Original Studies

Bacterial Populations in the Vaginas of Healthy Adolescent Women

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Abstract. Given that the microbiota of the healthy vagina plays an important role in the maintenance of health, it follows that an understanding of its composition and development may offer insights into the etiology and prevention of disease. In contrast to previous studies, this study exclusively investigated the structure and composition of adolescent vaginal bacterial communities. In this report, the vaginal bacterial communities of 90 menarcheal adolescents, ages 13–18y, were characterized using terminal restriction fragment length polymorphisms (T-RFLP) of 16S rRNA genes. Further characterization involved cluster analysis of the T-RFLP data to identify the number of different kinds of microbial communities found among the adolescents sampled, and phylogenetic analysis of 16S rRNA gene sequences cloned from samples representative of each cluster. We report the identification of four major clusters that accounted for 96.7% of the cohort. In general, these clusters could be divided into those dominated by Lactobacillus spp. and those dominated by a variety of lactic acid producing, anaerobic bacterial types such as Atopobium vaginae and Streptococcus spp. The compositional and structural similarity of the vaginal microbiota of menarcheal adolescents and adults suggests that the vaginal microbiota does not change significantly after the onset of menarche.

Introduction

Vaginal bacterial communities are an important first line of defense for the body. Disruption of this microbial barrier can result in a number of urogenital diseases such as bacterial vaginosisis (BV), yeast infections, sexually transmitted infections (STI), pelvic inflammatory disease (PID), and human immunodeficiency virus infection (HIV).1–5 Given its protective benefits, it is worthwhile to characterize the microbial constituents of the vaginal microbiota in order to gain insight into disease susceptibility, pathogenesis, and avenues of treatment.

The vaginal microbial ecosystem is unique in that it undergoes significant changes, which correspond with the maturational stages of a woman’s life. During the first 6 weeks of infancy maternal estrogen induces thickening of the vaginal epithelium and, in this process, provides a glycogen rich substrate for glucose fermenting microorganisms.6,7 In the weeks postpartum, subsequent metabolism of maternal estrogen results in a thinning of the mucosa, a reduction of glycogen substrate, and an increase in vaginal pH, which encourages the proliferation of a wide range of aerobic bacteria and facultative anaerobes.8 With the onset of puberty, the vaginal epithelium, under estrogenic control, once again thickens. As before, this glycogen rich environment selects for glucose fermenting microorganisms. The dynamic character of this ecosystem underscores the importance of resolving its microbial constituents at different stages of biological maturation.

The majority of studies have focused on the characterization of microbial populations found in the adult vagina (ages 18–35). These studies report an abundance of lactic acid producing bacteria (LAB). The most frequently reported species include L. crispatus, L. gasseri, and L. jensenii.9–10 Molecular approaches have also frequently detected L. iners and Atopobium vaginae.11–15 These LABs maintain the stability of the vaginal microbial ecosystem by creating an environment that precludes many other organisms. The precise control mechanisms exerted by these bacteria are still poorly understood; however, it is thought that the ability to modulate pH, the production of bacteriostatic and bactericidal compounds, and competitive exclusion play a significant regulatory role.5,16,17 Previous studies have also isolated several other Lactobacillus spp. such as L. casei, L. fermentum, L. delbrueckii, and L. salivarius, but these members are considered to be minor constituents of the vaginal microbiota.18–20 Other aerobic and anaerobic bacterial types that are also commonly isolated include Gardnerella vaginalis, Peptostreptococcus spp.,...
Streptococcus spp., Peptococcus spp., Bacteroides spp., Clostridium spp., and Staphylococcus epidermidis. High numbers of these organisms are thought to reflect an imbalance in the microbial ecosystem, and therefore, from a clinical perspective, an abundance of these minor bacterial types is a marker for disease. Accordingly, this altered equilibrium is characteristic of the polymicrobial disorder BV, which has been associated with an increased risk to PID, HIV, and other infectious diseases. 

Although this association suggests that anaerobic bacterial types have a role in disease induction, this has not been shown. Clearly, further study is required in order to understand the microbial underpinnings of disease pathogenesis.

