Effect of antibiotic treatment on *Oxalobacter formigenes* colonization of the gut microbiome and urinary oxalate excretion

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Abstract

Background: The incidence of kidney stones is increasing in the US population. Oxalate, a major factor for stone formation, is degraded by gut bacteria reducing its intestinal absorption. Intestinal *O. formigenes* colonization has been associated with a lower risk for recurrent kidney stones in humans. In the current study, we used a clinical trial of the eradication of *Helicobacter pylor*i to assess the effects of an antibiotic course on *O. formigenes* colonization, urine electrolytes, and the composition of the intestinal microbiome.

Methods: Of 69 healthy adult subjects recruited, 19 received antibiotics for *H. pylori* eradication, while 46 were followed as controls. Serial fecal samples were examined for *O. formigenes* presence and microbiota characteristics. Urine, collected serially fasting and following a standard meal, was tested for oxalate and electrolyte concentrations.

Results: *O. formigenes* prevalence was 50%. Colonization was significantly and persistently suppressed in antibiotic-exposed subjects but remained stable in controls. Urinary pH increased after antibiotics, but urinary oxalate did not differ between the control and treatment groups. The *O. formigenes*-positive samples had higher alpha-diversity and significantly differed in Beta-diversity from the

O. formigenes-negative samples. Specific taxa varied in abundance in relation to urinary oxalate levels.

Conclusions: These studies identified significant antibiotic effects on *O. formigenes* colonization and urinary electrolytes and showed that overall microbiome structure differed in subjects according to *O. formigenes* presence. Identifying a consortium of bacterial taxa associated with urinary oxalate may provide clues for the primary prevention of kidney stones in healthy adults.

Introduction

Nephrolithiasis (kidney stones) affect up to 9 percent of the US population[1], affecting both males and females, with incidence increasing in children and adults[2, 3]. Most stones are composed of calcium oxalate (CaOx)[4, 5]. Oxalate is absorbed in the gut from the diet and also produced endogenously as an end-product of amino acid metabolism[6, 7]. Human intestinal bacteria that degrade oxalate and contribute to its metabolism are considered the o*xalobiome*[8]. Increasing evidence suggests gut microbiota roles in nephrolithiasis pathogenesis [9–11].

Prior antibiotic use has been associated with kidney stone development months or even years later[12, 13]. Although multiple bacterial taxa are able to degrade oxalate in the gut[8, 14], *Oxalobacter formigenes* is the only commensal known to use oxalate as its sole energy and carbon source and may be the only specialist oxalate degrader in humans[15]. In rodent models, colonic colonization with *O. formigenes* significantly reduced urinary oxalate excretion[16–18]. Intestinal *O. formigenes* colonization has been associated with lower risk for recurrent kidney stones in humans[19].

Epidemiologic studies provide evidence that *O. formigenes* prevalence in developed countries such as the USA is lower than in developing countries [20, 21]. *O. formigenes* is susceptible to commonly used antibiotics [22]; an antibiotic course for *H. pylori* eradication led to loss of colonization for at least six months post-treatment [23], but post-treatment examination of urinary analytes and microbiome were not done.

In the current study, we used a study (ESSAY) of the eradication of *Helicobacter pylori* to assess the effect of an antibiotic course on *O. formigenes* colonization and on urine electrolytes, including oxalate excretion. We also asked whether microbiome structure and composition differed over time with respect to both *O. formigenes* status and antibiotic exposure.

Results

Recruitment

Of 69 eligible participants who returned for the baseline visit, 23 (33%) tested positive for *H. pylori* while 46 tested negative; 65 of the 69 participants also provided fecal samples at the baseline visit. *H. pylori*-positive participants (n = 19) who consented to the antibiotic treatment received antibiotics based on the standard-of-care. Of those, 17 completed the final study visit at 6 months. Four subjects who underwent a baseline assessment and tested positive for *H.pylori* withdrew from the study before receiving any antibiotics (Fig. 1).

O. formigenes **colonization status and stability over time.**

Based on the *O. formigenes* positivity score, the prevalence of *O. formigenes* was 50% in the 64 participants at baseline (Fig. 1,2), similar to prior findings in the USA [19, 21, 26, 29]. The *O. formigenes*-positive and negative participants were similar in distributions of age, sex, ethnicity, BMI, mode of birth-delivery, *H. pylori* status, and place of birth (**Table S2**). We evaluated *O. formigenes* colonization status stability over time in both the antibiotic-treated participants and controls. Among the controls, of the 21 *O. formigenes*-positive subjects at baseline assessed at 6 weeks, 19 (90%) remained positive, vs. 0/20 *formigenes*-negative participants at baseline becoming positive ($p < 0.001$). In contrast, in the antibiotic-treated group, of the 8 initially *O. formigenes*-positive participants, only 2 (25%) remained positive at 6 weeks vs. 19/20 (95%) who were untreated, and only three (37.5%) at 24 weeks vs. 20/24 (83.3%) in the untreated controls. All 11 of the baseline *O. formigenes*-negative group remained negative (Fig. 2A, B). Thus, treatment to eradicate *H. pylori* had a strong effect, largely eliminating *O. formigenes*, confirming prior studies [23].

