AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 28, Number 10, 2012

DOI: 10.1089/aid.2011.0382

Longitudinal Assessment of Pigtailed Macaque Lower Genital Tract Microbiota by Pyrosequencing Reveals Dissimilarity to the Genital Microbiota of Healthy Humans

Gregory T. Spear,[1] Ellen Kersh,[2] Patricia Guenthner,[2] Sundaram Ajay Vishwanathan,[2] Douglas Gilbert,[1] M. Reza Zariffard,[1] Paria Mirmonsef,[1] Alan Landay,[1] Luyang Zheng,[1] and Patrick Gillevet[3]

Abstract

Vaginal bacterial communities play an important role in human health and have been shown to influence HIV infection. Pigtailed macaques (*Macaca nemestrina*) are used as an animal model of HIV vaginal infection of women. Since the bacterial microbiota could influence retrovirus infection of pigtailed macaques, the genital microbiota in 10 cycling macaques was determined by pyrosequencing. The microbiota of all macaques was polymicrobial with a median of 13 distinct genera. Strikingly, the genera *Sneathia* and *Fusobacterium*, both in the phylum Fusobacteria, accounted for 18.9% and 13.3% of sequences while the next most frequent were *Prevotella* (5.6%), *Porphyromonas* (4.1%), *Atopobium* (3.6%), and *Parvimonas* (2.6%). Sequences corresponding to *Lactobacillus* comprised only 2.2% of sequences on average and were essentially all *L. amylovorus*. Longitudinal sampling of the 10 macaques over an 8-week period, which spanned at least one full ovulatory cycle, showed a generally stable presence of the major types of bacteria with some exceptions. These studies show that the microbiota of the pigtailed macaques is substantially dissimilar to that found in most healthy humans, where the genital microbiota is usually dominated by *Lactobacillus* sp. The polymicrobial makeup of the macaque bacterial populations, the paucity of lactobacilli, and the specific types of bacteria present suggest that the pigtailed macaque microbiota could influence vaginal retrovirus infection.

Introduction

Vaginal infection of pigtailed macaques (*Macaca nemestrina*) with SIV or SHIV is used as a primate model of HIV sexual transmission in women as well as for testing microbicides and other treatments for prevention of mucosal HIV infection.[1–4] The bacterial microbiota in the lower genital tract of pigtailed macaques could have an impact on vaginal infection with SIV or SHIV since in humans, epidemiological studies indicate that women with a genital microbiota that has the characteristics of bacterial vaginosis have a significantly increased susceptibility to sexual transmission of HIV.[5–7] Bacterial vaginosis (BV) is a condition in women in which the predominant genital bacterial types are a polymicrobial and variable mixture of bacteria including *Gardnerella vaginalis*, *Prevotella*, bacteria in the Order Clostridiales, and other anaerobes.[8] This spectrum of bacteria is quite different from that commonly found in women without BV, where the predominant type of bacteria is in most cases *Lactobacillus* species.

Several previous studies of pigtailed macaque genital microbiota have been performed, but those studies used cultivation to characterize the bacteria.[9–11] Cultivation biases are well known,[12] and recent studies that used molecular techniques to identify genital bacteria indicate that many of the bacteria that are present in the genital tract of women are not cultivable or are difficult to cultivate.[13,14] For example, bacteria from the genus *Sneathia*, in the phylum Fusobacteria, cannot be cultured using current methods, but make up a substantial proportion of the microbiota in some women with BV.[15,16] Furthermore, the type of culture medium used for isolation of genital bacteria can bias the types of cultivatable bacteria found.[17]

To avoid possible culture bias, a recent study used Multitag Pyrosequencing (MTPS) of the 16S ribosomal RNA (rRNA) gene to characterize the genital microbiota of rhesus macaques (*Macaca mulatta*).[18] That study found the rhesus vaginal microbiome had relatively low levels of *Lactobacillus* and had a relatively polymicrobial microbiota. Some of the

[1]Department of Immunology/Microbiology, Rush University Medical Center, Chicago, Illinois.
[2]Laboratory Branch, Division of HIV/AIDS Prevention, NCHHSTP, Centers for Disease Control and Prevention, Atlanta, Georgia.
[3]Microbiome Analysis Center, Department of Environmental Science and Policy, George Mason University, Prince William Campus, Manassas, Virginia.

genera of bacteria present in the rhesus macaques included *Peptoniphilus*, *Sneathia*, *Porphyromonas*, *Mobiluncus*, *Atopobacter*, *Dialister*, *Thioreductor*, *Prevotella*, and *Streptococcus*.