Few studies have investigated the composition of adolescent vaginal microbiota. These studies report isolating Staphylococcus epidermidis, Staphylococcus aureus, diphtheroids, Escherichia coli, Enterococcus faecalis, Proteus mirabilis, Streptococci spp., and Lactobacilli spp. In general, these studies report the same bacterial types but not in the same abundances. This inconsistency suggests that these studies may be incomparable because of the different age ranges that characterized the cohorts. In addition, because these studies did not culture for strict anaerobes they were unable to detect a significant fraction of vaginal microbial diversity. In order to specifically resolve the bacterial communities of adolescents we report on the age group 13–18y, an epidemiologically significant period considering that this group has a higher age-specific risk for acquiring STIs than adults >24 years of age (CDC 2005). To our knowledge, there have been no studies that have used culture-independent methods to characterize the vaginal bacterial communities of women in this age group. By using these methods it is possible to obtain a less biased picture of microbial community structure and composition because it is possible to avoid the inherent selectivity of cultivation techniques that often overlook fastidious microorganisms.

The aim of the present study was to characterize the predominant vaginal microbial populations of 90 adolescents between the ages of 13 and 18 (mean age, 15.8 ± 1.6y) using cultivation-independent techniques and statistical methods as described in Abdellah et al. An auxiliary aim was to compare these adolescent findings with the findings from a previous study performed on adults.

Materials and Methods

Sample Collection and Genomic DNA Extraction

Vaginal swabs were taken from 46 healthy, adolescent Caucasian and Black girls between the ages 13–18 from 5 different geographic locations: Ohio, Florida, Arizona, New Jersey, and Canada (Table 1). The girls in the present study were originally recruited by Procter & Gamble Co. as part of a study on the prevalence of Staphylococcus aureus carriage. They were required to meet all the inclusion criteria, which included having regular menstrual cycles, refraining from douching prior to the study, refraining from bathing within two hours of the clinic visit, and abstaining from sexual intercourse for 48 hours prior to clinic visit. Girls were excluded from the study if they had used antibiotics during the prior 6 weeks, were immunocompromised or pregnant, had piercings in the urogenital area, or any self-reported any abnormal symptoms. Samples were stored in 3 ml of liquid dental transport medium (LDTM, Anaerobe Systems, Morgan City, CA) and remained on dry ice or at −80°C until analysis procedures could be performed. Genomic DNA was isolated using a combination of freeze-thaw and enzymatic treatments using Wizard DNA purification kits (Promega, Madison, WI, USA) as described in Zhou et al.

Analysis of 16S rRNA Gene Terminal Restriction Length Polymorphisms

The 16S rRNA genes in genomic DNA from samples were amplified by polymerase chain reaction (PCR) in a PTC-100 Programmable Thermal Controller (MJ Research, Inc.). Each PCR reaction contained 100ng (1 μl) of DNA sample, 2U AmpliTaq DNA polymerase (Roche Molecular System, Inc., Branchburg, New Jersey, USA), 1X AmpliTaq reaction buffer, 3mM MgCl₂, 200μM dNTPs, 5% DMSO,
and 0.1 μM fluorescently labeled primers, Eub 8fm-VIC (5′-AGAGTTTGATCMTGGCTCAG-3′) and Eub 926r-FAM (5′-CCGTCATTGCTTTRAGTTT-3′) or Eub 49r-fluor 5′-3′ and Eub 926r-FAM. Initial DNA denaturation was performed at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min. Final extension was at 72°C for 10 min. To confirm amplification of the genes, an aliquot of the PCR reaction was analyzed by electrophoresis in a 1.5% agarose gel followed by staining with ethidium bromide and visualization under ultraviolet light. The PCR products of the 8fm and 926r were digested using MspI while the PCR products of 49r and 926r were digested using HaeIII. Digested products were pooled and the terminal restriction fragment length polymorphisms (T-RFLP) of 16S rRNA genes were determined by DNA fragment analysis using a 3100 PRISM Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).55

Analysis and Clustering of T-RFLP data

Methods used for the analysis and clustering of T-RFLP data were as described in Abdo et al.17 From each group an algorithm was used to identify the fewest number of samples in each cluster (group) that accounted for 85% of the diversity within the group.17 In total, clone libraries of 16S rRNA genes were prepared from at least four samples from each group, and there were 29 libraries in total. These constituted 32% of the entire cohort.

