Colonization of the Rectum by *Lactobacillus* Species and Decreased Risk of Bacterial Vaginosis

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Lactobacilli colonizing the rectum may be a reservoir for vaginal lactobacilli. In a cross-sectional study of 531 females, vaginal and rectal colonization by lactobacilli were assessed by culture methods. A subset of isolates was identified to the species level by use of whole-chromosomal DNA probes. *Lactobacillus crispatus* (16%), *L. jensenii* (10%), and *L. gasseri* (10%) were the prevalent lactobacilli colonizing the rectums of 290 females. Only 13 (9%) of 147 females colonized by *L. crispatus* or *L. jensenii* vaginally and/or rectally had bacterial vaginosis (BV), compared with 12 (44%) of 27 females colonized by other H₂O₂-producing lactobacilli (P < .001). Cocolonization of the vagina and rectum by H₂O₂-producing lactobacilli was associated with the lowest prevalence of BV (5%), whereas females colonized only vaginally, only rectally, or at neither site had a successively increased risk of BV (P < .001). *Lactobacillus* species in the rectum may contribute to the maintenance of vaginal microflora.

Several bacterial species are known to colonize both the gastrointestinal and the reproductive tract, and the rectum may play an important role as a source or reservoir for organisms that colonize the vagina. For example, group B *Streptococcus* (GBS) colonizes both the vaginas and the rectums of many women of reproductive age, but females with vaginal lactobacilli that produce hydrogen peroxide (H₂O₂) are more likely to become infected with GBS only in the rectum [1]. Researchers who have promoted the ingestion of *Lactobacillus*-containing products for the treatment or prevention of genital infections have suggested the possible role of the gut as a reservoir for vaginal colonization by lactobacilli [2, 3]. Nevertheless, the concordance of vaginal and rectal colonization by *Lactobacillus* species has not been extensively studied.

*L. plantarum* has been identified in both the vagina and the rectum by DNA hybridization [4, 5]. Although *L. crispatus*, *L. gasseri*, *L. fermentum*, *L. jensenii*, *L. oris*, *L. reuteri*, *L. ruminis*, *L. salivarius*, *L. iners*, and *L. vaginalis* have been identified in the vagina by use of genomic methods [5–9], it is unknown whether these species also colonize the rectum. One study that identified rectal *Lactobacillus* species by use of DNA hybridization techniques detected *L. plantarum*, *L. rhamnosus*, and *L. paracasei* in rectal samples from both women and men [4].

Studies of vaginal lactobacilli have demonstrated that *L. crispatus*, *L. jensenii*, *L. gasseri*, and *L. vaginalis* are the most commonly recovered species of H₂O₂-producing lactobacilli [5, 6, 8, 9]. The absence of H₂O₂-producing lactobacilli in the vagina is associated with an increased acquisition of bacterial vaginosis (BV) [10, 11], a condition that is associated with increased shedding of HIV in the female genital tract [12], increased acquisition of HIV [11] and herpes simplex virus type 2 in women [11, 13, 14], and preterm birth [15]. The presence of vaginal lactobacilli producing high levels of H₂O₂ has also been associated with a decreased occurrence of BV and chorioamnionitis during pregnancy [9]. It is possible that rectal colonization by *Lactoba-
cillus species may be important as a reservoir for the maintenance of a normal vaginal flora and that this may be associated with a decreased incidence of BV-associated adverse effects. The goals of the present study were to evaluate the variability and concordance of Lactobacillus species colonizing the vagina and rectum in a cohort of sexually active females and to assess the association between vaginal and/or rectal colonization by Lactobacillus species and the presence of BV.

