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Research Letter

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Antibiotic treatment disrupts bacterial communities in the colon and rectum of simian immunodeficiency virus-infected macaques

Alexander S. Zevin[1,2,†], Tiffany Hensley-McBain[1,2], Charlene Miller[1,2], Elise Smith[2], Stanley Langevin[2] and Nichole R. Klatt[1,2,*]

[1]Department of Pharmaceutics, Washington National Primate Research Center, University of Washington, Seattle, WA 98121, USA, [2]Washington National Primate Research Center, University of Washington, Seattle, WA 98121, USA and [3]Division of Systems Biology, Washington National Primate Research Center, University of Washington, Seattle, WA 98121, USA

*Corresponding author: Department of Pharmaceutics, Washington National Primate Research Center, University of Washington, Seattle, 3018 Western Ave, Seattle, WA 98121, USA. Tel: 206-221-0254; E-mail: klattnr@uw.edu

One sentence summary: Antibiotics alter bacterial communities in the colon, stool and rectal swabs of SIV-infected macaques, leading to a reduction of commensal bacteria and an increase in potentially pathogenic bacteria, including *Enterobacteriaceae* species.

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†Alexander S. Zevin, http://orcid.org/0000-0001-7811-4663

ABSTRACT

Antibiotic therapies are known to disrupt gastrointestinal (GI) bacterial communities. HIV and pathogenic simian immunodeficiency virus (SIV) infections have also been associated with disrupted GI bacterial communities. We administered a combination antibiotic therapy to six SIV-infected rhesus macaques and collected colon biopsies, stool samples and rectal swabs before and after antibiotics, and evaluated the bacterial communities at each sample site using high-throughput 16S rRNA gene sequencing. The colon mucosa and stool samples displayed different bacterial communities, while the rectal swabs showed a mixture of the mucosal and stool-associated bacteria. Antibiotics disrupted the native bacterial communities at each sample site. The colon mucosa showed depleted abundances of the dominant *Helicobacteraceae*, while we found depleted abundances of the dominant *Ruminococcaceae* sp. in the stool. The rectal swabs showed similar trends as the colon mucosa, but were more variable. After the antibiotic treatment, there were increased abundances of similar taxa of facultative anaerobic bacteria, including *Lactobacillaceae* and *Enterobacteriaceae* at each sample site.

Keywords: macaque; microbiome; antibiotics; SIV; HIV; rectal swab

INTRODUCTION

Antibiotics are widely used in human health and veterinary practices, and are known to disrupt the gastrointestinal (GI) microbiota (Becattini, Taur and Pamer 2016). Simian immunodeficiency virus (SIV) infection of non-human primates (NHP) is the leading animal model to accurately recapitulate the pathogenesis of HIV (Hatziioannou and Evans 2012). Dysbiosis of the intestinal microbiota and translocation of microbes from the colon into the periphery are important processes that drive inflammation in HIV and SIV infections (Brenchley *et al.* 2006; Klase *et al.*

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Table 1. Primers used in this study.

Name	Sequence (5′→3′)	Purpose	Reference
341F	CCTACGGGNGGCWGCAG	Illumina 16S rRNA gene sequencing	Klindworth *et al.* (2013)
805R	GACTACHVGGGTATCTAATCC	Illumina 16S rRNA gene sequencing	Klindworth *et al.* (2013)
8F	AGAGTTTGATCCTGGCTCAG	Cloning whole 16S rRNA genes	Galkiewicz and Kellogg (2008)
1542R	AAGGAGGTGATCCAGCCGCA	Cloning whole 16S rRNA genes	Galkiewicz and Kellogg (2008)
1055yF	ATGGYTGTCGTCAGCT	Total bacterial 16S qPCR	Ritalahti *et al.* (2006)
1392R	ACGGGCGGTGTGTAC	Total bacterial 16S qPCR	Dionisi *et al.* (2003)
16STaq1115	FAM-CAACGAGCGCAACCC-BHQ1	Total bacterial 16S qPCR	Dionisi *et al.* (2003)
HelicoF	ACCAAGGCWATGACGGGTATC	*Helicobacter*-specific 16S qPCR	Huijsdens *et al.* (2004)
HelicoR	CGGAGTTAGCCGGTGCTTATT	*Helicobacter*-specific 16S qPCR	Huijsdens *et al.* (2004)
HelicoTaq	FAM-AACCTTCATCCTCCACGCGGC-BHQ1	*Helicobacter*-specific 16S qPCR	Huijsdens *et al.* (2004)

2015), and these processes are likely affected by antibiotic administration. However, the impact of antibiotics on colonic and rectal microbiota in SIV-infected NHP has not been investigated.