O. formigenes **detection by high throughput 16S rRNA sequencing.** Our prior studies showed that *O. formigenes* abundance can vary in carriers over a range > 3-$\log_{10}$, with some individuals having minimally detectable numbers [26, 27], which intrinsically limits interpreting positivity.

Using 16S rRNA sequencing, we detected *O. formigenes* in 10 (22%) of the 45 controls at baseline, vs. 24(53%) using the above *O. formigenes* scoring system. At follow-up, we detected *O. formigenes* in six other controls by 16S analysis, but positivity fluctuated for individual subjects (Fig. 3). However, across all the control subjects, the mean % relative abundance was stable over time (0.06 ± 0.0 at baseline and 0.03 ± 0.04 at week 24). At baseline in the antibiotic-treated group, *O. formigenes* was detected in 4 (21.1%) of 19 participants; after antibiotic treatment, *O. formigenes* was significantly and persistently suppressed in all four.

Urinary Parameters according to antibiotic treatment and O. formigenes **status.**

The urinary oxalate to creatinine ratio (UOx/Cr mg/g) before and after the standard meal was measured at baseline and follow-up visits in the antibiotic-treated participants and untreated controls. In both the controls and treatment group, pre- and post-meal Uox/Cr did not change over time ($p > 0.1$) **(Suppl Fig. 2).** Urine pH levels were unchanged in the untreated controls, as expected, but increased in the treated patients in both the pre- and post-meal comparisons ($p = 0.04$ and 0.06, respectively). Urine ammonia/Cr differed in their direction of change in controls and treated subjects in pre-meal samples but after the meal increased in both control and antibiotic-treated participants. Urine citrate/Cr was stable over time in pre-meal samples from controls and in post-meal samples from both controls and treated subjects but significantly increased in the treatment group. The ratio to creatinine of urinary sodium, phosphorus, calcium, and urea nitrogen increased pre-meal in both the control and antibiotic-treated subjects, but were unchanged in post-meal comparisons over time (**Table S3**). These results indicate the effects of antibiotic exposures on urinary electrolyte excretion, pertaining particularly to acid-base homeostasis.

Microbial Community structure

Alpha-diversity is used to measure the bacterial diversity within a sample, whereas Beta-diversity provides a measure of the dissimilarity between samples. First, we asked whether the microbiome differed in the study subjects according to *O. formigenes* status. Fecal samples from the *O. formigenes*-positive subjects had higher α-diversity ($p < 0.01$) than those from *O. formigenes*-negative subjects (Fig. 3A). As expected, in the untreated controls, α-diversity remained stable, but after antibiotic treatment, decreased at 6 weeks and later returned to baseline (Fig. 4A). To assess the communities for β-diversity, we examined UniFrac distances in 173 samples from 64 participants according to *O. formigenes* baseline-status; β-diversity significantly differed in the samples based on *O. formigenes* status (Fig. 3B). Similar to α-diversity, β-diversity was stable over time in untreated controls, but changed ($p = 0.06$) immediately following antibiotic exposure, then returning to baseline (Fig. 4B). These studies provide evidence that *O. formigenes* status correlates with and clinical antibiotic regimen affects overall microbiome community characteristics.

Association between UOx/Cr and specific bacterial taxa

To assess whether any taxa other than *O. formigenes* were directly or inversely associated with UOx/Cr, we first analyzed the controls (41 participants with 108 measurements at four time points), using a centered log-ratio (CLR) transformation to standardize relative abundances of individual microbial taxa

before fitting to a linear mixed model (Table 1). The analyses showed that relative abundance of the phylum Proteobacteria was directly associated with urinary oxalate levels. Genus Dysgonomonas and species *D. gadei* were inversely associated with post-meal urinary oxalate level, adjusted for age, gender, ethnicity, BMI, and calcium level. Based on our model, in the antibiotic-treated subjects (Table 2), the clr transformed relative abundance of genera Dysgonomonas, Epulopiscium, and Providencia and species *Bacteroides plebeius* were inversely associated with pre-meal urinary oxalate level. whereas genus Lachnobacterium and species *Faecalitalea cylindroides* were directly associated. Family Barnesiellaceae and species *Streptococcus gordonii* were inversely associated with post-meal urinary oxalate levels, whereas order Turicibacterales and family Turicibacteraceae were directly associated.

Discussion

In this study, we confirmed that antibiotic treatment has a strong effect on *O. formigenes* status [23, 30]. Although most antibiotic-treated participants had durable suppression, lasting > 24 weeks, *O. formigenes* persisted in some, suggesting either heterogeneity in antibiotic resistance of *O. formigenes* strains or inability of the active agent(s) to achieve sufficient colonic concentrations. Since *O. formigenes* colonization has been associated with lower oxaluria[31], our observation of treatment effects provides one explanation for why antibiotic treatments have been associated with a higher kidney stone incidence[12, 32].

