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## Influence of Acid Blockade on the Aerodigestive Tract Microbiome in Children With Cystic Fibrosis

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### Abstract

**Background:** Acid blockade is commonly prescribed in patients with cystic fibrosis (CF). Growing concerns, however, exist about its possible role in the pathophysiology of pulmonary infections. We aimed to investigate if acid blockade alters esophageal and respiratory microbiota leading to dysbiosis and inflammation.

**Methods:** We performed a cross sectional study of children with CF who were either prescribed acid blockade or not. Samples from the gastrointestinal and respiratory tracts were obtained and microbiome analyzed. Mixed effect models were used to compare outcomes between cohorts and across sampling sites. A random subject intercept was included to account for the multiple sampling sites per individual.

**Results:** A cohort of 25 individuals, 44% girls with median age of 13.8 years [IQR 11.2–14.8] were enrolled. Alpha diversity, total bacterial load, and beta diversity were similar across anatomic

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compartments, across the upper gastrointestinal tract, and in respiratory samples. Similar alpha diversity, total bacterial load, and beta diversity results were also observed when comparing individuals on versus those off acid blockade. IL-8 was elevated in the distal versus proximal esophagus in the whole cohort ( $P<0.01$ ). IL-8 concentrations were similar in the distal esophagus in patients on and off acid blockade, but significantly greater in the proximal esophagus of subjects on treatment ( $P<0.01$ ).

**Conclusions:** On the basis of these data, acid blockade use does not appear to influence the microbiome of the aerodigestive tract in children with cystic fibrosis suggesting a complex interplay between these medications and the bacterial composition of the esophagus and lung.

### Keywords

acid blockade; cystic fibrosis; inflammation; microbiome

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The close anatomical juxtaposition shared by the lungs and esophagus has led to increasing interest in whether diseases of the esophagus, such as gastroesophageal reflux disease (GERD) may contribute to problems in the respiratory tract. This is particularly true for patients with cystic fibrosis (CF) who are prone to develop both pulmonary infections as well as GERD. In fact, clinical studies of patients with CF identify the association between GERD and pulmonary disease but the mechanisms related to these observations are not clear (1–3). Complicating this issue is the fact that CF patients are also treated with proton pump inhibitors (PPI), medications that are associated with increased gastrointestinal as well as pulmonary infections (4,5).

As chronic pulmonary infections in patients with CF represent a major source of morbidity and mortality (6–8), further studies that define their underlying mechanisms as well as those that will determine novel therapeutic approaches are essential. A number of studies indicate that acid blockade, specifically PPI use, poses increased risk of community-acquired pneumonias and pulmonary exacerbations in adult and pediatric CF (4,5,8–11). As gastric acid reduces the microbial load in the stomach, increase in gastric pH can change the bacterial populations and load. Changes in these gastric patterns may alter the esophageal microbiome, and thus contribute to pulmonary disease. No study, however, to our knowledge, has determined the esophageal microbiome in patients with CF, nor the impact of acid blockade on the aerodigestive microbiome.

To begin to address these issues, we aimed to determine if acid blockade use in patients with CF was associated with dysbiosis and alterations in inflammation in the esophageal and the respiratory sites. We hypothesized that acid blockade medications are associated with alterations of esophageal and respiratory microbiota via microaspiration, allowing proliferation of pathogenic bacteria and increased inflammation. To test this hypothesis, we performed a prospective study utilizing a novel minimally invasive technique, the esophageal string test (EST) (12), to capture the esophageal microbiome in children with CF who were treated or not with acid blockade.

## METHODS

### Patient Selection

We performed a prospective study in children between the ages of 10 and 21 years with a known diagnosis of CF at Children's Hospital Colorado (CHCO) from November 2017 to February 2020. Individuals cared for at the CHCO CF Center were approached for enrollment if they had: a diagnosis of CF based on sweat chloride greater than 60 mEq/L or 2 known cystic fibrosis transmembrane conductance regulator mutations; clinically stable pulmonary disease defined by clinical impression of the primary CF provider, no newly prescribed antibiotic treatments in the 30 days before enrollment, and stable lung function (forced expiratory volume in 1 second [FEV1] percentage predicted within 10% of baseline and greater than 40%); either been treated with a PPI or histamine 2 receptor antagonist (H2RA) for a minimum of 6 weeks or not been treated with a PPI or H2RA for at least 6 weeks; and been able to swallow a capsule. Exclusion criteria included: a history of meconium ileus, distal intestinal obstructive syndrome, any gastrointestinal surgery, intestinal stricture; a history of CF liver disease with cirrhosis, gastric or esophageal varices; and starting a new course of antibiotics, antifungals or antivirals within 30 days of their visit. The initial cross over study design that sought to enroll patients on and off acid blockade was changed to a cross sectional design because of enrollment concerns.

This study was approved by the Colorado Multiple Institutional Review Board (COMIRB), Aurora, CO. Written informed consent and HIPAA authorization were obtained for all enrolled individuals. Demographic data was collected along with clinical outcomes, including lung function and growth parameters, comorbidities, and concomitant medications.