We previously reported that oral administration of vancomycin for 7 days followed by a combination of vancomycin and enrofloxacin for 12 days disrupted colonic mucosal bacterial communities of six SIV-infected rhesus macaques treated with antiretroviral therapy (ART), and that a fecal microbiome transplant (FMT) using donor material from a healthy rhesus macaque restored these communities to a pre-antibiotics state. Furthermore, we demonstrated that the FMT was associated with statistically significant increases in peripheral IL-17 and IL-22-producing CD4 + T cells, and statistically significant decreases in activated CD4 + T cells in the jejunal and rectal mucosae (Hensley-McBain *et al.* 2016).

In the previous study, we did not report the bacterial communities detected in the colonic lumen (i.e. stool) or those associated with rectal swabs. Furthermore, the previous study was mostly focused on the effects of FMT on host immunity and did not exclusively evaluate the effects of the antibiotic treatment on the microbiota. Here, we compare the bacterial communities from each site (colon biopsy, stool and rectal swab) before and after antibiotic treatment. We demonstrate that the colonic mucosa and lumen in these macaques had unique bacterial communities, and that the community structure determined from rectal swab samples shared similarities with the mucosal and luminal communities. We further demonstrate that the antibiotic treatment disrupted the normal bacterial communities at each sample site, leading to a reduction in fermentative bacteria and increased abundances of facultative anaerobic bacteria.

MATERIALS AND METHODS

Study animals and antibiotic treatment

Animals were housed and cared for in Association for the Assessment and Accreditation of Laboratory Animal Care international accredited facilities, and all animal procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee of University of Washington (Protocol 4314–01) as previously described (Hensley-McBain *et al.* 2016). Briefly, six male rhesus macaques (*Macaca mulatta*) were infected intrarectally with $SIV_{MAC239X}$ and started on ART 130 days after infection, which consisted of tenofovir (PMPA; 20 mg kg^{-1}), emtricitabine (FTC; 30 mg kg^{-1}) and raltegravir (50 mg b.i.d). The animals were then treated with an antibiotic regimen consisting of oral vancomycin (15 mg kg^{-1} b.i.d) for 7 days followed by a combination of oral vancomycin and enrofloxacin (10 mg kg^{-1} b.i.d.) for 12 additional days. Antibiotics were given with a treat and were monitored to ensure the entire dose was taken. Biopsies of the colon (immersed in RNALater Solution (ThermoFisher Scientific, Waltham, MA)), rectal swabs and stool were collected before and after the antibiotic treatment and were placed at –80°C after collection.

DNA extraction and sequencing

Genomic DNA was extracted from the colon biopsies, rectal swabs and stool using the Omni Bead Ruptor (Omni International, Kennesaw, GA) with 2.8-mm ceramic beads, followed by purification using the RNA/DNA All-Prep Kit (Qiagen, Germantown, MA). We used 5 ng of DNA to generate 460 bp amplicons by targeting the V3-V4 hypervariable regions of the 16S rRNA gene. To achieve this, we utilized the previously described 341F-805R primer pair targeting the V3-V4 (Table 1) region of the bacterial 16S rRNA gene (Herlemann *et al.* 2011; Klindworth *et al.* 2013). PCR conditions were as follows: 95°C for 3 min followed by 25 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 5 min. Nextera XT dual index adaptors were incorporated by performing 12 PCR cycles using a FailSafe PCR system, cleaned using 1.1X AMPure XP Beads (Beckman Coulter, Brea, CA) and quantified using DNA High Sensitivity Qubit (ThermoFisher). The samples were then mixed in equimolar ratios. These final libraries were then loaded onto a 600-cycle Illumina MiSeq Kit and sequenced using an Illumina MiSeq (Illumina, San Diego, CA) at 2 pM concentration with 5% PhiX control and sequenced using Nextera sequencing read and index primers (Hensley-McBain *et al.* 2016).

Analysis of 16S rRNA gene sequencing data

Paired-end reads were joined using the PANDAseq assembler (Masella *et al.* 2012) and the resulting reads were analyzed using the QIIME V1.9.0 pipeline (Caporaso *et al.* 2010). We used the split_libraries_fastq.py script to demultiplex and quality filter the sequence reads using a quality score cutoff of 20. After quality filtering, we obtained 3214 697 total reads with an average of 91 848 reads per sample and a median sequence length of 443 base pairs. We clustered sequences into operational taxonomic units (OTUs) with an open-reference approach at 97% similarity using the UCLUST algorithm and assigned taxonomy to each OTU based using the SILVA database (Pruesse *et al.* 2007), as implemented by the pick_otus.py script. This resulted in an average of ~45 000 OTUs per sample. We identified potentially chimeric sequences using the ChimeraSlayer algorithm as implemented in the identify_chimeric_seqs.py script and removed those