The goal of the present study was to use MTPS of the 16S gene to determine the types of bacteria that constitute the genital microbiota of cycling pigtailed macaques. The microbiota was sampled and characterized six to eight times over an 8-week period to determine its stability. We hypothesized that the genital microbiota of pigtailed macaques would be more highly dominated by *Lactobacillus* than was the microbiota of rhesus macaques since pigtailed macaques and humans have the reproductive similarity of having cycles that take place throughout the year whereas rhesus macaques do not.[1]

Materials and Methods

Animals and specimen collection

All animal studies were reviewed and approved by the Centers for Disease Control and Prevention (CDC)'s Institutional Animal Care and Use Committee. The pigtail macaques were purchased from the breeding colony of Yerkes National Primate Research Center ($n=2$) and from New Iberia Research Center ($n=8$). Upon arrival at CDC, macaques were given complete physical examinations, blood work (CBC chemistries), and an evaluation of the female reproductive tract including colposcopic examination for signs of inflammation. All were deemed to be normal, healthy, cycling mature females 10–16 years of age at sample collection, and weighing 6–11 kg. All macaques tested negative for simian immunodeficiency virus (SIV), simian retroviruses, and simian T cell leukemia viruses. Animals were not Depo-provera treated or synchronized for menstrual cycle.

Ten mature, cycling pigtailed macaques were anesthetized using standard doses of ketamine once per week for 12 weeks. Progesterone and estrogen were measured in blood each week to confirm cycling (data not shown). Cervical vaginal lavage (CVL) samples were collected on weeks 4 through 12 by infusing 8 ml of phosphate-buffered saline into the vaginal vault via a sterile 10-ml syringe attached to a sterile gastric feeding tube, followed by immediate recovery and freezing at $-80°C$.

Pyrosequencing and identification of bacteria

Bacteria in CVL were pelleted by centrifugation and DNA was isolated using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH). The Multitag Pyrosequencing has been described previously and used 12 bar-coded primer sets each containing the 27F and 355R 16S rRNA gene primers.[18] The Bayesian Classifier provided by the Ribosomal Database II Project (RDP 10) was used to identify bacteria using forward reads only. An average of 2137 sequences were obtained for each sample (min = 797, max = 4394). Total numbers of bacteria in samples were estimated using real time PCR with primers F340 and R514.[19] To identify the species of *Lactobacillus*, *Lactobacillus* 16S rRNA gene sequences were assembled with reference sequences into phylogenetic trees with a 96% overlap identity and 80% confidence threshold using Geneious Pro 4.6.1 software (Auckland, New Zealand). Reference sequences were AF257097 (*L. crispatus*), M58820 (*L. gasseri*), AF243176 (*L. jensenii*), AF243177 (*L. vaginalis*), and AY526083 (*L. iners*).

Results

Lower genital tract bacterial microbiota were identified by MTPS of the 16S rRNA gene in 67 samples collected from 10 pigtailed macaques sampled approximately weekly over an 8-week period. At the phylum level, samples contained on average 36% Fusobacteria sequences, 29.6% Firmicutes sequences, 17.2% Bacteroidetes sequences, 10.1% Actinobacteria sequences, and 5.3% Proteobacteria sequences (Fig. 1). Together those five phyla comprised >97% of the sequences in all samples except for one sample that had 6% of its sequences from the phylum Spirochaetes (macaque #025, week 10).

Firmicutes sequences were detected at all time points in all 10 macaques (Fig. 1). Bacteriodetes sequences were present at all time points in all macaques except for one time point in macaque 017. Fusobacteria sequences were found in all samples except two time points from macaque 014 and one time point from macaque TD6. Actinobacteria were present at all time points from four macaques (025, 053, 056, and TC4), but undetected at one or two time points in the other six. Proteobacteria were present at all sampling times in only two macaques (014 and 058). Thus, the phyla Firmicutes, Bacteriodetes, and Fusobacteria were relatively consistent in their presence throughout the 8-week period in all 10 macaques while the presence of Actinobacteria and Proteobacteria was variable.