This study highlights the difficulty in assessing patients' *O. formigenes* status due to the wide range in colonization density that may reflect both diet and competing organisms. This variation confirms our prior work [26, 27], which provided the rationale for using a formula based on multiple tests to detect colonization. This biological variation is likely also in part responsible for the continued uncertainty about whether *O. formigenes* status is associated with nephrolithiasis or not. Our results may more accurately represent colonization of individual subjects than previously since prior studies used a single assessment types done cross-sectionally, which can underestimate colonization prevalence. Giving subjects an oxalate-rich diet that selects for *O. formigenes* growth may enhance detection in future cross-sectional analyses. Finding that 16S high throughput sequencing has low sensitivity for *O. formigenes* detection, due to its often low abundance, may be useful in future studies where colonization needs to be confirmed using more sensitive methods.

The standard meal used in this study did not contain a substantial oxalate load because the original trial was not designed to measure our primary outcome. Therefore, urinary oxalate changes might not reach those observed with moderate-high oxalate diets that healthy individuals typically consume. Differences in UOx/Cr (indicated by the standard deviations in **Suppl Fig. 2)** emphasize the need for larger trials with high oxalate, low calcium, and controlled diets. However, urinary pH increased after antibiotic treatment but not in controls, indicating possible effects beyond oxalate metabolism affecting acid-base homeostasis. The recent associations between increased stone risk and antibiotic courses did not include urinary chemistry data in one report[12], and in the second, the temporal relationship between antibiotic use and urine collection was not standardized [13]. Our findings provide potential mechanisms for the

linkage between prior antibiotic use and nephrolithiasis risk. Higher urinary pH is associated with increased risk for calcium phosphate stones[33].

Our community structure findings indicate that *O. formigenes* presence is a marker for a richer microbiome (consistent with prior studies [20, 26]) and its loss is a marker of important antibiotic-induced microbiome alterations. That no significant recolonization was detected by 6 months, while the overall diversity recovered, is consistent with persistent microbiome disturbance.

We identified a group of bacteria directly or inversely correlated with urinary oxalate level, consistent with the hypothesis that oxalate degradation in the gut is affected by the putative *oxalobiome* that facilitates oxalate degradation [8]. This study provides the first analysis of such associations in healthy subjects to potentially identify a microbiome associated with differential potential for stone formation. Since our findings indicate taxa with abundance associated with lower urinary oxalate levels, these could be protective against hyperoxaluria; species from genus Providencia are known to be oxalate-degraders[34]. Similarly, we identified bacteria associated with higher urinary oxalate levels whose presence could potentially increase kidney stone risk [9]. Proteobacteria were associated with higher urine oxalate in our study and were enriched in stone formers in two other studies[9, 11].

The lack of an association of *O. formigenes* colonization with lower urinary oxalate excretion could reflect its very low abundance in subjects without dietary oxalate enrichment and insensitive detection using 16S rRNA sequencing. Future studies with deeper coverage will facilitate identifying low-abundance bacterial taxa.

Our study was limited by the small sample size for the antibiotic-treated participants and lack of information about baseline diets. The standard meal did not contain oxalate, and the 3-hour post-meal urine collection did not permit study of less acute colonic bacterial actions on dietary components.

In conclusion, our study found substantial antibiotic effects on *O. formigenes* colonization, its relationship with other taxa and overall microbiome community structure, and with several urinary parameters, and identified a consortium of bacterial taxa associated with urinary oxalate levels. In the future, understanding metabolic activities of the intestinal taxa may be useful for primary kidney stone prevention.

Materials And Methods

Recruitment and subject enrollment. Participants were recruited for the ESSAY study (Eradication Study in Stable Adults/Youths) evaluating the effect of the standard-of-care practice of *H. pylori* eradication on metabolic profile and anthropometric measures of healthy adults. Participants were identified from the Bellevue Hospital primary care clinic, and community. Healthy young adults who were 18–40 years old were screened by research coordinators for eligibility criteria and then signed an informed consent if meeting those criteria. In total, 139 participants were screened for participation. Of the 87 participants who met the eligibility requirements and provided informed consent, 69 completed the baseline visit while

18 were lost to follow-up (Fig. 1). The clinical study was conducted between April 2012 and July 2016. We excluded participants with diabetes, hyper- or hypothyroidism, prior gastric or bariatric surgery, prior *H. pylori* treatment, steroid or other immunomodulatory drug use within 4 weeks of the first visit, and antibiotic use within the prior 6 months. Participants completed baseline questionnaires to provide their demographic information, medical history, and current medication use. The study was approved by the Institutional Review Board (IRB) at NYU Langone Health. All research was performed in accordance with the Declaration of Helsinki and our local IRB guidelines. Informed consent was obtained from all participants.

Determination of H. pylori **status at baseline and at follow-up and antibiotics regimen.** Subjects underwent a non-radioactive ^{13}C Urea Breath Test (Meretek Diagnostics, New York NY) to determine *H.pylori* status. Subjects (n = 23) who tested *H. pylori*-positive were offered treatment with a 14-day twice-daily regimen [amoxicillin 1000 mg, clarithromycin 500 mg, and proton pump inhibitor (PPI; omeprazole 20 mg, rabeprazole or esomeprazole 40 mg)], per the then-current standard of care [24]. In total, 19 subjects received a course of antibiotics, including three who received a second antibiotic course because they failed eradication with the first course, and four other subjects withdrew from the study before receiving antibiotics (Fig. 1). The 46 subjects who were *H.pylori* negative at baseline did not receive antibiotics and were followed serially as controls.