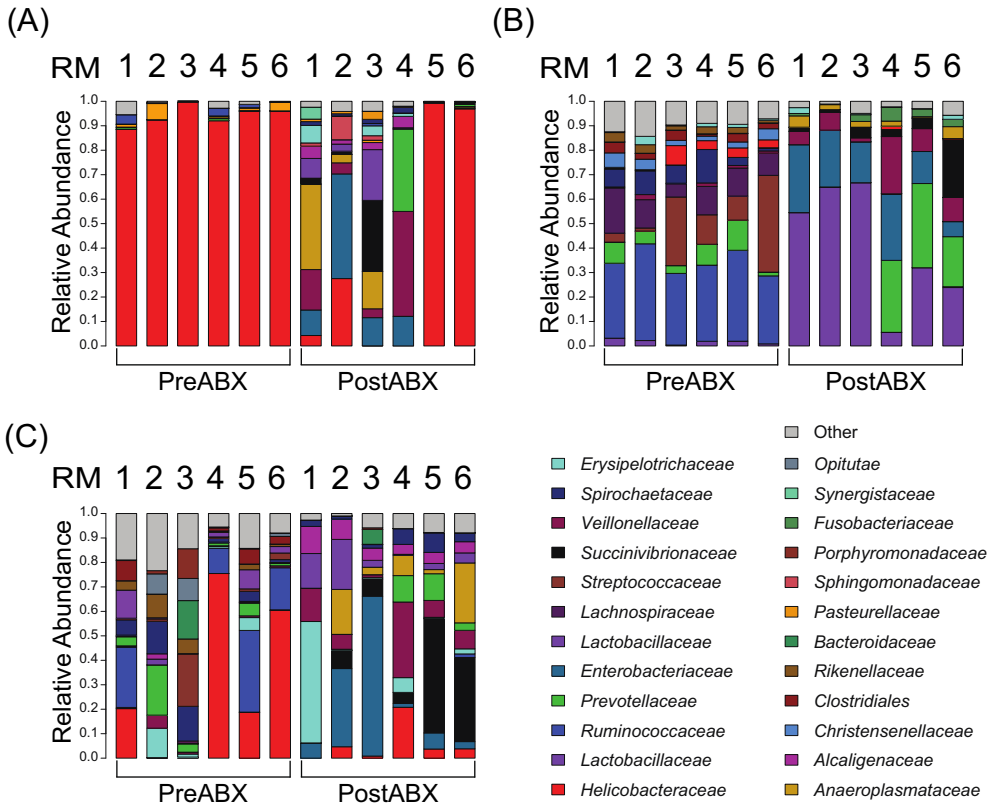

Figure 1. Bacterial community composition was altered by antibiotic treatment. Before the antibiotic treatment (PreABX), the colon biospies (**A**) were dominated by *Helicobacteraceae* while the stool communities (**B**) were composed of *Ruminococcaceae*, *Lachnospiraceae* and other anaerobic bacteria. The rectal swab (**C**) communities represented a mixture of the bacteria from the mucosa and stool. After the antibiotic treatment (PostABX), there was an expansion of *Enterobacteriaceae, Lactobacillaceae* and other facultative anaerobic bacterial phylotypes at all three sites. However, each site displayed unique bacterial communities.

sequences from the analysis. To further analyze the species within the *Helicobacteraceae* family, we pulled all sequences assigned to the family *Helicobacteraceae* and searched these against the NCBI 16S rRNA gene database using the BLAST algorithm. We subsampled 40 000 sequences per sample from the resulting OTU tables and calculated the Shannon diversity, and performed principal coordinate analysis (PCoA) based on Bray-Curtis distances using the *vegan* and *ape* packages in R. Wilcoxon signed-rank tests were performed using Graphpad Prism. Linear discriminant analysis effect size (LEfSe) analysis was performed using online tools (Segata *et al.* 2011). All sequence data from this study have been uploaded to the NCBI SRA under BioProject Accession PRJNA385206.