The genera present in the samples were also determined and analysis was limited to genera found at ≥1% of the total community in each sample with the premise that the most abundant bacteria contribute most significantly to the functionality of the microbial community. Sequences corresponding to 100 different genera of bacteria were found in at least one of the 67 samples at ≥1% of the sequences from each macaque. The 20 most predominant of the genera are shown in Table 1 and in Fig. 2. Strikingly, the genera *Sneathia* and *Fusobacterium*, both in the phylum Fusobacteria, accounted for an average of 18.9% and 13.3% of sequences in all the samples (Table 1). The next most frequent sequences were those corresponding to *Prevotella*, *Porphyromonas*, *Atopobium,* and *Parvimonas* (5.6%, 4.1%, 3.6%, and 2.6%, respectively).

All macaques had a bacterial microbiota that was polymicrobial with a median of 13 genera found in the 67 samples (Table 2). Only two samples had as few as two genera while the highest number of genera in a sample was 24. Macaque 017 had the lowest number of genera (median of 6) over the 8-week period while 058 had the highest (median of 17) (Fig. 2). While this difference was significant using the Mann–Whitney test ($p=0.014$) there were no significant differences between any of the macaques using a multiple group comparison (Kruskal–Wallis), indicating that overall, the number of genera in macaques over this time period was not a stable parameter.

Since *Sneathia* was the genus with the highest average number of sequences in this group of animals it was analyzed in further detail. *Sneathia* levels were relatively stable in five of the macaques over the 8-week period (Fig. 2). For example, macaque 017 consistently had relatively high levels

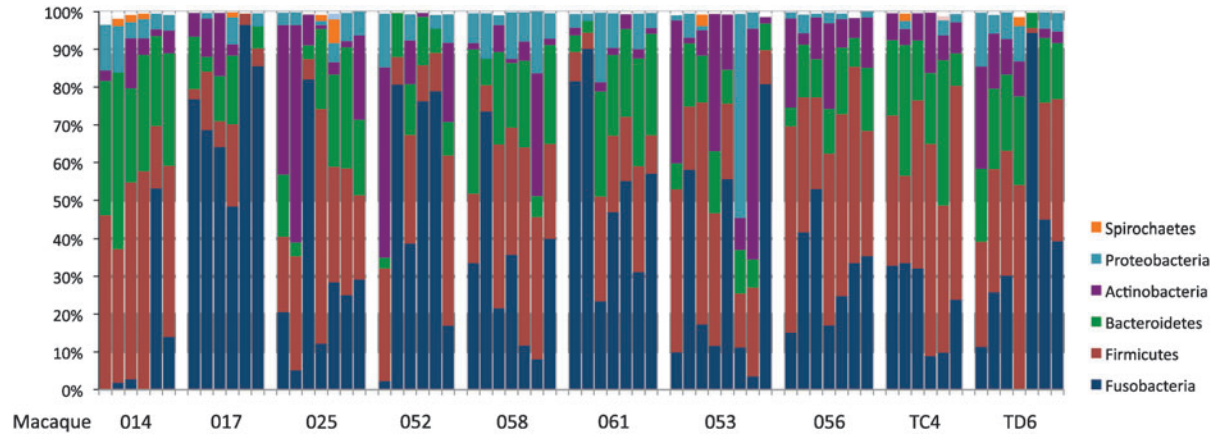

FIG. 1. Phyla of bacteria found in macaques. Each bar represents the relative proportions of 16S sequences corresponding to bacterial phyla in the macaque genital tract at one time point. Time points are displayed chronologically from left to right. For example, the six bars for macaque 014 are weeks 4, 5, 8, 9, 11, and 12. All macaques were sampled at weeks 4, 8, 9, 11, and 12.

of *Sneathia* sequences (48–96%) at all time points while macaques 056 and TC4 had lower *Sneathia* levels (5–25% of sequences) of *Sneathia* at all time points (except for week 7 of animal 056). Animals 014 and 061 did not have any *Sneathia* sequences over the 8 weeks. In contrast, the other animals had more variation in the levels of *Sneathia* with two animals (052 and TD6) varying over the sampling period from no detectable *Sneathia* to relatively high levels. Total copy numbers of *Sneathia* 16S rRNA were also analyzed (see Supplementary Table S1; Supplementary Data are available online at www.liebertonline.com/aid). Total copy numbers of *Sneathia* were significantly higher in animal 017 than in 053 and TD6 ($p < 0.05$, Dunn's Multiple Comparisons Test, excluding 014 and 061 from the analysis), but all other comparisons were not significant. Taken together these data indicate that the relative abundance of bacteria from the genus *Sneathia* was relatively stable in most of the macaques.