Construction of Clone Libraries and Sequencing

16S rRNA genes were amplified using unlabeled primers FD1 and RD1 and subsequently cloned into pCR2.1-TOPO (Invitrogen) using procedures recommended by the manufacturer. Ligation mixtures consisting of vector/insert ratio of 1:1 were used to transform E. coli TOP10 cells (Invitrogen). The transformed cells were plated on Luria-Bertani (LB) agar plates containing 100μg kanamycin ml−1 and incubated for approximately 12 h overnight at a constant temperature of 37°C. For each library, 96 white, distinct clones were randomly selected and grown in 96-deep well microtiter plates containing 1.5 mL LB broth and 100μg kanamycin ml−1 for 48–72 h. Cells from the 96-deep well microtiter plates were harvested, plasmid DNAs were isolated, sequenced and analyzed as previously described in Zhou et al.15

Comparison of Adolescents and Adults

To increase the sample number, the T-RFLP data from 46 Black and Caucasian adolescents (present study) were combined with T-RFLP data from 44 Black and Caucasian adolescents from a previous study28 in which identical procedures for sample analysis were used (Table 1). The T-RFLP data for this cohort of 90 adolescents was then combined with T-RFLP data from 97 Black and Caucasian adults28 and analyzed according to Abdo et al.27 In order to determine if the distribution of adolescent samples (13–18 years of age) was different from adults (>18 years of age) a likelihood ratio test was performed using the procedure described in Schütte et al (in preparation). Q2 Briefly, the likelihood ratio test compares a reduced model, which assumes no age-based differences, with a full model, which accounts for age-based differences in its explanation of microbial diversity. The rejection of the former for the latter would indicate that age partially explains the different types of microbial communities observed among the samples analyzed. Since the adolescent cohort was not a homogeneous population we tested if the distribution of age groups of the adolescent cohort were different. For this purpose we performed the Pearson chi-square test using the age groups 13–14 and 15–18.

Results

To determine the number of different kinds of vaginal microbial communities found in adolescent women, we performed cluster analysis on profiles of terminal restriction site polymorphisms in 16S rRNA genes. The analysis showed there were six clusters of microbial communities observed among the samples analyzed. Since the adolescent cohort was not a homogeneous population we tested if the distribution of age groups of the adolescent cohort were different. For this purpose we performed the Pearson chi-square test using the age groups 13–14 and 15–18.
differed between these two groups in that the single woman harbored a vaginal microbial community that was significantly less diverse.

To determine if the vaginal microbial communities of adolescent women were significantly different from that of older women, a dendrogram composed of 90 adolescents (present study) and 97 adults, which were characterized by Zhou et al., was constructed (data not shown). Log-likelihood analysis, as described in Schütte et al., indicated that there was no significant difference in the rank abundances of adolescents (13–18 y) compared with older women ($P = 0.522$), suggesting that the composition of vaginal communities in these two age groups were similar to one another. Although no difference was detected between the adolescent and adult women’s community proportions, for these sample sizes the power of the statistical test places some limits on what we can infer from a finding of no statistical difference. As one way to investigate the power of this test, the groups were collapsed into the two most abundant communities and the remaining communities, to allow power calculations to be conducted for the Fisher exact test for these data. For these sample sizes, only a difference in the proportion of the grouped two most abundant communities of 20% or more could be detected with high power ($> 80\%$). Thus, the finding of no significant difference in community proportions between adolescent and adult women only implies that large differences between these communities are unlikely.

Given the age range of the adolescent subjects (13–18 years of age) and the importance of estrogen-induced physiological changes in the development of the vaginal microbiota, we wanted to resolve any microbial community differences (if any) that distinguished the younger subjects (13–14 y of age) from the older subjects (15–18 y of age). Pearson’s chi-square test indicated that the distribution of the microbial communities of the younger subjects was not significantly different from the older subjects ($P = 1.0$).

### Discussion

To our knowledge, this is the first study to characterize the vaginal microbiota of adolescents using cultivation-independent molecular methods. The use of cultivation-independent methods improves upon cultivation-dependent methods which are inherently biased. In this study, lactic acid producers *L. iners* and *L. crispatus* frequently constituted >50% of isolates from individual vaginal samples; in fact, a number of samples consisted of near monocultures of *L. iners* or *L. crispatus*. This was not observed for other indigenous species, therefore exemplifying the superior