Quantitative polymerase chain reaction

We used quantitative polymerase chain reaction (qPCR) to quantify total bacterial 16S rRNA genes at each sample site. We prepared triplicate 10 μL reactions of each sample and standard containing 1X SsoAdvanced Universal Probes Supermix (BioRad, Hercules, CA), and 300 nM each of forward primer, 1055yF (Ritalahti *et al.* 2006); reverse primer; 1392R, and TaqMan probe, 16STaq1115 (Dionisi *et al.* 2003) (Table 1). The TaqMan probe carried a 5′ FAM dye and a 3′ BHQ1 quencher. To generate a standard curve, we PCR amplified the entire 16S rRNA gene of *Lactobacillus rhamnosus* ATCC53103 using the universal bacterial primers 8F and 1542R and cloned this amplicon into a plasmid using the TOPO TA Cloning Kit (ThermoFisher Scientific). We then serially diluted this plasmid such that final standard curve ranged from 7.1–7.1 $\times$ 10^6 16S rRNA gene copies per microliter. Reactions were initially denatured at 95°C for 2 min followed by 40 cycles of 95°C for 10 s, 56°C for 20 s and 68°C for 20 s. We performed qPCR using a BioRad CFX96 Real-Time PCR Detection System (BioRad, Hercules, CA). We performed *Helicobacter* genus specific qPCR using previously described primers (Table 1) and qPCR methods (Huijsdens *et al.* 2004). For this, we prepared a qPCR standard as described above, but instead used the PCR-amplified 16S rRNA gene from *Helicobacter pylori* in the TOPO-TA reaction. For the colon biopsy and stool samples, we normalized copy numbers to the amount of input biomass. As this information was not available for rectal swabs, we reported total copies detected.

RESULTS

Prior to the antibiotic treatment, the colon biopsies (Fig. 1A) were dominated by bacteria from the family *Helicobacteraceae*, on average representing 94.1 $\pm$ 3.9% of the total community. This high abundance of *Helicobacteraceae* in the colonic mucosa was unexpected, as mucosal bacterial communities in humans often display a greater degree of diversity (Stearns *et al.* 2011; Dillon *et al.* 2014). Thus, we further evaluated the bacterial species within the family *Helicobacteraceae* across each sample site and found that it was composed mostly of *Helicobacter fennalliae* (53.2%) and *H. macacae* (45.7%). We also detected a variety of other *Helicobacter* species as well as several *Campylobacter* species, but these totaled <1% of the *Helicobacteraceae* species (Table 2).

Table 2. Species detected within the family *Helicobacteraceae*.

Accession no.	Number of sequences	Fraction of *Helicobacteraceae* sequences	Nearest BLAST hit species
NG_042881	581 758	0.532471384	*Helicobacter fennelliae*
NG_042884	499 625	0.457296703	*Helicobacter macacae*
NR_146694	5270	0.004823525	*Helicobacter canicola*
NR_135861	3064	0.002804418	*Helicobacter himalayensis*
NR_044761	789	0.000722156	*Helicobacter pylori*
NR_116595	334	0.000305703	*Helicobacter pullorum*
NG_042880	304	0.000278245	*Helicobacter fennelliae*
NR_115104	268	0.000245295	*Helicobacter canadensis*
NR_025941	225	0.000205938	*Helicobacter cinaedi*
NR_074476	144	0.0001318	*Helicobacter cetorum*
NR_114557	124	0.000113495	*Helicobacter winghamensis*
NR_024836	100	9.1528E-05	*Helicobacter ganmani*
NR_026074	98	8.96974E-05	*Helicobacter rodentium*
NR_043799	93	8.5121E-05	*Helicobacter brantae*
NR_118528	80	7.32224E-05	*Campylobacter upsaliensis*
NR_025124	77	7.04765E-05	*Helicobacter aurati*
NR_044169	45	4.11876E-05	*Helicobacter suis*
NR_029182	32	2.9289E-05	*Helicobacter bilis*
NR_025939	31	2.83737E-05	*Helicobacter muridarum*
NR_118526	27	2.47126E-05	*Campylobacter showae*
NR_041825	18	1.6475E-05	*Helicobacter marmotae*
NR_118513	16	1.46445E-05	*Campylobacter curvus*
NR_028019	9	8.23752E-06	*Helicobacter pametensis*
NR_043052	7	6.40696E-06	*Helicobacter canis*
NR_043108	6	5.49168E-06	*Helicobacter cholecystus*
NR_115105	6	5.49168E-06	*Helicobacter equorum*
NR_025952	4	3.66112E-06	*Helicobacter hepaticus*
NR_025935	3	2.74584E-06	*Helicobacter felis*
NR_118516	3	2.74584E-06	*Campylobacter gracilis*
NG_042882	2	1.83056E-06	*Helicobacter mastomyrinus*

In contrast, the stool bacterial community (Fig. 1B) showed a more even distribution of *Ruminococcaceae* (32.6 ± 4.7%), *Streptococcaceae* (15.7 ± 15%), *Lachnospiraceae* (11.3 ± 4.3%) and *Prevotellaceae* (6.6 ± 4.0%), among a variety of other bacterial families. The bacteria detected on the rectal swabs (Fig. 1C) were similar to those from the colon biopsy and stool samples, including *Helicobacteraceae, Ruminococcaceae* and *Prevotellaceae*. However, the rectal swab communities for each animal were distinct from the corresponding biopsy and stool samples, and the rectal swabs were more variable between the animals. Indeed, PCoA of the bacterial abundance and diversity in the samples taken before the antibiotic treatment revealed that the rectal swab samples appeared to share features with the mucosal and fecal communities (Fig. 2A). This grouping of the rectal swab samples also emphasized that the rectum is a unique compartment.