Sequences corresponding to *Lactobacillus* comprised only 2.2% of all sequences and were found in 31% of all samples (Table 3). Only five of the macaques had *Lactobacillus* sequences at >2% of total sequences at one or more sampling times. Animal 014 had the highest levels of

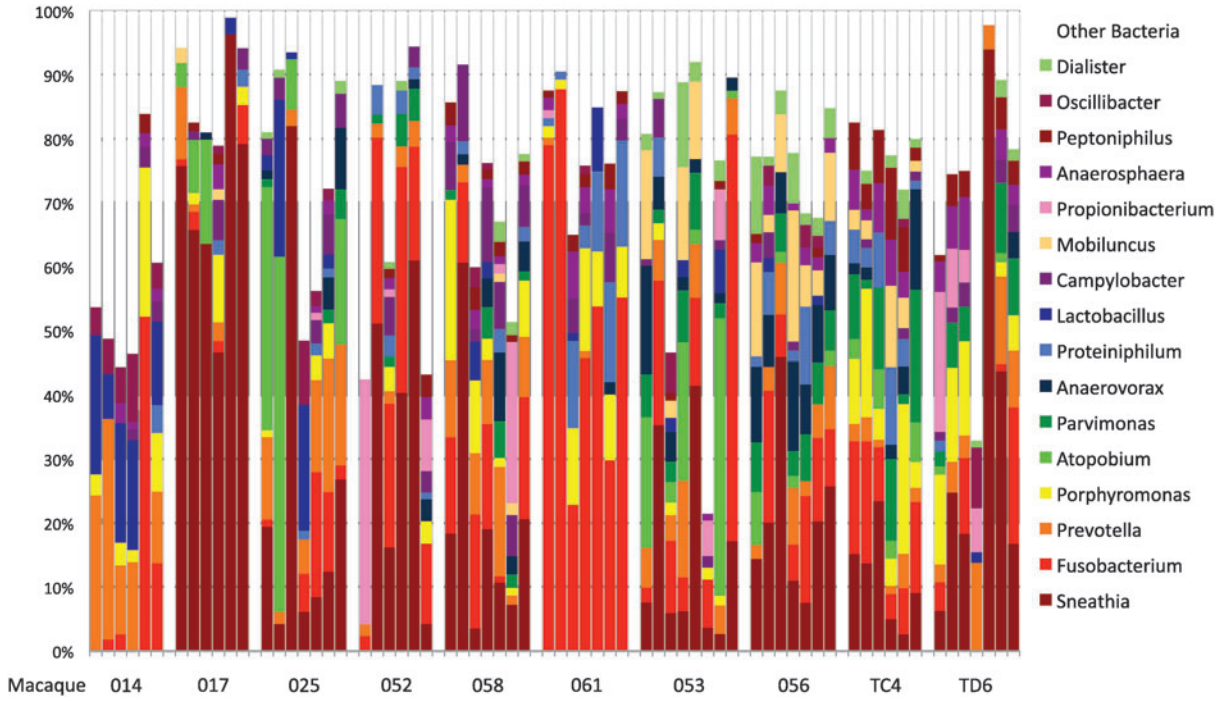

FIG. 2. Genera of bacteria found in macaques. Each bar represents the relative proportions of 16S sequences indentifying bacterial genera in the macaque genital tract at one time point. Time points are displayed the same as in Fig. 1. Only the 16 most predominant genera are displayed for clarity.