### Esophageal String Test

At the time of the research visit, individuals swallowed the EST as previously described (12,13). Briefly, individuals fasted for 1 hour prior and swallowed the EST that was then left in place for 1 hour. Following removal, the string was divided using a combination of pH indicator testing and using standardized measurements as previously described (14). Segments (2 cm) including oral, proximal and distal esophagus, gastric and duodenal strings were collected and either flash frozen dried or placed immediately in 0.5 mL PBS buffer containing the protease inhibitors ethylenediaminetetraacetic acid (EDTA) and phenylmethylsulfonyl fluoride (PMSF) before freezing. Samples collected were kept at  $-80^{\circ}\text{C}$  until DNA extraction or inflammatory marker testing was performed.

### Spirometry and Airway Samples

Individuals performed spirometry according to the American Thoracic Society guidelines (15). Either expectorated or induced sputum samples were collected as previously described (16). If a sputum sample was not obtained (unable to expectorate, sputum induction failed), an oropharyngeal swab was collected from the posterior oropharyngeal wall and tonsillar pillars. Samples were snap frozen and stored at  $-80^{\circ}\text{C}$  until DNA extraction was performed.

**Cytokine Analysis**—IL-8 measurement from the EST was determined by ELISA (Luminex Multiplex beads R & D system, Minneapolis, Minnesota, USA) according to manufacturer's instructions.

### Microbiota Identification

**DNA Extraction and Bacterial Load Assessment**—DNA was prepared using the Qiagen EZ1 extraction platform using the Tissue Extraction Kit and bacterial DNA card after enzymatic digestion (17,18). DNA was used to determine the bacterial load present using the quantitative PCR (qPCR) assay described by Nadkarni et al (19), and assessed for CF samples (20). The qPCR assay is a TaqMan design that targets highly conserved regions (~357F/805R) for amplification and uses a probe sequence near the highly conserved 515F region of the *16S rRNA* gene.

**16S Amplicon Library Construction**—Bacterial profiles were determined by broad-range amplification and sequence analysis of *16S rRNA* genes following our previously described methods (21–23). In brief, amplicons were generated using primers that target approximately 300 base pairs of the V1V2 variable region of the *16S rRNA* gene. The V1V2 region was selected based on performance in taxonomic evaluations relative to full-length sequences (24,25). PCR products were normalized by agarose gel densitometry, pooled in approximately equimolar amounts, gel-purified, and concentrated using a DNA Clean and Concentrator Kit (Zymo, Irvine, CA). Pooled amplicons were quantified using Qubit Fluorometer 2.0 (Invitrogen, Carlsbad, CA). The pool was diluted to 4 nmol/L and denatured with 0.2 N NaOH at room temperature. The denatured DNA was diluted to 20 pmol/L and spiked with 10% of the Illumina PhiX control DNA before loading the sequencer. Illumina paired-end sequencing was performed on the Miseq using a 500 cycle version 2 reagent kit.

**Analysis of Illumina Paired-end Reads**—Illumina MiSeq paired-end reads were aligned to human reference genome Hg19 with bowtie2 and matching sequences discarded (26,27). As previously described, the remaining nonhuman paired-end sequences were sorted by sample via barcodes in the paired reads with a python script (23). Sorted paired end sequence data were deposited in the NCBI Short Read Archive under accession number PRJNA629006. The sorted paired reads were assembled using phrap (28,29). Pairs that did not assemble were discarded. Assembled sequence ends were trimmed over a moving window of 5 nucleotides until average quality met or exceeded 20. Trimmed sequences with more than 1 ambiguity or shorter than 200 nt were discarded. Potential chimeras identified with Uchime (usearch6.0.203\_i86linux32) (30) using the Schloss (31) Silva reference sequences were removed from subsequent analyses. Assembled sequences were aligned and classified with SINA (1.3.0-r23838) (32) using the 418,497 bacterial sequences in Silva 115NR99 (33) as reference configured to yield the Silva taxonomy. Sequences with identical taxonomic assignments were clustered to produce operational taxonomic units (OTUs). This process generated 9,499,069 sequences for 176 samples (average sequence length: 316 nt; average sample size: 53,972 sequences/sample; minimum sample size: 7624; maximum sample size: 145,522). The median Goods coverage score was 99% at the rarefaction point of 7624. The software package Explicitet (v2.10.5, [www.explicitet.org](http://www.explicitet.org)) (34) was used for display, analysis, and visualization of sequencing results. Species calls are based on pre-

computed differentiation criteria for each genera of interest based on genomic reference sequences in Silva using the primer-specific region. Species classification is computed within each genus to make species assignments, and any sequence that is not assigned to a species remains classified to the genus level.

### Statistical Analyses

Descriptive data are presented as medians [interquartile range] or percentages. Linear mixed models were performed to evaluate changes in total bacterial load, alpha diversity and IL-8 (log base 10 transformed). An interaction between the binary acid blockade variable and string location was included to fit a means model. The model also included a random subject intercept and the comparisons between all the sites and treatment within sites were adjusted for multiple comparisons using the Šidák adjustment. The Morisita-Horn beta diversity was used as a distance metric in a multidimensional scaling (MDS) plot to visualize the distance between samples. All analyses were performed using SAS version 9.4 (The SAS Institute, Cary, NC).