Following the antibiotic treatment, there were dramatic shifts in the bacterial communities detected in all three of the sample sites. Interestingly, the colon biopsy bacterial communities from RM5 and RM6 did not show a response to the antibiotic treatment, and instead retained high abundances of *Helicobacteraceae*. However, the stool and rectal swab samples from these animals did show altered bacterial community composition after the antibiotic treatment (Fig. 1). A further interesting trend revealed by PCoA of the samples from before and after the antibiotic treatment was that the bacterial communities from all three of the sample sites were more similar after the antibiotic treatment than they had been before the antibiotic treatment, as demonstrated by clustering of the post-antibiotic samples in PCoA plots (Fig. 2B). PCoA of the samples taken after the antibiotic treatment confirmed this trend (Fig. 2C) demonstrated by less distinctive clustering of the samples based on the sampling site. This indicated that the antibiotic treatment led to increased abundances of similar bacteria without regard to the biogeographic location. We also found that the antibiotic treatments altered bacterial species diversity at each sample site. The colon biopsy samples showed significantly increased diversity after the antibiotic treatment, while that of the stool samples was significantly decreased. There was no significant change in the species diversity of the rectal swabs after the antibiotic treatment (Fig. 2C).

We used LEfSe analysis to determine which bacterial families were most differentially abundant before and after the antibiotic treatment at each sample site as well as across all of the sample sites (Fig. 3). Because the colon biopsies from RM5 and 6 did not show a response to the antibiotics, we omitted these samples from the analysis and only performed LEfSe analysis using animals that responded to the antibiotic treatment. The LEfSe analysis indicated that, across all sample sites, *Enterobacteriaceae, Lactobacillaceae, Veillonellaceae* and *Succinivibrionaceae* were most significantly expanded while *Ruminococcaceae, Streptococcaceae, Lachnospiraceae* and *Treponema* were most significantly depleted. *Helicobacteraceae* was identified as being significantly depleted after the antibiotic treatment only when the colon biopsy samples were analyzed independently by LEfSe analysis.

Finally, we estimated the total bacterial load and *Helicobacter* load at each sample site before and after the antibiotic treatment using qPCR targeting either all bacterial 16S rRNA genes or only *Helicobacter* 16S rRNA genes (Fig. 4). We found that the stool