Study time points and assessments. Subjects fasted overnight at home and then underwent basic assessment at the NYU Clinical and Translational Science Institute (CTSI) at Bellevue Hospital at the baseline and 6, 12, and 24-week timepoints. And height and weight obtained. Stool was collected at home the day prior to the assessments using a stool collection kit, brought to NYU, and stored immediately at -80^{o}C. In total, we obtained fecal samples from 65 subjects at baseline (Fig. 1).

Test meal and urine collections. To assess the differences in urinary electrolytes before and after *H. pylori* eradication, we collected a fasting urine sample and then administered a standard 16-oz liquid meal totaling 700 calories (2 cans of Ensure Plus®) (**Table S1**). Urine was collected for three hours after the meal. In total, we collected urine samples (pre and post) from 32 controls and 14 treated subjects, (Fig. 1).

DNA isolation and PCR. DNA was extracted from fecal samples and PCR was performed as described[21].

Quantitative PCR. qPCR was performed using the LightCycler 480 SYBR Green I Master Mix, primers targeting *oxc*, in the LightCycler 480 system (Roche, Pleasanton CA). The oligonucleotide primers were: forward, 5'-GTGTTGTCGGCATTCCTATC-3' and reverse 5'-GAAGCAGTTGGTGGTTGC-3'. Melting peak analysis was performed from 65^{o}C to 95^{o}C to confirm amplicon specificity. A positive result was defined by amplification greater than 1.0E2, with melting peak between 86-87^{o}C.

16S rRNA sequencing. For amplicon library generation, the V4 region of the 16S rRNA gene was amplified with gene-specific primers, as described[21]. The 254 bp V4 region was sequenced using the Illumina

MiSeq 2 × 150 bp platform at NYU Langone Health. Operational taxonomic units (OTUs) were picked using GreenGenes 13_8 for reference, using the DADA2 pipeline [25].

Sample diversity analyses. Intra-sample α-diversity was calculated using QIIME2, using phylogenetic diversity, observed OTU number, Chao1, and Shannon indices at rarefaction depths of 8,000 sequences/sample. Beta-diversity was assessed using the unweighted UniFrac distance metric [26].

Oxalobacter formigenes **status determination.** We tested each fecal sample for the presence of *O. formigenes* using PCR in duplicate, qPCR, and 16S rRNA sequencing. Based on the known several log-fold biological variations in *O. formigenes* abundance in human fecal specimens[26, 27], given the ascertainment variability using different methods, we developed a positivity score based on the 42 control subjects who did not receive antibiotics and who were tested at baseline and at least once in follow-up. The maximum number of assessments for each test subject was 16 if the participant was tested at all 4 time points (baseline, weeks 6, 12, 24) in all four assays; and a minimum of 8 if only tested at two time points. For the 42 control participants, the mean ± SD number of determinations was 14.7 ± 2.6. We computed the positivity score by dividing the number of positive assessments by the total number of assessments for that subject. Since the results were bimodal, as expected (**Suppl. Figure 1**) we assigned baseline *O. formigenes* status as negative with score ≤ 0.2 and positive with score ≥ 0.4. Only one subject had a score of 0.3, which we considered indeterminate and we removed this participant from further analysis (**Suppl. Figure 1**).

Urine testing. Urine aliquots were mixed with either HCl (for oxalate measurements) or with thymol (for all other assessments) and then stored at -80^{o}C. Pre-meal (fasting) and the 3-hour post-meal urinary samples were analyzed by Litholink Corporation (Chicago IL). In each urine sample, we measured calcium, chloride, creatinine, magnesium, sodium, potassium, phosphate, and ammonium concentrations by standard techniques by Beckman Synchron AU680 (Beckman Instruments, Brea CA), as described [28]; pH was measured by glass electrode. Oxalate was measured by enzyme assay using oxalate oxidase (Trinity Biotech, Bray, Ireland). Citrate was measured by enzyme assay using citrate lyase (Mannheim Bohringer, Mannheim, Germany). All urine parameters except pH were normalized by dividing by creatinine concentration to account for hydration status.

Longitudinal analysis on urine parameters. We compared urine parameters and their changing rates according to the subjects' baseline treatment status (control, treatment group) and *O. formigenes* colonization status during the period of 0–24 weeks. A linear mixed model was fitted to data over that time interval in which the changing rates in urine parameters were compared. The covariates age, sex, and body mass index (BMI) were adjusted in the model.

Association between Uox/Cr and bacterial taxa. We used the linear mixed effect model to identify which microbial taxa were associated with pre/post-meal urinary oxalate level, adjusted for age, gender, ethnicity, BMI, and calcium level. We included data for the participants in (i) the control group, we analyzed a total of 108 pairs of microbial measurements and urinary oxalate level (pre-/post- dietary) at

baseline, 6, 12 and 24 weeks; and (ii), in the antibiotic-treatment group, we analyzed a total of 51 pairs of microbial measurements and urinary oxalate level (pre-/post- dietary) at 6, 12, and 24 weeks. In the mixed effects model, intercept and slope of the linear time trend for each subject are regarded as random effects. ANOVA results indicate that no correlation exists between random intercept and slope. We used the pre- and post-meal urinary oxalate levels as a dependent variable, respectively, while the relative abundance of individual microbial taxa after centered log-ratio (CLR) transformation as the independent variable. To calculate adjusted p-values, the Benjamini-Hochberg procedure was applied for each taxonomic level. Unclassified microbial taxa were not included in the analyses.