Table 1. Predominant Genera in Macaque Samples

Taxa	Percent of sequences[b]	Present in (%)[a]	
		Samples	Macaques
Sneathia	18.9	75	90
Fusobacterium	13.3	82	100
Prevotella	5.6	64	100
Porphyromonas	4.1	61	90
Atopobium	3.6	34	70
Parvimonas	2.6	52	70
Anaerovorax	2.3	48	90
Proteiniphilum	2.2	49	100
Lactobacillus	2.2	31	80
Campylobacter	2.1	58	100
Mobiluncus	1.9	30	50
Propionibacerium	1.8	19	60
Anaerosphaera	1.6	54	100
Peptoniphilus	1.3	46	90
Dialister	1.1	40	70
Oscillibacter	1.0	28	80
Butyricicoccus	0.9	33	70
Corynebacerium	0.9	24	70
Streptococcus	0.8	25	90
Soehngenia	0.8	37	90
Other	31.0		

[a]Present at ≥1% of sequences in a sample, or in any one sample from a macaque.
[b]Average number of sequences in the 67 samples from 10 macaques.

Lactobacillus on average, although levels dipped below 1% of sequences on week 11. The *Lactobacillus* sequences from all the animals corresponded to *L. amylovorus* (see Supplementary Fig. S1).

To further assess the stability of the microbiota in the macaques over the 8-week period, principle coordinates analysis (PCO) was performed. PCO analysis of the genera in the samples showed clustering of the time points from four animals (Fig. 3). The most notable clustering was in animal 061 with all six time points closely arranged followed by 014 and 017 with four time points closely arranged. Animal TC4 also had some clustering of the data points. The other six animals' points were not closely clustered indicating less stability by this measure.

Table 2. Number of Genera in Macaque Genital Samples

Week	Number of genera (≥1% of sequences)									
	014	017	025	052	058	061	053	056	TC4	TD6
4	10	6	12	18	10	9	14	16	15	21
5	13			7		5	11	14		
7		9	6		8		24	9	18	12
8	14	4	4	19	13	13	11	15	14	16
9	22	14	16	7	17	13	9	14	15	20
10			18		23	7	17			2
11	9	2	15	7	21	13	15	16	17	11
12	15	6	10	17	18	7	5	13	16	13

A blank indicates that a sample was not obtained.

Table 3. Proportion of Sequences Corresponding to *Lactobacillus*

Week	% of sequences corresponding to *Lactobacillus*									
	014	017	025	052	058	061	053	056	TC4	TD6
4	22	<1	2	<1	<1	<1	<1	<1	<1	<1
5	7			<1		<1	<1	2		
7		2	27		<1		2	<1	<1	<1
8	19	<1	1	<1	6	1	3	<1	<1	<1
9	17	<1	20	<1	3	<1	<1	<1	<1	2
10			<1		<1	10	<1			<1
11	<1	<1	<1	<1	<1	<1	7	1	<1	<1
12	13	<1	<1	<1	<1	<1	<1	<1	<1	<1

A blank indicates that a sample was not obtained at that time point.

Analysis of Shannon diversity scores (see Supplementary Fig. S2) showed a large variability in diversity over the 8-week period for all animals except macaque TC4. TC4 had the highest median Shannon score (2.37) while macaque 017 had the lowest (0.84). The Shannon scores of macaques TC4 and 017 over the 8-week period were significantly different ($p < 0.05$, Dunn's Multiple Comparisons Test) but all other comparisons were not significant. These data indicate that the diversity of the genital microbiota in most of the macaques was variable over the 8-week period.

Discussion

This study found that the genital microbiota in this group of pigtailed macaques over an 8-week period was quite different from a *Lactobacillus*-dominated type of microbiota commonly found in women.[13,20–23] There were several characteristics of the macaque microbiota that were substantially different from a healthy microbiota in women.

First, levels of lactobacillus were relatively low. Thus, only two macaques had a level of *Lactobacillus* sequences >10% with the highest level reaching 27% of sequences at one time point. In contrast, women with a *Lactobacillus*-dominated genital microbiota typically have >75% *Lactobacillus* sequences.[13–16,22]

Second, the macaque genital microbiota was relatively polymicrobial, which is a feature not usually found in healthy microbiota in women, but that is common in BV.[13–16] Thus, the number of genera found in the macaques ranged from a median of 6 to a median of 17. In contrast, women without BV typically have one to four genera that constitute ≥98% of the microbiota.[13,14,16] Interestingly, many of the genera that were found at high levels in the pigtailed macaques are equivalent to those present in women with BV; *Sneathia*, *Prevotella*, *Porphyromonas,* and *Atopobium* sequences were found in the macaques frequently and are also often found in women with BV.[13, 5] However, there were also several notable differences between the genital microbiota in the pigtailed macaques and BV. First, *Gardnerella vaginalis* is present at relatively high levels in many women with BV (average of approximately 5% of sequences), but was found in only three samples from two macaques and at relatively low levels (1.2%, 2.1%, and 6.5% of the sequences in those samples). Second, sequences from two genera, *Fusobacterium*