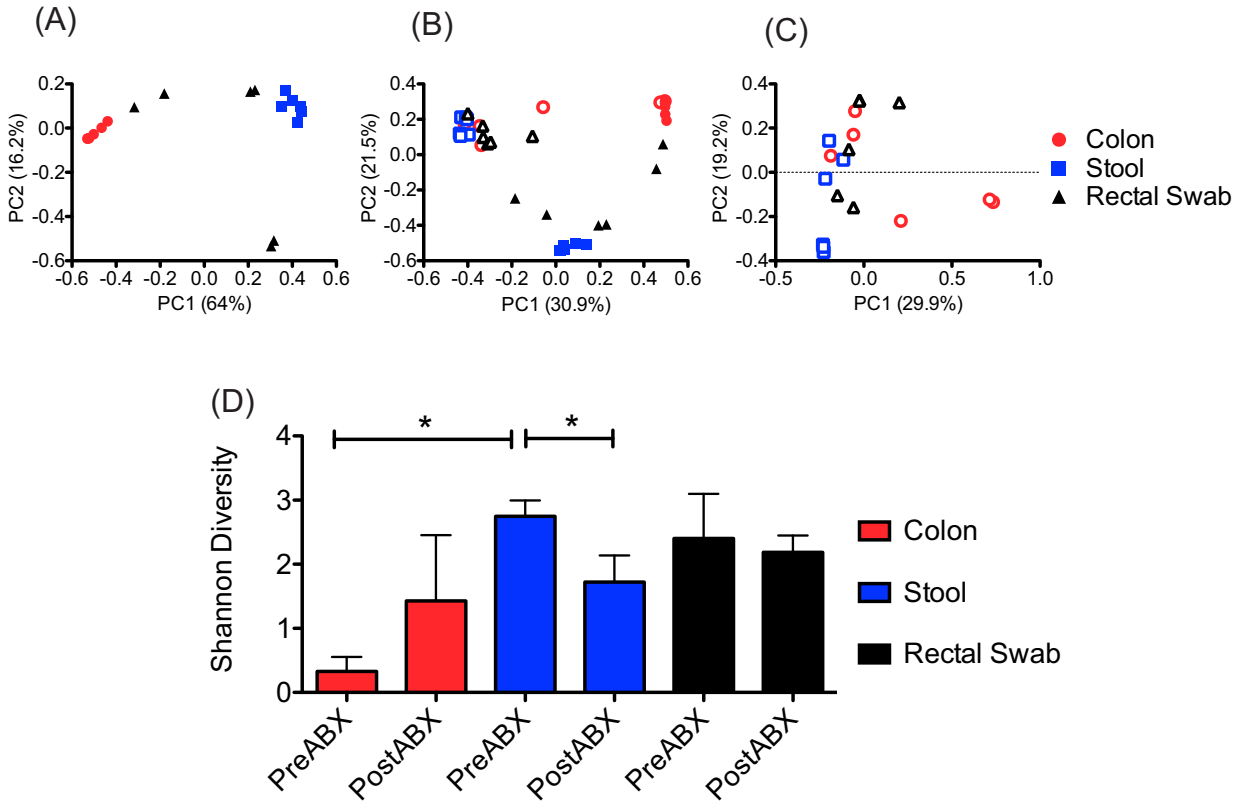

Figure 2. Antibiotics altered bacterial community diversity. PCoA of the samples taken before antibiotic treatment demonstrated that the mucosal (red circles) and stool-associated (blue squares) communities clustered separately, while those from the rectal swabs (black triangles) formed an intermediate grouping (**A**). Comparing all of the samples from before (closed symbols) and after (open symbols) the antibiotic treatment demonstrated that there was a greater degree of similarity between the bacterial communities at each site after the antibiotic treatment (**B**). This trend was further demonstrated by PCoA of samples taken after the antibiotic treatment, which did not show distinctive clustering (**C**). The antibiotic treatment led to increased species diversity in the colon mucosa, but decreased diversity in the stool-associated communities, while rectal swabs showed a minor, but not statistically significant, decrease in diversity. A Wilcoxon signed-rank test was used to determine statistical significance $* = P < 0.05$ (**D**).

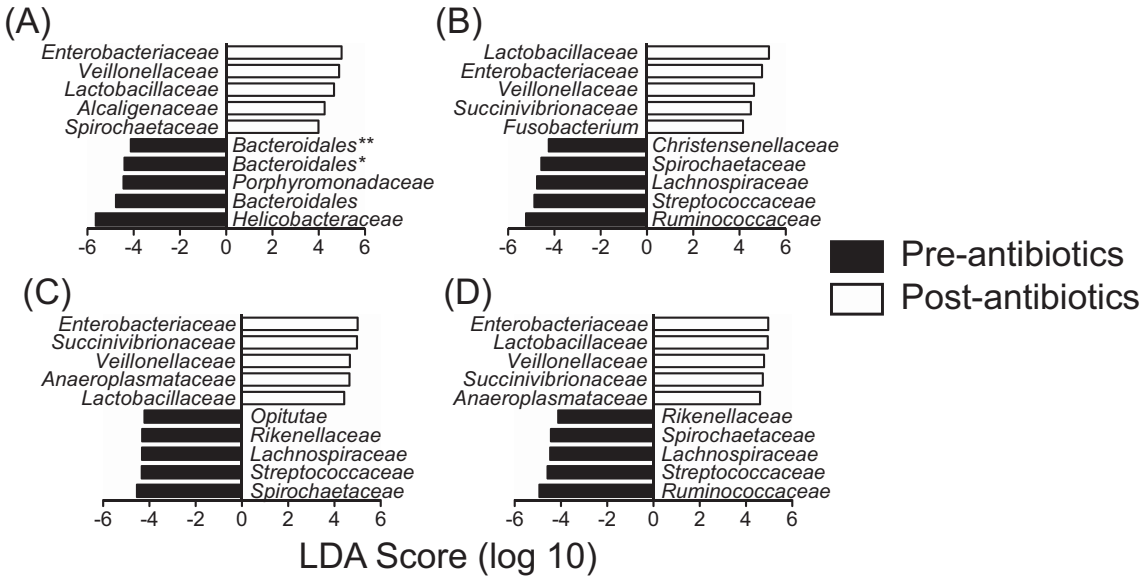

Figure 3. Facultative anaerobic bacteria were enriched after antibiotic treatment. LEfSe analysis of the bacterial communities of the colon biopsies (**A**), stool (**B**), rectal swabs (**C**) and across all sites (**D**) before and after the antibiotic treatment demonstrated that facultative anaerobic bacterial taxa, including *Enterobacteriaceae* and *Lactobacillaceae*, were significantly expanded after the antibiotic treatment, while many fermentative obligate anaerobes such as *Ruminococcaceae* were depleted.