## RESULTS

### Subject Characteristics

Initial screening identified 102 patients who met entry criteria for the study. Of these, 25 individuals completed all study-related components and were included in the final analysis. (Figure S1, <http://links.lww.com/MPG/C88>). The cohort was 44% girls with a median age of 13.8 years [IQR 11.2–14.8]. Further demographic and clinical characteristics at the time of the baseline research visit are summarized in Table 1. As a part of the initial crossover design, 2 individuals had samples collected at 3 time points, 2 while on acid blockade, and once off acid blockade. These 2 individuals were included in the acid blockade group for the purposes of describing the cohort.

A total of 176 samples were collected from 25 individuals. Twenty-six oral samples, 29 from each the proximal and the distal esophagus, 28 gastric samples, and 14 duodenal samples were included in the final analysis of the gastrointestinal tract. Otherwise, 27 samples from the nares, 13 oropharyngeal samples, and 10 sputum samples were available from the airway. IL-8 concentration was measured in 50 proximal and distal esophageal EST samples from 21 individuals. For individuals on acid blockade, 8 had appropriate acid suppression exhibited by a basic pH in the stomach, whereas 3 did not have pH change. Nine individuals were on chronic antibiotics; sensitivity analysis with these individuals excluded did not alter results displayed below.

### Impact of Acid Blockade on Microbial Load and Diversity

The average total bacterial load per site regardless of acid blockade treatment is displayed in Figure 1A. The nares have statistically lower bacterial load than all other sites ( $P < 0.01$  for all pairwise comparisons). No differences were found in the average total bacterial load at any site when comparing individuals who were treated with acid blockade compared with those who were not (Fig. 1B, Table S1, <http://links.lww.com/MPG/C92>). Notably, the

proximal and distal esophageal portions of the string had comparable bacterial load to oropharyngeal and sputum samples in both cohorts.

The Shannon alpha diversity index per site of all individuals regardless of treatment is displayed in Figure 1C. The nares had a statistically lower diversity than all other sites ( $P < 0.01$  for all pairwise comparisons). When comparing samples from individuals on acid blockade treatment versus those off acid blockade, Shannon alpha diversity was not different for any site (Fig. 1D, Table S1, <http://links.lww.com/MPG/C92>). Specifically, Shannon diversity index was similar between those on versus those off acid blockade treatment with regards to respiratory microbiome sampled via the oropharyngeal swabs and sputum samples.

### Comparison of Microbial Taxa in Aerodigestive Compartments

The individual esophageal and respiratory taxa were also compared across groups (Fig. 2). Specifically, relative abundance of taxa is visualized in oropharyngeal, sputum, proximal, and distal esophageal samples according to treatment. The aggregate compilation of data from all individuals in each group were analyzed and also showed virtually identical patterns of relative abundance in the predominant taxa present in these compartments.

MDS using Morisita-Horn beta diversity for all samples showed that only the nares had a community distinct from other sites (Fig. 3). The gastrointestinal segments and the respiratory specimens all had communities that shared similar taxa. MDS was repeated after excluding the nares samples to assess if the large difference observed for nares was influencing relationships within the more similar airway and esophageal communities. The results, however did not change (Figure S2, <http://links.lww.com/MPG/C89>). Further comparison of sputum composition to oral, proximal, and distal string was done for 10 individuals with sputum samples. The agreement appears to be similar between sputum composition and the different string locations (Figure S3, <http://links.lww.com/MPG/C90>). In the 3 individuals with decreased overlap (individuals 4, 6, 22) the low agreement between locations is largely because of the prominence of *Staphylococcus aureus* in the sputum sample, which was absent from the string samples (Figure S4, <http://links.lww.com/MPG/C91>).

### Influence of Acid Blockade on Esophageal Inflammatory Marker

IL-8 concentration in the distal esophagus was significantly greater than the proximal esophagus in all individuals included in the cohort ( $N = 25$ ) (Fig. 4A). Average log IL-8 protein concentration was 3.34 pg/mL (SE = 0.08 pg/mL) in the distal esophagus and 3.04 pg/mL (SE = 0.08pg/mL) in the proximal esophagus ( $P < 0.01$ ). For individuals on acid blockade treatment, no differences were measured in IL-8 concentrations between the proximal and distal esophageal locations ( $P = 0.24$ ) but a difference was observed between those off acid blockade ( $P < 0.01$ ) (Fig. 4B). The IL-8 concentration was greater within the proximal esophagus in those subjects on acid blockade treatments compared with those off acid blockade (3.19 pg/mL, SE = 0.10 vs 2.89 pg/mL, SE = 0.10 pg/mL,  $P = 0.03$ ) (Fig. 4B). Within the distal esophagus, IL-8 concentrations were, however, similar across groups (3.32 pg/mL, SE = 0.10 vs 3.36 pg/mL, SE = 0.10 pg/mL) ( $P = 0.76$ ).