Declarations

Author contributions:

LN wrote the main manuscript text and prepared the figures. FF, MJB, DSG, GPP, and LN designed the study. LN and NH performed the study. LN, ML, HL, HK, CW, and ZG performed the analysis. JRA tested the urine samples. All authors reviewed the manuscript.

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Tables

Table 1. Microbial taxa associated with pre/post-dietary urinary oxalate levels in untreated controls*

Microbial Taxon	Pre-meal urinary oxalate level				Post-meal urinary oxalate level			
	Coef. Est.	Std. Error	Unadj. p	Adj. p	Coef. Est.	Std. Error	Unadj. p	Adj. p
Phylum Proteobacteria	0.02	0.04	0.01	**0.05**	0.01	0.01	0.54	0.94
Genus Dysgonomonas	-0.04	0.05	0.51	0.94	-0.04	0.01	<0.01	**<0.01**
Dysgonomonas gadei	-0.02	0.05	0.67	0.96	-0.05	0.01	<0.01	**<0.01**

*The estimated regression coefficients, standard errors, and unadjusted/adjusted p-values for the effect of the relative abundance of individual microbial taxa with centered log-ratio transformation in fitted mixed effect models. Analysis adjusted for age, gender, ethnicity, BMI, and calcium level.

Table 2. Microbial taxa associated with pre/post-dietary urinary oxalate level in antibiotic-treated subjects*

Microbial Taxon	Pre-meal urinary oxalate level				Post-meal urinary oxalate level			
	Coef. Est.	Std. Error	Unadj. p	Adj. p	Coef. Est.	Std. Error	Unadj. p	Adj. p
Order Turicibacterales	0.05	0.02	0.05	0.31	0.02	0.01	<0.01	**0.01**
Family Barnesiellaceae	-0.10	0.04	0.04	0.50	-0.04	0.01	<0.01	**0.01**
Family Turicibacteraceae	0.04	0.02	0.08	0.60	0.02	0.01	<0.01	**0.01**
Genus Dysgonomonas	-0.40	0.11	<0.01	**0.02**	-0.04	0.02	0.03	0.25
Genus Epulopiscium	-0.43	0.12	<0.01	**0.02**	-0.05	0.02	0.03	0.25
Genus Lachnobacterium	0.08	0.01	<0.01	**0.02**	0.01	<0.01	0.03	0.25
Genus Providencia	-0.43	0.12	<0.01	**0.02**	-0.05	0.02	0.02	0.25
Bacteroides plebeius	-0.12	0.03	<0.01	**0.03**	-0.01	<0.01	0.21	0.72
Streptococcus gordonii	-0.07	0.06	0.22	0.78	-0.04	0.01	<0.01	**0.05**
Faecalitalea cylindroides	0.24	0.05	<0.01	**<0.01**	0.02	0.02	0.33	0.77