SUBJECTS AND METHODS

The study population consisted of 561 nonpregnant females, 14–35 years old, who visited an adolescent medicine clinic, 2 sexually transmitted disease clinics, a student health clinic, and a low-income primary-care clinic for females. Written, informed consent was obtained from all participants. One vaginal swab and 1 rectal swab, collected from each subject, were placed into separate Amies transport medium (MML Diagnostics) and delivered to the research laboratory within 12 h. Vaginal smears were gram stained and evaluated for BV by use of the criteria of Nugent et al. [16]. Because of a lack of vaginal or rectal swabs or vaginal smear collection, 30 females were excluded, leaving 531 evaluable subjects. The study population was 61% white, 44% were <20 years old, 29% reported the use of oral contraceptives, and 10% reported the use of douching products during the preceding month. Most of the females (95%) were unmarried. In the conduct of clinical research, human experimentation guidelines of the US Department of Health and Human Services were followed.

Direct non–culture-based methods for species identification of lactobacilli have not yet been validated. Therefore, lactobacilli were initially recovered by culture and then identified by use of DNA hybridization to American Type Culture Collection (ATCC) strains, as described below.

Each swab specimen was inoculated onto a Difco Rogosa SL agar (Becton Dickinson) plate and a human-blood bilayer Tween agar (Becton Dickinson Microbiology Systems) plate. The plates were streaked into 4 quadrants and then incubated anaerobically for a minimum of 48 h at 36°C. Semiquantitative growth of suspect lactobacilli into the first, second, third, or fourth quadrants was reported as “1+,” “2+,” “3+,” or “4+,” respectively. Lactobacilli were identified to the genus level by Gram stain and colony morphologic examination, negative catalase test, and production of a major lactic acid peak as assessed by gas-chromatographic analysis [17]. All lactobacilli were tested for the production of H2O2 in a qualitative assay on a tetramethylbenzidine agar plate [18, 19]. The Lactobacillus strains were frozen and stored at −70°C in reconstituted litmus milk (Becton Dickinson Microbiology Systems) for subsequent DNA hybridization studies.

The vaginal and rectal lactobacilli isolated were identified to the species level on the basis of whole-chromosomal DNA hybridology, as described elsewhere [6]. DNA isolated from the lactobacilli were slot-blotted onto nylon membranes and tested for hybridization to whole-chromosomal probes made from ATCC Lactobacillus strains. The Random Primer DNA Labeling Kit (Boehringer Mannheim) was used to make whole-chromosomal probes to 18 ATCC Lactobacillus strains: L. acidophilus 521 and 4356, L. buchneri 11577 and 11579, L. casei 4646, L. crispatus 33197, L. delbrueckii delbrueckii 9649, L. delbrueckii bulgaricus 11842, L. fermentum 23271, L. gasseri 4963, L. jensenii 25258, L. oris 49062, L. parabuchneri 49374, L. reuteri 23272, L. rhamnosus 21052, L. ruminis 25644, L. salivarius salivarius 11741, and L. vaginalis 49540. A probe was also made to an isolate that had been previously designated Lactobacillus 1086V [6], which is now known to have positive whole-chromosomal hybridization against CCUG (Culture Collection, University of Göteborg, Göteborg, Sweden) L. iners 28746. Control filters included DNA from the Lactobacillus ATCC strains listed above and additional strains to ensure probe specificity, including L. acidophilus 4357, L. brevis 14869, L. buchneri 4005, L. casei 393, L. delbrueckii lactis 4797 and 12315, L. fermentum 11739 and 14931, L. gasseri 9857, L. johnsonii 33200, L. paracasei paracasei 27216, L. plantarum 14917, L. rhamnosus 7469, and L. salivarius salicinus 11742.

Relationships among H2O2 production status, frequency of vaginal and rectal colonization of Lactobacillus species, prevalence of BV, and density of colonization were evaluated by use of Fisher’s exact test or the χ2 test for linear trend, where appropriate. All tests were evaluated at the P < .05 significance level.