## DISCUSSION

As previous studies provide an association of PPI use with increases in pulmonary comorbidity in patients with (10,11) and without CF (9), our study provides interesting but unexpected findings. First, acid blockade does not appear to be associated with alterations in the microbiome in any site examined. Comparisons of samples from the nasal, oropharyngeal, pulmonary, esophageal, gastric, and duodenal sites obtained from children treated with acid blockade or not, revealed no differences in bacterial load, alpha diversity, or microbial composition. Second, there appears to be a high degree of similarity between the bacterial load and abundance across the sites examined except for the nares. Third, patients with CF appear to have a baseline level of inflammation present that is not affected by the use of acid-blocking medications. Taken together, our results indicate that the aerodigestive tract in patients with CF contains a highly complex microbiome that is not influenced by the use of acid-blocking medications.

Previous work has shown that esophageal microbiome differences can be detected between states of health and disease. Our work has shown that the microbiome detected using the EST is consistent with the mucosal microbiome obtained from esophageal biopsies and distinct from oral and nasal microbiomes (12). Specifically, in pediatric and adult cohorts of normal individuals compared with those with GERD or eosinophilic esophagitis, our earlier work using the EST showed that the esophageal bacterial load was increased in both disease states (13), and that individuals with GERD on a PPI have decreased levels of *Streptococcus* in the esophagus when compared with normal controls, a finding consistent with others (35). Rosen et al (36) investigated the effect of PPI medications on gastric, lung, and oropharyngeal microbiota of pediatric patients and showed that there were dose-dependent changes in the microflora in PPI-treated children, and evidence of microflora exchange between the respiratory and the gastrointestinal tract that is independent of the oropharyngeal microflora. Goetz et al (8) demonstrated an increase in the incidence of *Pseudomonas* in respiratory cultures in infants with CF on PPI and H2RA. In the present study, we anticipated that acid blockade would similarly be associated with alterations of the esophageal microbiota by increasing the pH of refluxed gastric contents of patients with CF leading to esophageal dysbiosis and secondary pulmonary dysbiosis that is distinct from documented alterations to both gut and airway microbiomes in patients with CF (16,22,37). The bacterial load, and community composition were, however, similar across cohorts regardless of treatment, and similar across anatomic compartments. These findings are consistent with prior work by Al-Mormani et al (38), which showed similar bacterial profiles of CF sputum and gastric juice samples in adult patients with CF who had gastrostomy tubes suggesting the presence of a distinct aerodigestive microbiome in individuals with CF. This study, however, did not investigate the effect of acid blockade on the microbiome in patients with CF. Our work indicates that perhaps the thick mucosal secretions that are the hallmark of CF disease, and the abundance of airway mucus that may be swallowed into the upper gastrointestinal tract may be the dominant source of microbiota in the aerodigestive tract. Perhaps the thickness of the secretions, whether from the airway or the upper intestinal tract, blunts the anticipated impact of acid blockade on the aerodigestive microbiome and inflammation as anticipated.

As IL-8 is increased in CF lung infections (37) and in esophagitis (39–42), we used measurements of this cytokine as an inflammatory biomarker. In our previous work, we have shown concordant measurements from the string and concurrently collected biopsy for both microbiome and protein biomarkers in healthy controls (12). In this study, esophageal IL-8 concentrations were elevated in all sections examined, an unanticipated finding in those on and off acid blockade treatment. In a study of adults with GERD compared with healthy normals, average esophageal IL-8 levels obtained from biopsy specimens was  $0.32 \pm 0.09$  pg/mg (equivalent log IL-8 was  $-0.49 \pm -1.04$  pg/mg) in healthy individuals, which was significantly ( $P < 0.01$ ) lower than that the average IL-8 of  $23.44 \pm 2.14$  pg/mg in individuals with reflux esophagitis (equivalent log IL-8 was  $1.37 \pm 0.33$  pg/mg) (43). This study further showed that treatment with a PPI for 8 weeks, specifically lansoprazole, significantly decreased IL-8 concentrations in patients with GERD ( $P < 0.01$ ). In another study by Huo et al (40), omeprazole, another PPI, inhibited IL-8 expression in esophageal epithelial cells exposed to acid bile salt independent of effects on gastric acid secretion. Our results, however, suggest that the protective effect of acid blockade on esophageal inflammation is blunted in patients with CF, especially with significantly increased proximal esophageal IL-8 concentrations in those on acid blockade versus those off treatment. Furthermore, when comparing esophageal IL-8 concentrations in our cohort to previously reported sputum log IL-8 concentrations in individuals with CF at times of health (4.7–4.8 pg/mL) (44), our results indicate that the level of inflammation albeit elevated is less than levels reported in the respiratory tract. Strings were, however, eluted in buffer, which adds an additional dilution factor that cannot be measured directly. Our data could suggest that inflammation of the lungs in CF may beget inflammation of the upper intestinal tract and vice versa, perhaps secondary to the cross-talk between the gut microbiota and the lungs, known as the gut-lung axis. Both these compartments share a mucosal immune system, which is only now being realized. Prior research has shown that when inflammation in the lung exists, the lung-gut axis can induce changes in the circulating biomarkers and gut microbiota that can be bidirectional and secondary to the effects of the pulmonary inflammation propagated by lymphocyte migration, and systemic inflammatory mediators (45). Esophageal inflammation may also be secondary to swallowed airway secretions by typical clearance, which subsequently alters the upper intestinal tract microbiota and results in not only similar microbiomes across sites despite acid blockade treatment but also higher levels of inflammation in the gastrointestinal tract. In addition, previous research has shown that pediatric patients with CF have significant gut inflammation measured via stool calprotectin, the source of which remains ambiguous (46). It is possible that this inflammation extends to the proximal intestinal tract. Further studies are required to delineate if the source of inflammation is gastrointestinal or pulmonary and to determine if the differences in IL-8 concentrations seen are a result of different sampling techniques as opposed to true differences in inflammation.