Figures

Fig.1

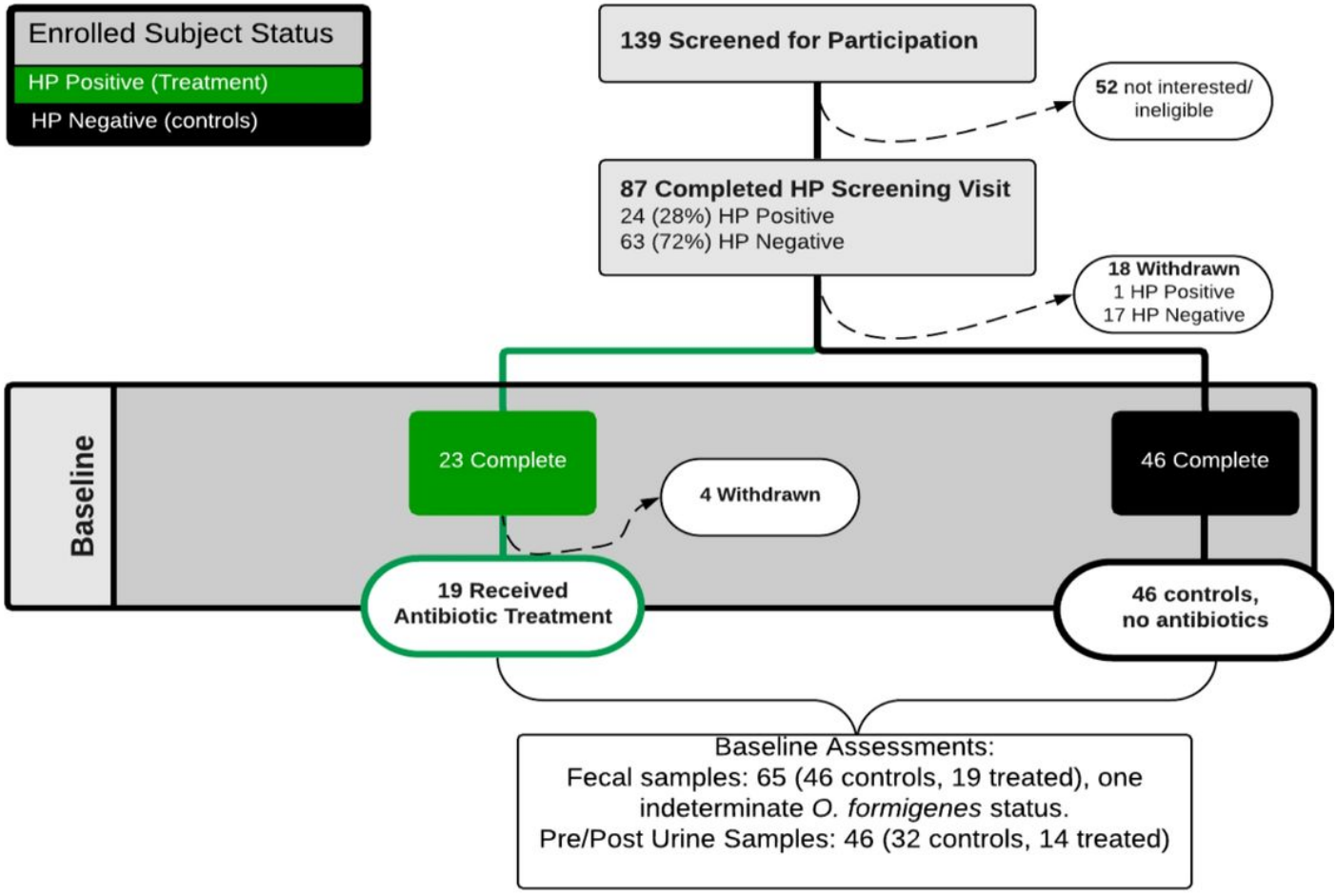


Figure 1

Study design and enrollment. Of 139 subjects screened for participation, 87 completed the screening, and 69 subjects underwent baseline assessment. 19 H.pylori-positive subjects were treated with antibiotics ,whereas 46 H.pylori-negative subjects served as controls.