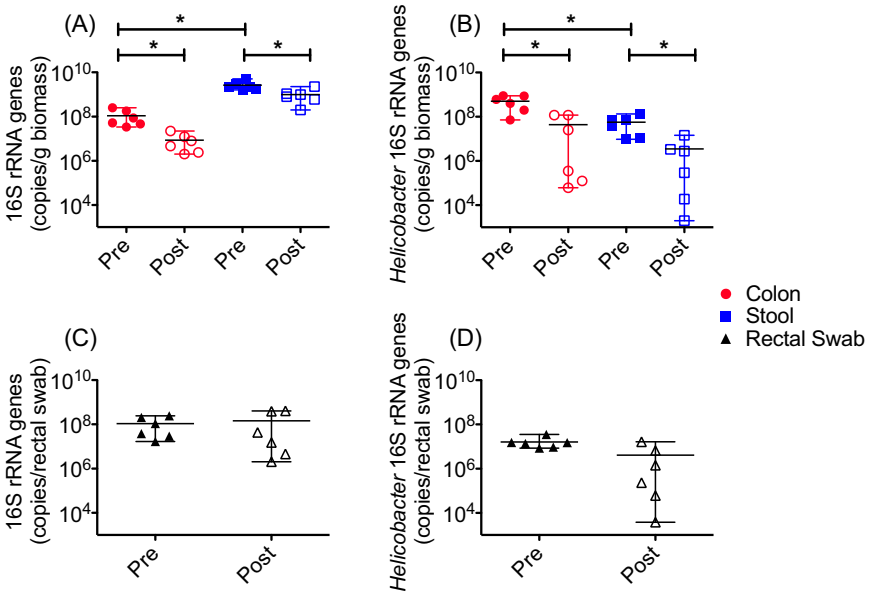

Figure 4. Antibiotics decreased total bacterial and *Helicobacter* load in colon mucsoa and stool. qPCR of total 16S rRNA genes revealed that the stool (blue squares) had a significantly higher bacterial load than did the colon mucosa (red circles), and that the total bacterial load before the antibiotic treatment (closed symbols) was significantly greater than the bacterial load after the antibiotic treatment (open symbols) in both compartments **(A).** In contrast, the colon mucosa showed a significantly higher load of *Helicobacter* 16S rRNA genes than did the stool, and the antibiotic treatment reduced the *Helicobacter* load at both sites **(B)**. The rectal swab samples (black triangles) showed reduction of both total bacterial **(C)** and *Helicobacter* **(D)** 16S rRNA genes after the antibiotic treatment, but this did not reach statistical significance. A Wilcoxon signed-rank test was used to determine statistical significance $* = P < 0.05$.

bacterial communities had significantly higher total copy numbers than did those from the colon biopsies and that the bacterial load at these sites was significantly decreased after the antibiotic treatment (Fig. 4A). In accordance with the high-throughput sequencing data, the colon mucosa showed significantly higher copies of *Helicobacter* 16S rRNA genes than did the stool, and these were also significantly decreased after the antibiotic treatment (Fig. 4B). Because there was no way to measure the amount of biomass collected on each rectal swab, we were unable to compare these communities with those from the colon biopsies or stool in terms of total bacterial load. Interestingly, the total 16S rRNA gene copies detected from the rectal swabs were similar to that of the colon mucosa before the antibiotic treatment. However, after the antibiotic treatment, the rectal swab samples did not show a significant reduction in the total bacterial load, although some rectal swab samples did show reduced bacterial loads after the antibiotic treatment (Fig. 4C). We also observed similar trends for the *Helicobacter* 16S rRNA genes (Fig. 4D).

DISCUSSION

Here, we reported on the effects of a combination antibiotic treatment on mucosal and luminal bacterial communities in six ART-treated, SIV-infected male rhesus macaques. As has been previously reported in healthy and SIV-infected macaques, the colonic mucosa of these macaques was heavily dominated by *Helicobacteraceae* (Yasuda *et al.* 2015), which we determined to be *H. fennalliae* and *H. macacae*. Several *Helicobacter* spp. have been isolated from the colon mucosa of rhesus macaques, including *H. cinaedi* (Fox *et al.* 2001) and *H. macacae* (Lertpiriyapong *et al.* 2014). While *Helicobacter* spp. are often associated with GI inflammation, the animals in this study did not display overt colonic inflammation, suggesting that the *Helicobacteraceae* sp. in these animals was not pathogenic. By comparison, the stool bacterial communities were more diverse and composed mostly of bacteria from the phyla *Firmicutes* and *Bacteroides*. This distinct compartmentalization of the mucosa and stool is in contrast to humans, which typically display similar bacterial taxa in these compartments (Stearns *et al.* 2011; Lozupone *et al.* 2013; Dillon *et al.* 2014). *Helicobacter* spp. are microaerophillic (Fox 2001), while the majority of the stool-associated bacteria, such as *Prevotellaceae* and *Ruminococcaceae*, are typically obligate anaerobes. This indicates that the mucosa possibly has a higher oxygen content than the lumen, which may be derived from oxygen flowing to host cells at the mucosal surface, as has been previously suggested (Yasuda *et al.* 2015). Indeed, oxygen gradients in the colonic mucosa have previously been linked to differential bacterial community composition when comparing mucosal and luminal communities (Albenberg *et al.* 2014; De Weirdt and Van de Wiele 2015). Such environmental factors combined with the ability of *Helicobacter* spp. to efficiently colonize intestinal mucosa (Kao, *Sheu and Wu* 2016) may help explain the high abundance of *Helicobacteraceae* in the mucosa of these animals. However, the specific factors contributing to this require further investigation.