### Table 2. Average species composition of vaginal communities in healthy adolescent women.

<table>
<thead>
<tr>
<th>Cluster (% clones)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1a</td>
<td>C1b</td>
<td>C2</td>
<td>C3</td>
</tr>
<tr>
<td><strong>Phyotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus iners</td>
<td>91.7</td>
<td>37.4</td>
<td>0.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Lactobacillus crispatus</td>
<td>2.9</td>
<td>8.2</td>
<td>96.7</td>
<td>56.9</td>
</tr>
<tr>
<td>Lactobacillus jensenii</td>
<td>0.0</td>
<td>0.0</td>
<td>2.2</td>
<td>8.8</td>
</tr>
<tr>
<td>Lactobacillus gasseri</td>
<td>0.4</td>
<td>1.8</td>
<td>0.0</td>
<td>32.3</td>
</tr>
<tr>
<td>Lactobacillus vaginalis</td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Actinobaculum spp.</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Aerococcus spp.</td>
<td>0.4</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Anaerococcus spp.</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Atoptobium vaginae</td>
<td>1.1</td>
<td>0.3</td>
<td>0.2</td>
<td>24.1</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Dialister spp.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.5</td>
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<td>Enterococcus faecalis</td>
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<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Finegoldia magna</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>0.0</td>
<td>3.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Gemella pallidans</td>
<td>0.0</td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Granulicatella elegans</td>
<td>0.0</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Lachnospiraceae spp.</td>
<td>1.0</td>
<td>1.4</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Megaphaera spp.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Micromonas spp.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Mycoplasma spp.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Peptococcus niger</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Peptoniphilus spp.</td>
<td>0.1</td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Peptostreptococcus spp.</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Prevotella</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>0.2</td>
<td>15.4</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>0.0</td>
<td>0.0</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Veillonella spp.</td>
<td>0.0</td>
<td>14.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Novel bacteria</td>
<td>0.8</td>
<td>14.5</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Number of women (per group)</td>
<td>28</td>
<td>10</td>
<td>20</td>
<td>14</td>
</tr>
</tbody>
</table>

1. The classification of clones was done by comparing their 16S rRNA gene sequences to those of known organisms. Clones were designated as novel if the sequence similarity to known organisms was <90%.
2. Mean relative abundances of populations in clone libraries analyzed.

that an ecological relationship exists between *L. crispatus* and *L. jensenii*. Microbial communities of the fourth group, which accounted for 17% of the women sampled, were not predominated by any particular phylotype. This group harbored a wide array of anaerobic bacterial species including the lactic acid-producing bacterium *A. vaginae* as well as clinically important microorganisms such as *Gardnerella vaginalis*, *Prevotella*, and genital mycoplasmas. Notably, the microbial communities of this category were characterized by the relative absence of *Lactobacillus spp*. In addition to these four groups, clones characterized from a single-woman group (1/90) revealed a microbial community structure predominated by *A. vaginae* (91.7% of clones). The numerical abundance of *A. vaginae* in this woman paralleled those women of group four; however, the microbial communities...
we did not observe a significant difference in the

established by adolescence.

suggest that the adult vaginal microbiota is already

different from those found in adults. Altogether, these findings

communities, by extension, did not differ significantly

merically minor species found in adolescent microbial

cent microbial communities we concluded that the nu-

merically minor species can also significantly contribute to

the overall vaginal microbial community structure, we
decided to investigate if these minor constituents
differed significantly between adolescents and adults.

Since similarity assessment using T-RFLP data did
not reveal any segregation between adult and adoles-
cent microbial communities we concluded that the nu-
merically minor species found in adolescent microbial
communities, by extension, did not differ significantly
from those found in adults. Altogether, these findings
suggest that the adult vaginal microbiota is already
established by adolescence.

There are several hypotheses that can explain why
we did not observe a significant difference in the

composition and structure of vaginal microbial com-

munities between adolescence and adulthood. The
most plausible explanation is that estrogen induced
physiological changes have already occurred in post-
menarcheal young women. In our study, the mean age
for the cohort was 15.8 ± 1.6 SD years. From this un-
derstanding, it is likely that changes in community
composition occur before or at the time of menarche
which is characterized by a significant increase of
serum estrogen. Rising estrogen levels increase the
levels of vaginal glycogen which provides adequate se-
lective pressure to favor glucose-fermenting microor-
ganisms such as Lactobacillus spp. While several
microorganisms have fundamental niches that would
allow them to exist under such conditions, competition
likely limits the abundance of many bacterial types that
could exist in the vaginal environment. This could ac-
count for the relative predominance of L. iners, L. cris-
patus, L. gasseri, L. jensenii, and A. vaginae, which are
presumably highly adapted to the vaginal environment,
and the relative absence of numerous other LABs.