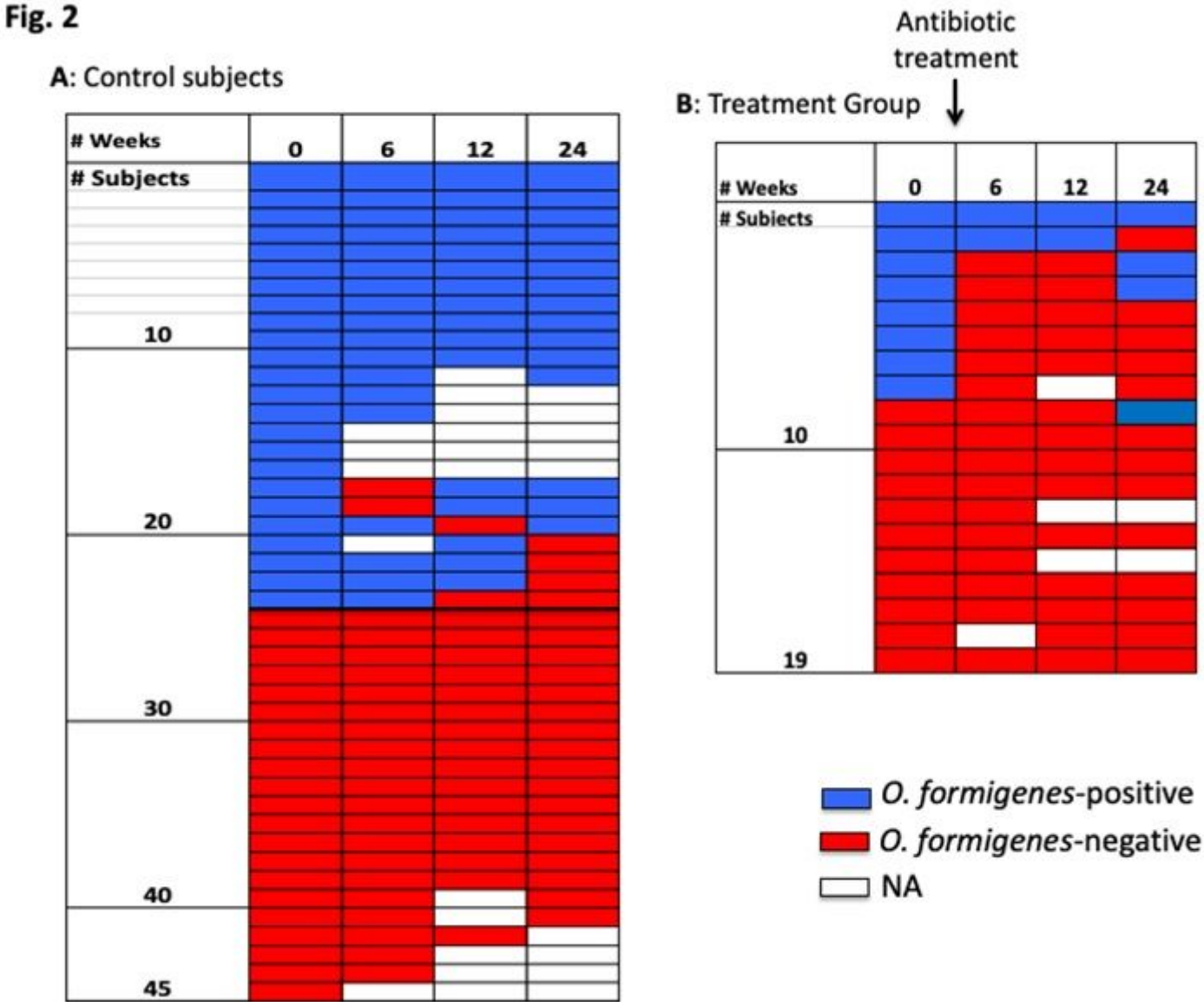


Figure 2

O. formigenes colonization status in 45 control subjects and 19 antibiotic-treated subjects over the study period. O. formigenes-positive samples are shown in blue, negative in red, and missing in white. Panel A: Untreated control subjects. Panel B: Antibiotic-treated subjects.

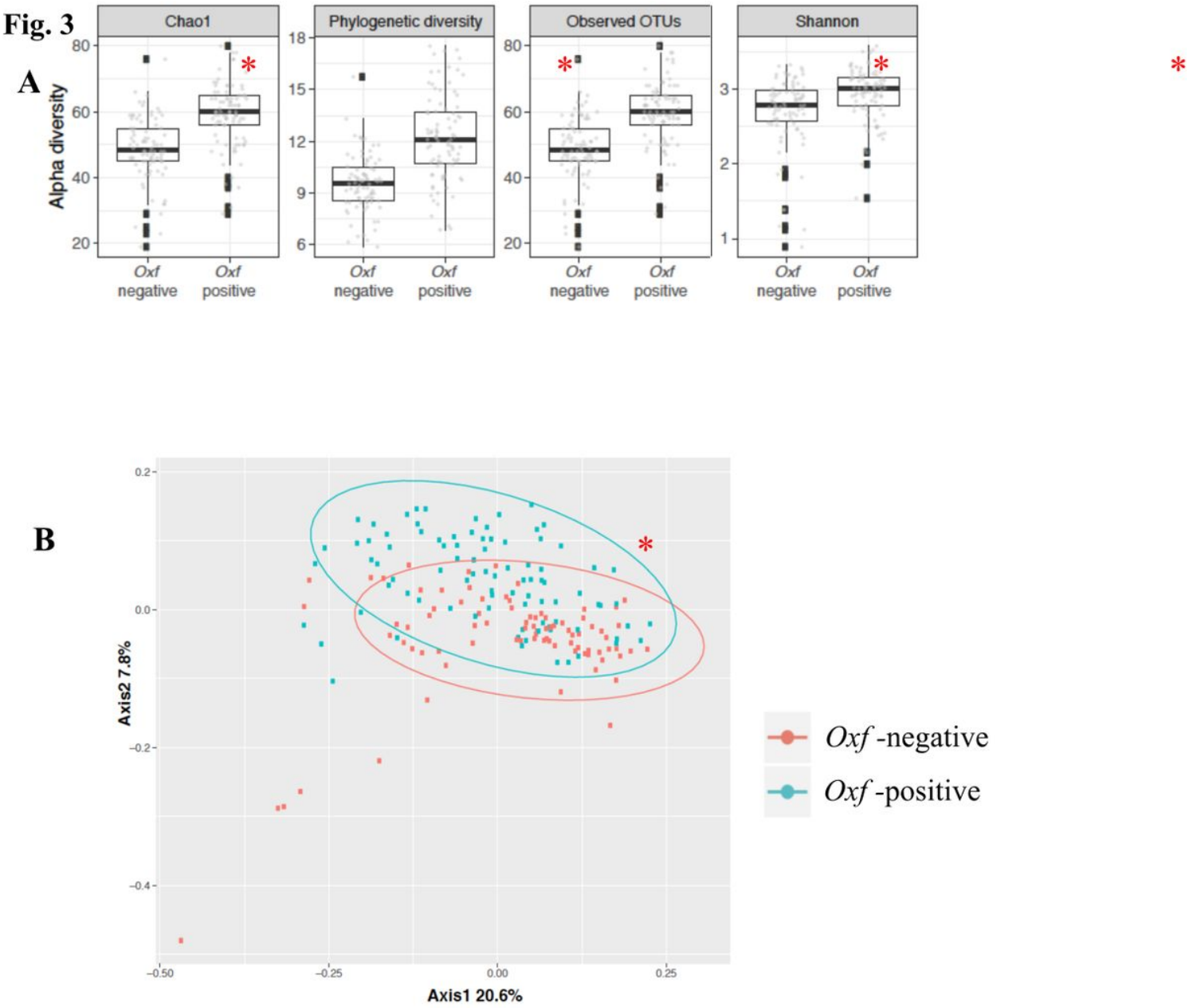
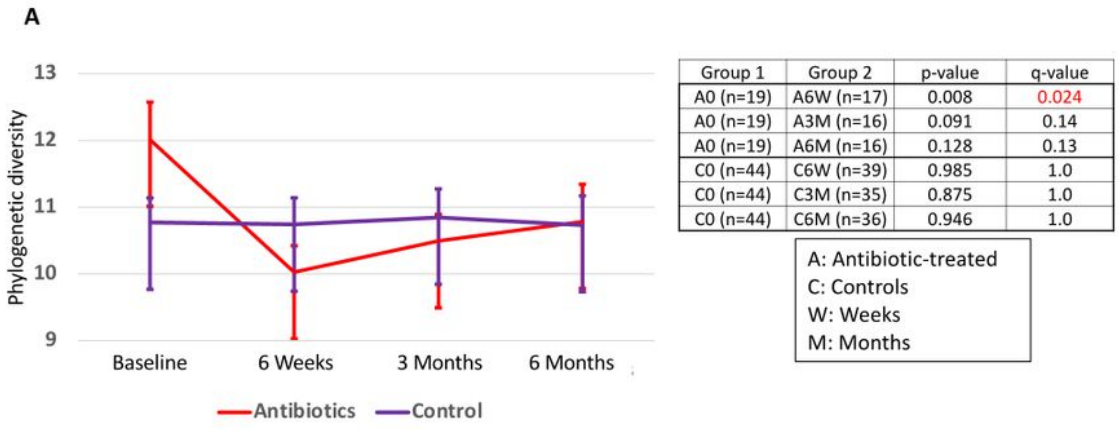