RESULTS

The frequency of vaginal and rectal colonization by lactobacilli and the H2O2 production status of these isolates are shown in table 1. Lactobacilli were recovered from the vaginas of 392 (74%) of 531 females and from the rectums of 270 (51%) of them. Overall, 424 (80%) of 531 females had been colonized vaginally and/or rectally by lactobacilli, and most females were colonized by H2O2-producing lactobacilli (356/531 [67%]). H2O2-producing lactobacilli

| Table 1. Vaginal vs. rectal colonization by Lactobacillus H2O2 production status in 531 females. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Vaginal Lactobacillus colonization              | Rectal Lactobacillus colonization              |
| Any H2O2 positive                               | Only H2O2 negative                            | None                                          |
| (n = 324)                                      | (n = 68)                                      | (n = 139)                                     |
| Any H2O2 positive                               | 198                                          | 10                                            | 22                                            |
| (n = 230)                                      |                                              |                                               |                                               |
| Only H2O2 negative                              | 25                                           | 5                                             | 10                                            |
| (n = 40)                                       |                                              |                                               |                                               |
| None                                          | 101                                          | 53                                            | 107                                           |
| (n = 261)                                      |                                              |                                               |                                               |

NOTE. Data are no. of females.

a Includes 27 females also vaginally colonized by H2O2-negative lactobacilli.

b Includes 12 females also rectally colonized by H2O2-negative lactobacilli.
were more commonly isolated from the vagina than from the rectum (61% vs. 43%; \(P < .001\)). Overall, 198 (37%) of 531 females were colonized both vaginally and rectally by \(H_2O_2\)-producing lactobacilli. Recovery of only \(H_2O_2\)-negative lactobacilli from both the vagina and the rectum of a woman was rare (table 1).

Identification of lactobacilli to the species level by use of whole-chromosomal DNA probes was performed on a subset of 363 isolates obtained from 290 consecutively enrolled females. These isolates were identified to the species level on the basis of DNA homology (table 2). Lactobacilli vaginally and/or rectally colonized nearly three-fourths (215/290 [74%]) of the females—a proportion similar to that seen in the whole population (table 1). Two or more Lactobacillus species were detected in 58 of the females. L. crispatus was the most frequently identified Lactobacillus species isolated from the vagina (31%), followed by L. jensenii (23%) and L. iners (15%). L. crispatus was also the most frequently isolated Lactobacillus species from the rectum (16%), followed by L. jensenii (10%) and L. gasseri (10%). L. ruminis, L. reuteri, and L. oris were each isolated from the vagina or rectum, at a frequency of \(\leq 1\%\). L. vaginalis was only isolated from the vagina, at a frequency of \(< 1\%\), and L. rhamnosus, L. casei, L. fermentum, L. parabuchneri, and L. salivarius were each only isolated from the rectum, at a frequency of \(\leq 1\%\). Of the 18 ATCC Lactobacillus strains that were used to make whole-chromosomal probes, the following did not yield any positive results: L. acidophilus 521 and 4356, L. buchneri 11577 and 11579, L. delbrueckii delbrueckii 9649, and L. delbrueckii bulgaricus 11842, which suggests that these species are not common constituents of the vaginal flora.

Overall, the 2 most prevalent Lactobacillus species, L. crispatus and L. jensenii, colonized the vaginas and/or the rectums of more than one-half (150 [52%]) of the 290 females. The dissimilarity of the third most prevalent Lactobacillus species in the vagina versus in the rectum may be explained by differences in anatomical site distribution among Lactobacillus species. The isolation frequencies of Lactobacillus species, by site, are shown in table 2. Nearly all of the females colonized by L. crispatus (91/95 [96%]) or L. iners (45/45 [100%]) carried the organism in the vagina. However, L. iners was rarely recovered from the rectum (2/45 [4%]). In contrast, nearly one-half (45/95 [47%]) of the females colonized by L. crispatus were rectally colonized. L. jensenii was also recovered with a higher frequency from the vagina (67/79 [85%]) than from the rectum (30/79 [38%]). Compared with colonization by L. crispatus, colonization of both sites by L. jensenii was nearly 2 times less frequent. In contrast to the first 3 species mentioned, L. gasseri was recovered at a higher frequency from the rectum than from the vagina—76% (29/38) versus 42% (16/38). These data suggest that L. crispatus, L. jensenii, and L. iners should be considered to colonize predominantly the vagina, whereas L. gasseri more frequently colonizes the rectum.