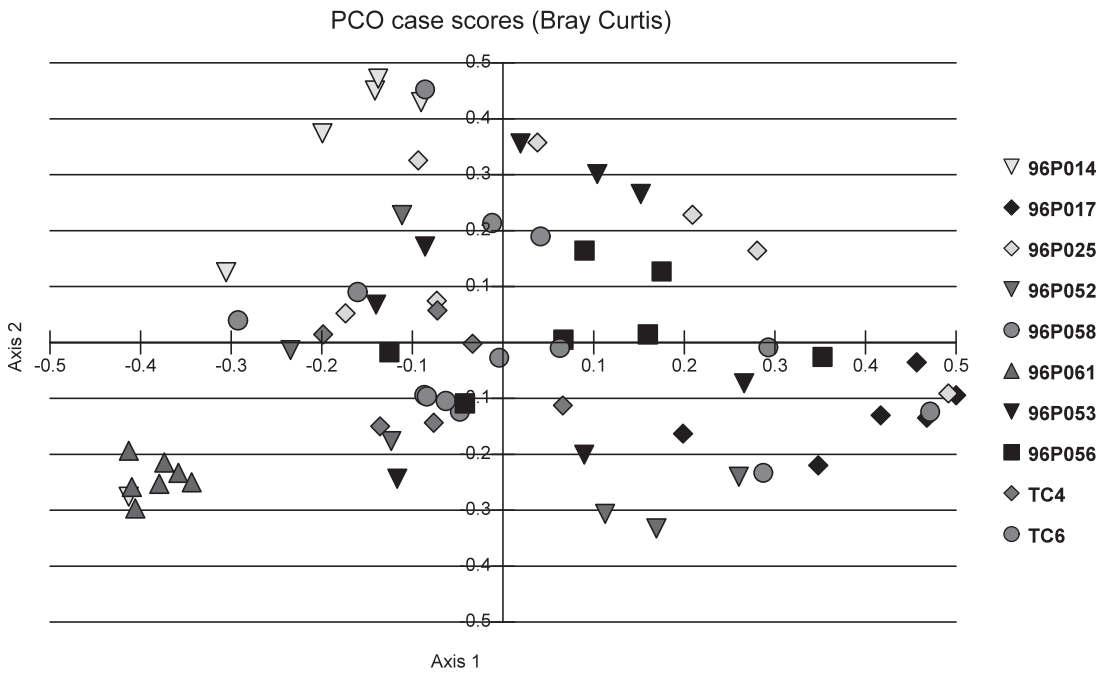

FIG. 3. Principal coordinate analysis of macaque microbiota. Each macaque is represented by one type of symbol and each point is a separate sampling time. For example, the six points representing the six sampling times for macaque 061 are closely clustered indicating the relative relatedness of the bacterial microbiota over time in this animal.

and *Parvimonas*, were found frequently in the macaques but are not frequent in BV.

Third, there were no major differences in proinflammatory cytokine–chemokine levels in macaque vaginal secretions (data not shown) that correlated with differences in the microbiota, as is observed when comparing humans with and without bacterial vaginosis.[24] However, this lack of notable differences in cytokine–chemokine levels between pigtailed macaques could be due in part to the fact that none of the macaques had microbiota that corresponded to what is regarded as healthy microbiota in humans.

A remarkable finding in this study was the very high levels of bacteria from the phylum Fusobacteria with the two genera *Sneathia* and *Fusobacterium* together accounting for more than a third of the sequences. It is interesting to compare this to a study that determined the genital microbiota of rhesus macaques.[18] In that study, *Sneathia* was found at >10% of sequences in 8 out of 11 of the rhesus macaques. In contrast, *Fusobacterium* was found at >10% of sequences in only one. Thus, while *Sneathia* was highly represented in both rhesus and pigtailed macaques, *Fusobacterium* was not. Another interesting comparison between the rhesus and pigtailed studies was that *Lactobacillus* was found at relatively low levels in both types of macaque when compared with humans, but was identified as *L. amylovorus* in the pigtailed macaques and *L. johnsonii* in the rhesus macaques. Yu *et al.* [25] reported that *L. johnsonii*, *L. amylovorus,* and *L. acidophilus* were all present in a group of Chinese rhesus macaques, but *L. johnsonii* was most prevalent. This is in contrast to humans where the most prevalent species are *L. iners*, *L. crispatus*, *L. jensenii,* and *L. gasseri*.[22,26]