The presence of relatively few hallmark species is
likely a function of the combined influence of bacterial,
environmental, and host selective forces which together
maintain a dynamic equilibrium in vaginal bacterial
communities. Many studies have focused on the bacte-
rial component of this ecosystem, looking specifically
at the ability of bacterial populations in the vagina to ex-
clude nonindigenous bacteria and maintain homeosta-
sis. The mechanisms that underlie this are thought to
include physical exclusion due to superior endothelial
adherence, modulation of vaginal pH, and production

Fig. 1. Similarity dendrogram of 90 adolescent samples showing the relationship of community profiles based on T-RFLP and Q5
clustering analysis. The dashed line indicates the statistically determined number of clusters. Asterisks indicate samples from
which clone libraries were constructed. The plus symbols (+) indicate adolescent samples from a previous study (Zhou et al.,
in-press).
of microbicides such as hydrogen peroxide and nisin. In addition, interspecies mechanisms are known to contribute to the stability of the vaginal microbiota. These include coaggregation, which promotes the survival of two or more bacterial types, and commensal relationships in which the byproducts of one species are used by another. Empirical evidence for these mechanisms comes from several studies. Kmet and Lucchini reported that certain Lactobacillus spp. strains coaggregate with uropathogenic E. coli presumably increasing the persistence of both species and, furthermore, vaginal species L. crispatus, L. gasseri, and L. jensenii have been observed to autoaggregate and/or coaggregate. In support of the second interspecies mechanism, Anukum et al speculate that the ammonium produced by A. vaginae may act as a substrate for BV associated microorganisms like Gardnerella vaginalis, Mobiluncus, and Pretovella. On a practical level, this second interspecies mechanism is commonly exploited for the production of fermented foods. Here, a proteolytic starter species such as L. delbrueckii, L. bulgaricus, or S. thermophilus is introduced in order to foster growth of nonstarter lactic acid bacteria. It is likely that the predominant bacterial types utilize a combination of these mechanisms in order to maintain the stability of the vaginal ecosystem.

Focus on the bacterial component of this ecological system understates the importance of environmental and host factors in the maintenance of the vaginal microbiota. Lactic acid bacteria are fastidious microorganisms that have specific nutrient requirements as illustrated by studies attempting to optimize culturing of Lactobacillus spp.. Glycogen alone is insufficient to support the proliferation of LABs, which, in addition, generally require a variety of amino acids, vitamins, and growth factors. For example, species of the group L. acidophilus which include L. crispatus, L. gasseri, and L. johnsonii are known to require supplements of pantetheine and vitamin B6 (in the form of pyridoxal, pyridoxamine, or pyridoxamine phosphate). These strict nutrient requirements suggest that vaginal exudates, and perhaps indirectly diet, may play a role in vaginal homeostasis, but currently, there is little support for this speculation. However, the importance of diet may become a salient issue in vaginal ecology if vaginal "seeding" from the gastrointestinal tract is found to significantly contribute to the establishment of the microbiota as suggested by Antonio et al., Reid et al., and others. In addition to these environmental factors, immune interactions are also thought to contribute to vaginal homeostasis. For example, a study by Caucci et al. associated extensive degradation of IgA and IgM with a subgroup of patients diagnosed with bacterial vaginosis. While this is far from definitive evidence for host-microbiota interactions, a study of the rat uterus suggests that uterine epithelial cells are able to selectively initiate the release of tumor necrosis factor alpha TNF-α and macrophage inflammatory protein 3α (MIP3α), both of which are known to play an important role in immune initiation in the female reproductive tract, in response to an encounter with pathogens.

The variety of selective pressures creates a highly restrictive vaginal environment that allows for the proliferation of relatively few bacterial types. From hundreds of potential bacterial colonizers only a handful of Lactobacillus spp. and acid-tolerant bacteria are known to be successful competitors. Our study expands on previous studies by its characterization of the vaginal microbial communities of adolescents and adults. Our identification of L. iners, L. crispatus, L. gasseri, L. jensenii, and other acid-tolerant species coincides with adult characterization studies and therefore we conclude that the vaginal microbiota does not change significantly after the onset of menarche. The similar microbial communities of these two populations are likely a result of similarities in the host-mediated environment (i.e., estrogenic control of glycogen substrate). This finding is clinically relevant because it suggests that it is reasonable to diagnose and treat both adolescents and adults in a similar manner. If we consider the paradigm of ecological succession, a concept often used to explain microbiota changes of the intestinal tract, the notion of adult climax microbiota would describe the vaginal microbiota from menarche to menopause and the notion of transitional microbiota would describe the vaginal microbiota prior to menarche. It has been speculated that the particular composition and structure of transitional microbiota is important for determining the adult climax microbiota but this has not been investigated. For future studies, further characterization of the vaginal microbiota at time points between birth and the onset of menarche is necessary in order to more clearly elucidate changes of the vaginal microbial ecosystem.

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