Nearly all L. crispatus and L. jensenii isolates produced \(H_2O_2\) (97% and 99%, respectively), whereas the production of \(H_2O_2\) was detected in only 6% of L. iners isolates. The group of 246 \(H_2O_2\)-producing lactobacilli was composed predominantly of L. crispatus (47%), L. jensenii (35%), and L. gasseri (13%). Colonization by \(H_2O_2\)-producing L. crispatus or L. jensenii isolates was associated with a decreased prevalence of BV (\(P < .001\)). Only 13 (9%) of 147 females colonized by \(H_2O_2\)-producing L. crispatus and/or L. jensenii were positive for BV, compared with 99 (69%) of 143 females not colonized by either species.

To determine whether rectal colonization by lactobacilli was simply a surrogate for high-density vaginal colonization, we analyzed the semiquantitative data of \(H_2O_2\)-producing L. crispatus and L. jensenii recovered from females who had been vaginally colonized by these species (table 3). The frequency of L. crispatus or L. jensenii from the rectum increased with a higher colonization density of the species in the vagina (\(P < .001\)). However, these species were not solely recovered from the rectums of females who were colonized at a high density in the vagina.
data suggest that rectal colonization by \textit{L. crispatus} or \textit{L. jensenii} can occur in the absence of vaginal colonization.

The independent contribution of vaginal versus rectal colonization by \(H_2O_2\)-producing lactobacilli to \textit{BV} was evaluated in the full data set of 531 females (table 4). In the reference group of females who had been colonized both vaginally and rectally by \(H_2O_2\)-producing lactobacilli, only 5\% had \textit{BV}. In comparison, females who were only vaginally colonized by \(H_2O_2\)-producing lactobacilli were 4 times more likely to have \textit{BV}, while females who were only rectally colonized were 10 times more likely to have \textit{BV}, and females lacking \(H_2O_2\)-producing lactobacilli at either site were 15 times more likely to have \textit{BV} (\(P < .001\)). Although the lowest prevalence of \textit{BV} was found among females who had been colonized both vaginally and rectally by \(H_2O_2\)-producing lactobacilli, colonization at either site was better than colonization at neither site, with respect to \textit{BV} risk.

**DISCUSSION**

Vaginal and rectal cocolonization by \(H_2O_2\)-producing lactobacilli was associated with a lower prevalence of \textit{BV}, compared with vaginal colonization alone. Identified risk factors for \textit{BV} have included exposure to new sex partners [10, 20–22], having female sex partners [22], use of an intrauterine contraceptive device [20, 22], smoking [22], douching [10], and an absence of \(H_2O_2\)-producing lactobacilli in the vagina [10]. Identified risk factors for a lack of \(H_2O_2\)-producing lactobacilli among females with \textit{BV} include douching and having had \(\geq 3\) sex partners during the preceding year [23]. However, this is the first study (to our knowledge) to suggest that rectal colonization by \(H_2O_2\)-producing lactobacilli may contribute to the maintenance of normal vaginal flora. The capacity of lactobacilli to colonize the rectum may be another criterion that should be considered in the choice of a probiotic strain for the reconstitution of vaginal lactobacilli.

Because of the close proximity of the rectum to the vagina, the isolation of \(H_2O_2\)-producing vaginal \textit{Lactobacillus} species from the rectum suggests that it may play a role as a reservoir for these microorganisms. Vaginal colonization by \textit{Lactobacillus} species was found to be transient in many females [8], and the rectum may be a source for vaginal recolonization by lactobacilli after a disturbance of the ecology that follows douching or sexual intercourse. In a previous study of 23 females who were not vaginally colonized by any lactobacilli at baseline, 18 (78\%) had acquired lactobacilli by the 4- or 8-month follow-up visit [8]. It is possible that these species were present in the rectum and subsequently colonized the vagina. The intermittent colonization of the rectum, as well as the vagina, may be characteristic of some species of lactobacilli, as has been reported for \textit{GBS} [1]. Longitudinal studies examining the concordance of rectal and vaginal \textit{Lactobacillus} species colonization may provide new insights.