It is of interest to compare the vaginal bacterial microbiota of pigtailed macaques with the gut microbiota at the phylum level. While the gut microbiota of pigtailed macaques has not been analyzed with culture-independent techniques, the gut microbiota of rhesus macaques and humans have been determined and are both composed mostly of the phyla Bacteroidetes and Firmicutes with little representation of Fusobacteria and Actinobacteria.[27–29] Relatively high proportions of Fusobacteria and Actinobacteria are a common characteristic of the genital microbiota of pigtailed macaques (Fig. 1), rhesus macaques,[18] and humans with BV.[13,20] This microbiota pattern is substantially different from gut microbiota, indicating that the genital microbiota samples do not merely reflect contamination with feces.

This study has the limitations that pigtailed macaques from only one primate center were analyzed and that Nugent scores and vaginal pH were not determined. Also, vaginal cultures were not obtained, which could have been used to compare cultivatable and noncultivatable microbiota. It is possible that different housing conditions or diets found at other primate centers could impact the makeup of the genital microbiota. However, all 10 macaques over the entire assessment period of 8 weeks showed a genital microbiota that was dissimilar to healthy microbiota in humans but had some similarities to BV in humans. Because in humans, women who lack a healthy genital microbiota have increased susceptibility to sexual transmission of HIV, these results suggest that the genital microbiota of pigtailed macaques in captivity could augment infection with SIV or SHIV. However, the relationship between pigtailed macaque microbiota, inflammation, and virus susceptibility needs further clarification.

Acknowledgments

We thank Drs. Ron Otten and Nat Promadej-Lanier for macaque samples. This work was supported by NIH Grants

U19 AI076981 and P30 AI082151, by the James B. Pendleton Charitable Trust, and by the Interagency Agreement Y1-AI-0681-02 between CDC and NIH. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Author Disclosure Statement

No competing financial interests exist.