Limitations to our study include the fact that it is a single center study, and thus the findings may not be generalizable to other locations. Individuals included in this study were pediatric patients with stable lung disease, results of similar comparisons may be different among adults or individuals with a worse lung function. The EST samples mucosal surfaces, which may vary from luminal contents (eg, gastric compartment), and could partially explain the



high degree of similarity observed across the upper gastrointestinal tract. We did not control for other possible mediators of microbial alterations including concomitant antibiotic use, prior intestinal surgery, underlying CFTR mutations, and use of probiotics. Also, as this is a cross-sectional study, we are unable to rule out the chance that there are within-person changes that occur associated with acid blockade use. Unfortunately, we were unable to complete the originally conceived prospective study, which would have helped delineate within-person changes. Furthermore, although we did inquire about medication adherence, it is possible that individuals may not have been taking acid blockade medications as reported. Future studies can explore whether the indication for acid blockade use, such as absorption or reflux disease affects the microbial communities. Additionally, although sputum and oropharyngeal swabs may offer some insight into the microbial communities in the lungs, a bronchoalveolar lavage (BAL) remains the gold standard for evaluating the respiratory microbiome but because of the invasive nature of this procedure, we were unable to obtain samples via a BAL. Lastly, although all of our specimens had identical handling, comparable yield of the nasal swab versus the EST may be different because of swab or EST volume and efficiency of sample collection.