Figure 3

Alpha- and beta-diversity of microbiota in 173 fecal samples from 64 study subjects, according to baseline O. formigenes status. Panel A: Alpha-diversity measurements. Chao1, phylogenetic diversity, observed OTUs, and Shannon diversity according to Oxf status at baseline. Panel B: Analysis of Beta-diversity, Unweighted UniFrac distances, according to O. formigenes status. * p<0.05

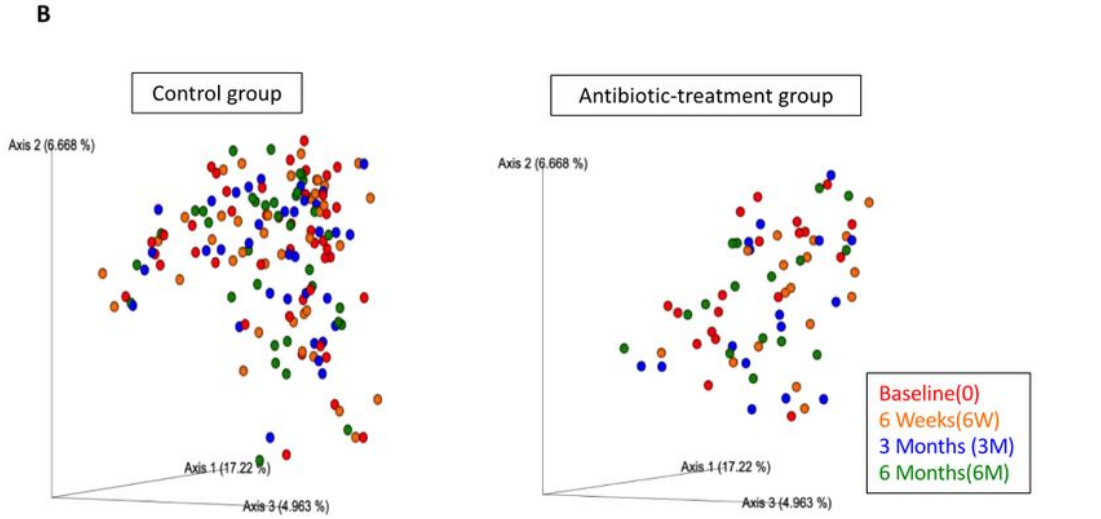
Fig. 4



Group 1	Group 2	p-value	q-value
A0 (n=19)	A6W (n=17)	0.008	0.024
A0 (n=19)	A3M (n=16)	0.091	0.14
A0 (n=19)	A6M (n=16)	0.128	0.13
C0 (n=44)	C6W (n=39)	0.985	1.0
C0 (n=44)	C3M (n=35)	0.875	1.0
C0 (n=44)	C6M (n=36)	0.946	1.0

A: Antibiotic-treated
C: Controls
W: Weeks
M: Months

Fig. 4



Group 1	Group 2	Sample size	# Perm	p-value
C0	C6W	83	999	1
C0	C3M	79	999	1
C0	C6M	80	999	0.999

Group 1	Group 2	Sample size	# Perm	p-value
A0	A6W	36	999	0.06
A0	A3M	35	999	0.12
A0	A6M	35	999	0.54

Figure 4

Alpha- and beta-diversity of microbiota in controls and antibiotic-treated subjects over time. Panel A: Phylogenetic diversity over time in the two groups of subjects; Kruskal Wallace analysis. Panel B: Unweighted UniFrac distances over time.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplmaterial123020.docx