### Table 4. Prevalence of bacterial vaginosis (BV) among females with vaginal vs. rectal colonization by facultative \(H_2O_2\)-producing lactobacilli.

<table>
<thead>
<tr>
<th>Site(s) positive for (H_2O_2)-producing lactobacilli</th>
<th>Females with BV present, no. (%)</th>
<th>RR (95% CI) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagina and rectum (n = 198)</td>
<td>9 (5)</td>
<td>Referent</td>
</tr>
<tr>
<td>Vagina alone (n = 126)</td>
<td>25 (20)</td>
<td>4.4 (2.1–9.0)</td>
</tr>
<tr>
<td>Rectum alone (n = 32)</td>
<td>15 (47)</td>
<td>10.3 (4.9–21.6)</td>
</tr>
<tr>
<td>Neither vagina nor rectum (n = 175)</td>
<td>123 (70)</td>
<td>15.5 (8.1–29.5)</td>
</tr>
</tbody>
</table>

**NOTE.** RR, relative risk; CI, confidence interval.

\(a P < .001\), Fisher’s exact test for each comparison with the referent group.

In table 1, vaginal colonization by lactobacilli was more common than rectal colonization (74\% vs. 51\%), and only 1 in 5 females lacked \textit{Lactobacillus} colonization at both sites. \(H_2O_2\)-producing lactobacilli were more prevalent than \(H_2O_2\)-negative lactobacilli in the present study. Some species of lactobacilli appeared to colonize an anatomic site preferentially. \textit{L. iners} was most frequently isolated from the vagina, whereas \textit{L. gasseri} was most frequently recovered from the rectum. This suggests that differences in these ecosystems may select for colonization by different lactobacilli. Thus, whereas both \textit{L. crispatus} and \textit{L. gasseri} colonize the rectum, \textit{L. crispatus} may be more likely to contribute to the maintenance of the normal vaginal ecosystem.

Song et al. [5] reported \textit{L. gasseri} (22\%), \textit{L. fermentum} (18\%), and \textit{L. paracasei} (16\%) species to be the most prevalent \textit{Lactobacillus} species isolated from stool specimens of Japanese females, whereas, in the present study, \textit{L. crispatus} (16\%), \textit{L. jensenii} (10\%), and \textit{L. gasseri} (10\%) were the most common rectal species. The differences in prevalence seen between the study of Japanese females and the present study may reflect the different specimen types evaluated (stool vs. rectal swab) or other factors—such as diet—that may influence \textit{Lactobacillus} species colonization. However, the distribution of vaginal species in Japanese females has also been reported to differ from that in US females, in that \textit{L. vaginalis} is more commonly recovered from Japanese females, whereas \textit{L. jensenii} is more commonly recovered from US females [5, 6]. Vásquez et al. [7] described the vaginal lactobacilli in 23 healthy females and reported that the most common species was \textit{L. crispatus} (48\%), followed by \textit{L. gasseri} (30\%), \textit{L. jensenii} (17\%), and \textit{L. iners} (17\%). Although the aforementioned genomic studies differed in protocol [5–7], all are in agreement that \textit{L. crispatus} is the most prevalent \textit{Lactobacillus} species colonizing the vagina.

Most commercial products that contain lactobacilli are sold as dietary supplements to be ingested. Many of these products contain lactobacilli derived for probiotic use from the food and dairy industries. Wood et al. [24] reported that vaginally derived lactobacilli greatly outnumbered yogurt-derived lactobacilli in numbers adherent per vaginal epithelial cell. In the
present study, the dairy-related lactobacilli *L. acidophilus* and *L. delbrueckii bulgaricus* were not isolated vaginally or rectally, which suggests that these food-derived lactobacilli may not establish colonization. If the capacity of a strain to adhere to the rectal epithelium predisposes it to colonize the vagina for a longer duration, *L. crispatus* and/or *L. jensenii* may be better suited for probiotic use.

References