The relationship between the lung and esophagus is of pathophysiological interest. In particular, the impact of acid blockade therapy, including PPI and H2RA, on respiratory and esophageal microbiota and respiratory inflammation in the especially vulnerable CF population has not been studied. Clinically, PPIs may benefit CF patients with GERD or who those need them to augment digestive enzyme actions. These initial results in small numbers of patients suggest that whenever indicated, the use of acid-reducing medications do not appear to be associated with alterations of the microbiota, thus reducing concerns about infectious consequences. The cross talk between the aerodigestive tract or the thick mucosal secretions of individuals with CF and the ongoing swallowing of airway mucus may account for this lack of change among groups and for the high bacterial load in the esophagus. Further work is needed to delineate the source of the esophageal inflammation, to determine whether it is indeed airway secretions being swallowed that leads to elevated bacterial loads and whether these findings alter the clinical outcome of pulmonary disease in CF overall.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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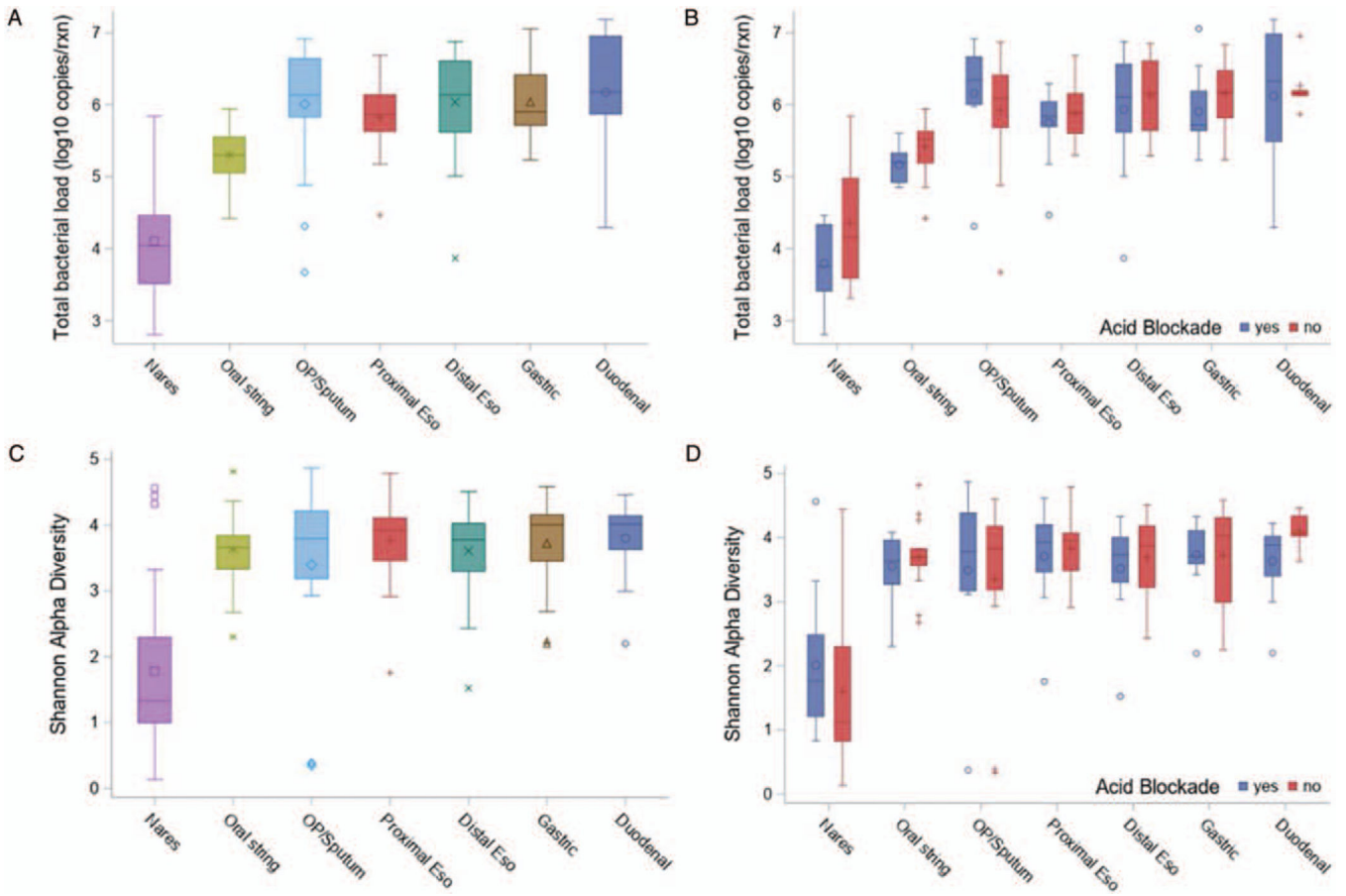
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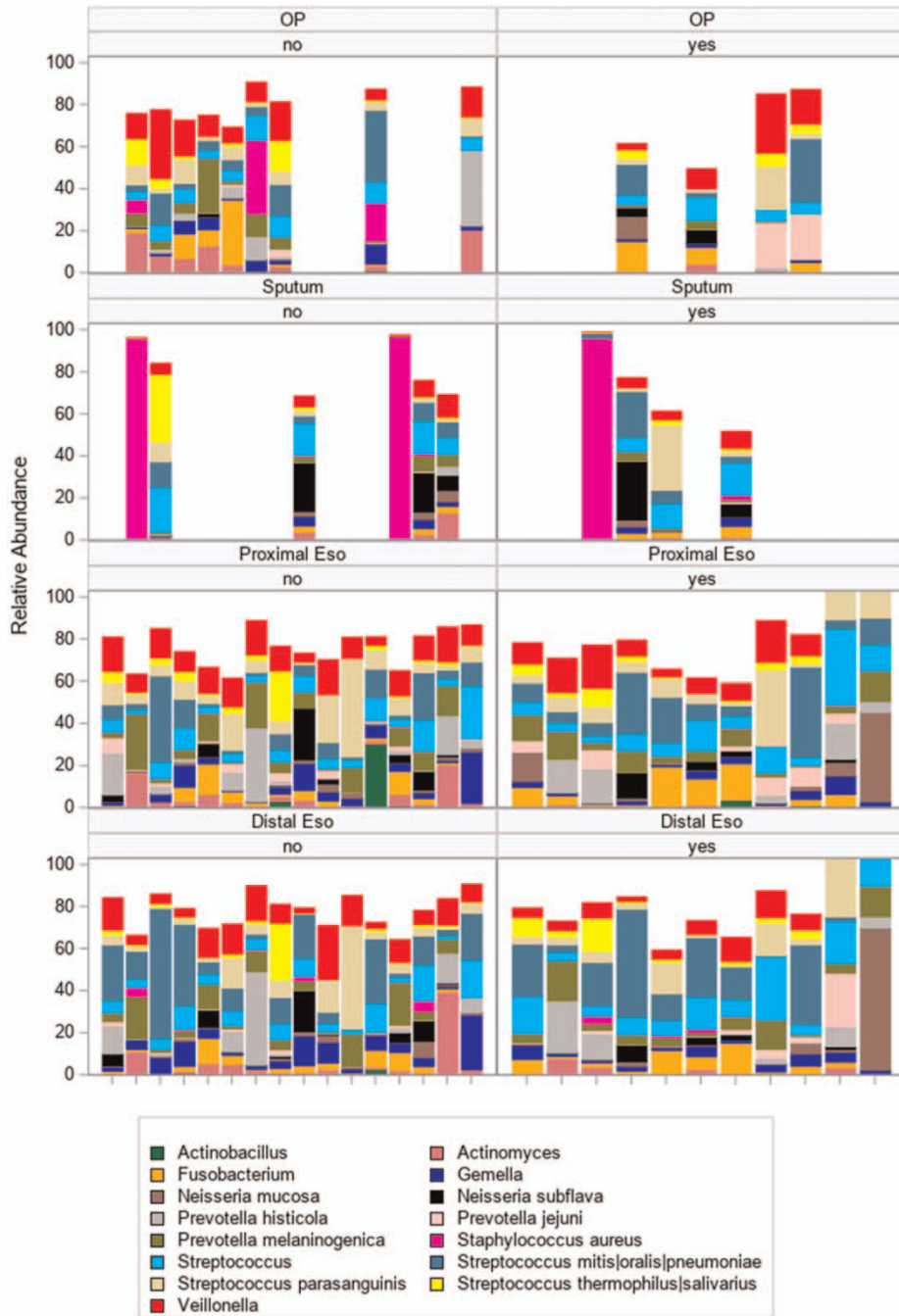
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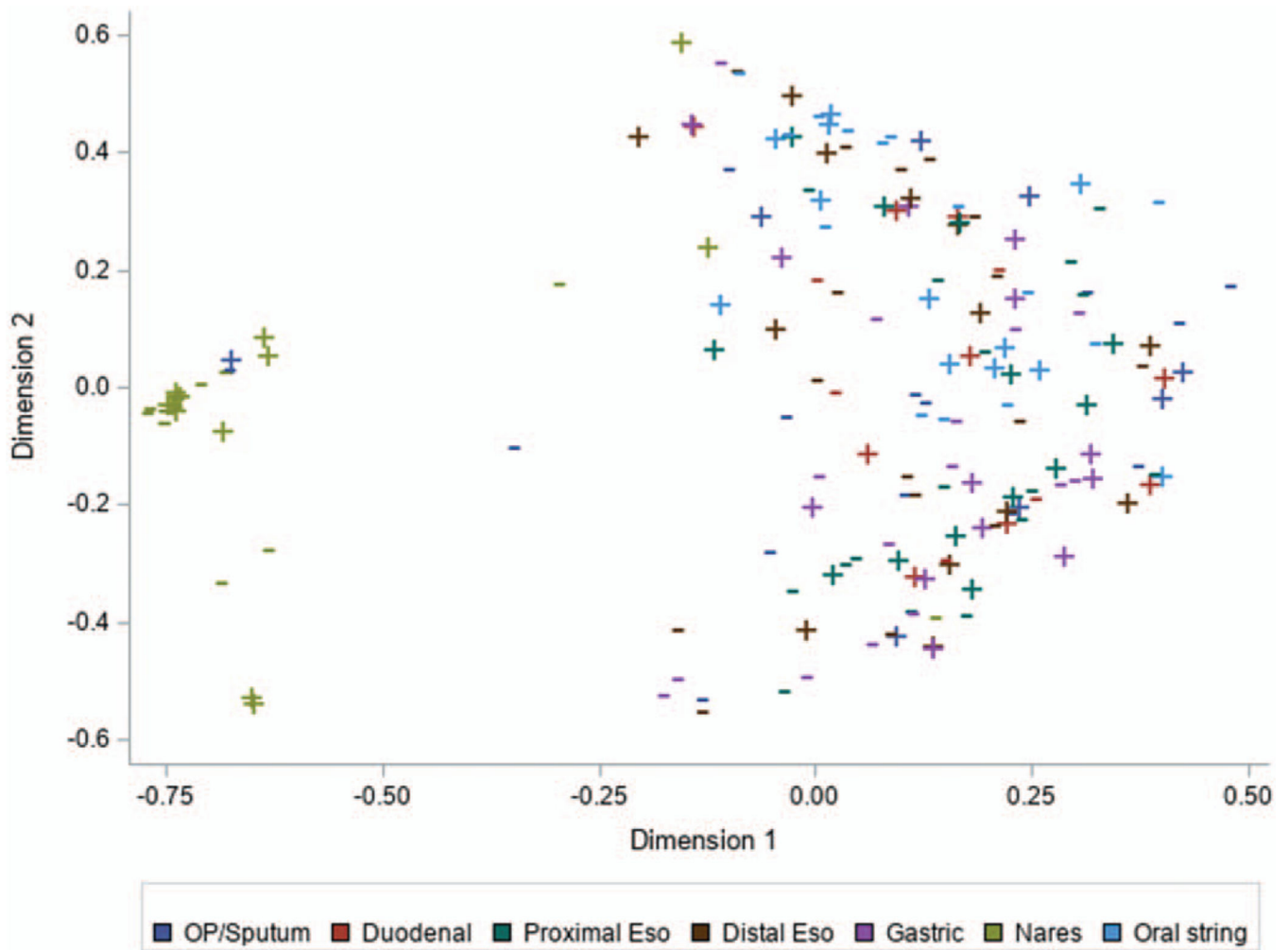


