was first characterized by Pavlova et al. (26), and it is phylogenetically distinct from known species of \(Lactobacillus\).
Fidel et al. proposed that the symptoms related to the presence of Candida are due to infiltration of polymorphonuclear neutrophils (9); that is, symptoms of Candida infection are not the result of a failure of a woman’s immune system but rather are a result of an overly aggressive innate immune response (8). It should be noted that our study, along with other previous studies, characterized the composition of the vaginal microbiota of women with frequent VVC in a cross-sectional fashion. Moreover, most subjects in our study were in the quiescent (silent) phase and were not experiencing acute frequent VVC at the time of sampling. Thus, we cannot exclude the possibility that the composition of the vaginal communities of these subjects may vary over time (even over very short time scales). It could be that the vaginal communities of women with frequent VVC vary more often or more extensively than those of women who do not have frequent VVC. It might be interesting and potentially important to set up longitudinal studies for assessment of the composition of the vaginal microbiota of women with frequent VVC. This may help us to better understand the dynamics of vaginal microbial communities in women with frequent VVC and to establish whether fluctuations in these communities are linked to the onset of acute frequent VVC.

The results of this study failed to provide evidence of altered or unusual vaginal bacterial communities in women with frequent VVC compared with women without frequent VVC. This suggests that the risk of recurrent yeast infections is not correlated with the composition of vaginal bacterial communities. Specifically, the results indicate that the vaginal communities of women with frequent VVC did not have reduced proportions of lactobacilli. These findings suggest that indigenous vaginal bacterial species, such as Lactobacillus species, may not be key players in the defense against Candida infections.

ACKNOWLEDGMENTS

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The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health. The results of this study failed to provide evidence of altered or unusual vaginal bacterial communities in women with frequent VVC compared with women without frequent VVC. This suggests that the risk of recurrent yeast infections is not correlated with the composition of vaginal bacterial communities. Specifically, the results indicate that the vaginal communities of women with frequent VVC did not have reduced proportions of lactobacilli. These findings suggest that indigenous vaginal bacterial species, such as Lactobacillus species, may not be key players in the defense against Candida infections.

REFERENCES


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