Some of the bacterial communities detected on the rectal swabs were more similar to the colon mucosa, while others were more similar to the stool communities, and this was likely influenced by the capture of stool on the rectal swab. Rectal swabs are often used to approximate colon mucosal bacterial communities in lieu of biopsies due to the non-invasive nature of sample collection. While this approach appears to work well in humans (Budding *et al.* 2014), the differences between mucosal and luminal bacterial communities in these macaques indicates that complete clearing of stool from the rectal mucosa (e.g. by enema) is necessary to ensure that rectal swabs accurately reflect the colon mucosal bacterial communities. These data also indicate that rectal bacterial communities may truly be unique from those of the colon mucosa and stool. Thus, the

rectum may be an important factor to consider in the context of transmission of certain viruses, such as HIV, as mucosal bacterial communities have been shown to affect processes related to mucosal homeostasis, such as wound healing rates (Zevin *et al.* 2016).

The antibiotic treatment disrupted the bacterial community structure of all three compartments, and significantly decreased bacterial load in the colon and stool. Intriguingly, the composition of the colon biopsy communities from RM5 and RM6 showed no response to the antibiotics, while the stool bacterial communities from these animals showed a response similar to the other animals. However, there was a decrease in the total bacterial load in the colonic mucosa of these animals. One potential explanation for this phenomenon is that the *Helicobacteraceae* sp. residing in the colon of these animals was resistant to the antibiotics used. Vancomycin acts by inhibiting cell wall synthesis and is thus ineffective against most Gram-negative bacteria, including *Proteobacteria*. Indeed, vancomycin is often used in growth media to select for *Helicobacter* spp. (Fox *et al.* 2001). Enrofloxacin is a fluoroquinolone antibiotic that acts by inhibiting DNA synthesis, but a variety of mechanisms, such as target site modification target site blockade, or increased efflux can reduce their efficacy (Redgrave *et al.* 2014). This may suggest that the vancomycin may have had no effect on the colonic *Helicobacteraceae* spp. and that those from RM5/6 were resistant to enrofloxacin. However, this study lacked the temporal resolution to attribute the observed disruption of microbiota to one specific antibiotic. Further investigation of the dynamics of intestinal bacterial communities during combination antibiotic therapies and assessment of antibiotic resistance genes will be valuable to understand these mechanisms.

At all of the sample sites, the antibiotic treatment led to increased abundances of similar bacterial phylotypes, especially *Enterobacteriaceae* and *Lactobacillaceae*. Multidrug-resistant *Enterobacteriaceae* are a major emerging public health threat (Savard and Perl 2012) and populations of these bacteria have been shown to increase following antibiotic treatment in a variety of settings, possibly by occupying metabolic niches that were previously occupied by bacterial groups that were reduced by the antibiotics (Ng *et al.* 2013; Keeney *et al.* 2014; Lankelma *et al.* 2017). Indeed, antibiotics have previously been linked to altered bacterial respiration and redox states, and expansion of enteric bacteria through metabolism of free monosaccharides liberated by the host or microbiota (Ng *et al.* 2013; Dwyer *et al.* 2014; Lobritz *et al.* 2015; Faber *et al.* 2016). The specific changes to microbiome function associated with antibiotic treatment in these animals are the subject of ongoing metagenomic studies.

Colonic microbiota play a key role in maintaining homeostais of the mucosal immune system (Belkaid and Hand 2014), and disruption of the microbiome by antibiotic treatment may also influence immunity. Indeed, current research suggests that antibiotics can induce mucosal inflammation (Knoop *et al.* 2016, 2017). Similarly, HIV infection is associated with disruption of colon mucosal microbiota and immunity independent of antibiotics (Dillon *et al.* 2014; Somsouk *et al.* 2015). Thus, antibiotic treatments may exacerbate these symptoms and further disrupt mucosal homeostasis in HIV-infected individuals, and further investigation of the possible effects of antibiotics on mucosal immunity in HIV infection is warranted to aid in the development of improved therapeutics. Finally, these data show that antibiotic use must be minimized in studies designed to evaluate the effects of colonic and rectal bacteria in the context of SIV infection models.

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Conflicts of interest. None declared.

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