**FIGURE 1.** Bacterial load and alpha diversity. (A) Total bacterial load for all samples by site determined by 16S qPCR. (B) Total bacterial load by site compared between those on versus those off acid blockade. (C) Shannon alpha diversity for all samples by site. (D) Shannon alpha diversity by site compared between those on versus those off acid blockade. The box indicates the interquartile range (IQR) (25th–75th percentile) and the median and mean are indicated by the line and marker, respectively. Whiskers indicate data within 1.5 times the IQR, and smaller points outside the whiskers indicate individual data values. Nares were significantly different from any other location for both TBL and Shannon (all *P* values <0.01). TBL=total bacterial load.



**FIGURE 2.** Esophageal and lung taxa across groups. The *y*-axis in the left-hand panels show relative abundance of taxa in the samples collected off acid blockade (n = 14) whereas the panels on the right show relative abundance in those on acid blockade (n = 13, 13 samples from 11 individuals on acid blockade). Horizontal panels from top to bottom are clustered by site from top to bottom: oropharyngeal swab (OP), sputum, proximal esophagus (proximal eso), and distal esophageal (distal eso) specimens. Each unique vertical bar graph indicates taxa detected in an individual sample grouped by location. Each taxon is indicated in a different

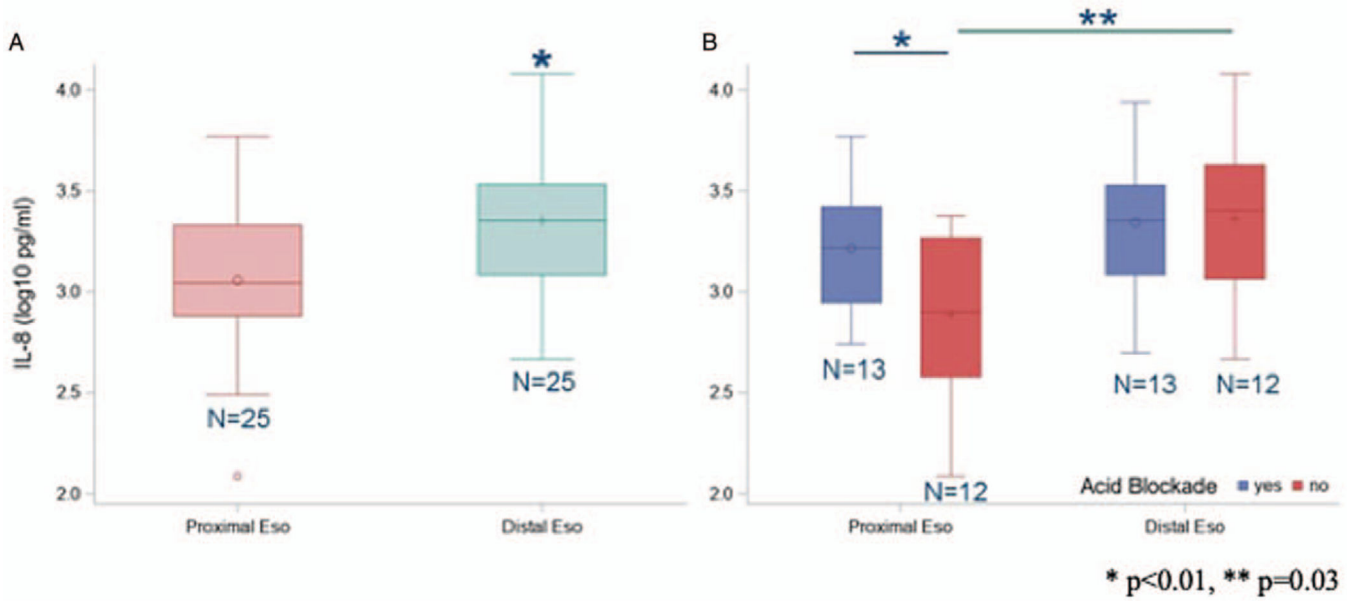
color. The height of the bar corresponds to the relative abundance. Samples from each individual are aligned along the *x*-axis between anatomical sites. Empty spaces indicate no specimen from the indicated site for that individual. No = individual off acid blockade; yes = individual on acid blockade.



**FIGURE 3.**

Multidimensional scaling. Morisita-Horn beta diversity was used as a distance metric in a multidimensional scaling plot to visualize the relationship between samples based on community composition. The nares samples clustered away from all other sample sites indicating that investigated sites had similar species composition irrespective of acid blockade treatment. Points indicated by plus signs correspond to samples collected while the individual was on acid blockade treatment and points with dashes were collected while not on treatment.





**FIGURE 4.**

Esophageal IL-8 concentrations. (A) Esophageal IL-8 concentration in proximal and distal esophagus. (B) IL-8 concentrations by site compared between those on versus those off acid blockade. The box indicates the interquartile range (IQR) (25th–75th percentile) and the median and mean are indicated by the lines and markers, respectively. Whiskers indicate data within 1.5 times the IQR, and smaller points outside the whiskers indicate individual data values. \* $P<0.01$ , \*\* $P=0.03$ .

TABLE 1.

## Individual demographics

	N = 25	Off acid blockade (N = 14)	On acid blockade (N = 11) *
Female sex	11 (44%)	7 (50%)	4 (36%)
Age, years; median [IQR]	13.8 [11.2–14.8]	13.5 [11.2–14.7]	13.8 [11.2–15.5]
Genotype			
F508/F508	11 (44%)	6 (43%)	5 (45%)
F508/other	12 (48%)	7 (50%)	5 (45%)
Other/other	2 (8%)	1 (7%)	1 (9%)
Race			
Caucasian	24 (96)	13 (93%)	11 (100%)
African American	1 (4%)	1 (7%)	0
Hispanic or Latino Ethnicity	3 (12%)	2 (14%)	1 (9%)
BMI percentile, median [IQR]	58 [37–78]	63 [38–78]	54 [33–76]
FEV1% predicted, median [IQR]	92 [85–100]	96 [88–102]	88 [85–94]
On CFTR modulators	15 (60%)	9 (64%)	6 (55%)
On PPI medications	11 (44%)		6 (55%)

\* Two individuals from the cross over study design are included.