References

1. Cole AM, Patton DL, Rohan LC, *et al.:* The formulated microbicide RC-101 was safe and antivirally active following intravaginal application in pigtailed macaques. PLoS One 5(11):e15111.
2. Jiang Y, Tian B, Saifuddin M, *et al.:* RT-SHIV, an infectious CCR5-tropic chimeric virus suitable for evaluating HIV reverse transcriptase inhibitors in macaque models. AIDS Res Ther 2009;6:23.
3. Promadej-Lanier N, Srinivasan P, Curtis K, *et al.:* Systemic and mucosal immunological responses during repeated mucosal SHIV(162P3) challenges prior to and following infection in pigtailed macaques. Virology 2008;375(2):492–503.
4. Kent SJ, Dale CJ, Ranasinghe C, *et al.:* Mucosally-administered human-simian immunodeficiency virus DNA and fowlpoxvirus-based recombinant vaccines reduce acute phase viral replication in macaques following vaginal challenge with CCR5-tropic SHIVSF162P3. Vaccine 2005;23(42):5009–5021.
5. Atashili J, Poole C, Ndumbe PM, Adimora AA, and Smith JS: Bacterial vaginosis and HIV acquisition: A meta-analysis of published studies. AIDS 2008;22(12):1493–1501.
6. Kaul R, Nagelkerke NJ, Kimani J, *et al.:* Prevalent herpes simplex virus type 2 infection is associated with altered vaginal flora and an increased susceptibility to multiple sexually transmitted infections. J Infect Dis 2007;196(11):1692–1697.
7. Cherpes TL, Meyn LA, Krohn MA, Lurie JG, and Hillier SL: Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. Clin Infect Dis 2003;37(3):319–325.
8. Cherpes TL, Hillier SL, Meyn LA, Busch JL, and Krohn MA: A delicate balance: Risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. Sex Transm Dis 2008;35(1):78–83.
9. Patton DL, Sweeney YC, Cummings PK, Meyn L, Rabe LK, and Hillier SL: Safety and efficacy evaluations for vaginal and rectal use of BufferGel in the macaque model. Sex Transm Dis 2004;31(5):290–296.
10. Patton DL, Sweeney YT, Balkus JE, *et al.:* Preclinical safety assessments of UC781 anti-human immunodeficiency virus topical microbicide formulations. Antimicrob Agents Chemother 2007;51(5):1608–1615.
11. Patton DL, Sweeney YC, Rabe LK, and Hillier SL: The vaginal microflora of pig-tailed macaques and the effects of chlorhexidine and benzalkonium on this ecosystem. Sex Transm Dis 1996;23(6):489–493.
12. Amann RI, Ludwig W, and Schleifer KH: Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 1995;59(1):143–169.
13. Fredricks DN, Fiedler TL, and Marrazzo JM: Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med 2005;353(18):1899–1911.
14. Hyman RW, Fukushima M, Diamond L, Kumm J, Giudice LC, and Davis RW: Microbes on the human vaginal epithelium. Proc Natl Acad Sci USA 2005;102(22):7952–7957.
15. Ravel J, Gajer P, Abdo Z, *et al.:* Microbes and Health Sackler Colloquium: Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA 2011;108(Suppl 1);4680–4687.
16. Spear GT, Sikaroodi M, Zariffard MR, Landay AL, French AL, and Gillevet PM: Comparison of the diversity of the vaginal microbiota in HIV-infected and HIV-uninfected women with or without bacterial vaginosis. J Infect Dis 2008;198:1131–1140.
17. Falsen E, Pascual C, Sjoden B, Ohlen M, and Collins MD: Phenotypic and phylogenetic characterization of a novel Lactobacillus species from human sources: Description of Lactobacillus iners sp. nov. Int J Syst Bacteriol 1999;49(Pt 1):217–221.
18. Spear GT, Gilbert D, Sikaroodi M, *et al.:* Identification of rhesus macaque genital microbiota by 16S pyrosequencing shows similarities to human bacterial vaginosis: Implications for use as an animal model for HIV vaginal infection. AIDS Res Hum Retroviruses 2010;26(2):193–200.
19. Barman M, Unold D, Shifley K, *et al.:* Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. Infect Immun 2008;76(3):907–915.
20. Hummelen R, Fernandes AD, Macklaim JM, *et al.:* Deep sequencing of the vaginal microbiota of women with HIV. PLoS One 2010;5(8):e12078.
21. Pepin J, Deslandes S, Giroux G, *et al.:* The complex vaginal flora of West African women with bacterial vaginosis. PLoS One 2011;6(9):e25082.
22. Spear GT, Gilbert D, Landay AL, *et al.:* Pyrosequencing of the genital microbiotas of HIV-seropositive and -seronegative women reveals Lactobacillus iners as the predominant Lactobacillus species. Appl Environ Microbiol 2011;77(1):378–381.
23. Ling Z, Liu X, Chen X, *et al.:* Diversity of cervicovaginal microbiota associated with female lower genital tract infections. Microb Ecol 2011;61(3):704–714.
24. Mitchell CM, Balkus J, Agnew KJ, *et al.:* Bacterial vaginosis, not HIV, is primarily responsible for increased vaginal concentrations of proinflammatory cytokines. AIDS Res Hum Retroviruses 2008;24(5):667–671.
25. Yu RR, Cheng AT, Lagenaur LA, *et al.:* A Chinese rhesus macaque (Macaca mulatta) model for vaginal Lactobacillus colonization and live microbicide development. J Med Primatol 2009;38(2):125–136.
26. Antonio MA, Hawes SE, and Hillier SL: The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. J Infect Dis 1999;180(6):1950–1956.
27. Dethlefsen L and Relman DA: Microbes and Health Sackler Colloquium: Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci USA 2011;108(Suppl 1):4554–4561.
28. McKenna P, Hoffmann C, Minkah N, *et al.:* The macaque gut microbiome in health, lentiviral infection, and chronic enterocolitis. PLoS Pathog 2008;4(2):e20.
29. Eckburg PB, Bik EM, Bernstein CN, *et al.:* Diversity of the human intestinal microbial flora. Science 2005;308(5728):1635–1638.

Address correspondence to:
Gregory Spear
Department of Immunology/Microbiology
Rush University Medical Center
Chicago, Illinois 60612

E-mail: gspear@rush.edu