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MAJOR ARTICLE

Acute Appendicitis in Children Is Associated With a Local Expansion of Fusobacteria

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Background. Lumenal obstruction has typically been regarded as the cause of acute appendicitis (AA). Recent evidence including data from "antibiotics first" trials suggests that this disease may result from invasion of the appendix by specific pathogens. Small studies have identified an abundance of bacteria from the genus *Fusobacterium* in appendixes from patients with AA. We aimed to validate these findings in a larger cohort of children with appendicitis in addition to profiling the appendiceal microbiota in a population of children without appendicitis.

Methods. Appendix swabs were collected from children undergoing appendectomy for AA (n = 60), incidental appendectomy for reasons other than appendicitis (n = 18), or ileocecectomy for inflammatory bowel disease (n = 7), in addition to samples from other sites. Bacterial 16S ribosomal RNA gene sequences from each sample were amplified, sequenced, and analyzed with the UPARSE and QIIME programs.

Results. We found that the normal human appendix harbors populations of Fusobacteria that are generally absent in fecal samples from healthy adults and children. In patients with AA, Fusobacteria populations proliferate and often persist despite several weeks of broad-spectrum antibiotics prior to surgery. Relative to non-AA samples, AA samples were depleted of sequences from the genus *Bacteroides*. Phylogenetic analysis of sequence data indicates that *F. nucleatum*, *F. necrophorum*, and *F. varium* are the species of *Fusobacterium* observed in AA samples.

Conclusions. These results indicate that the appendiceal niche harbors distinct microbial populations that likely contribute to the pathogenesis of appendicitis, which may one day be leveraged to improve the diagnosis and/or treatment of patients with AA.

Keywords. *Fusobacterium*; appendicitis; microbiome.

For generations, surgical removal of the appendix has been accepted as the preferred method to treat appendicitis and to prevent septic complications of the disease [1]. More recently, antibiotic administration has emerged as a reasonable first-line treatment strategy for patients with uncomplicated appendicitis [2]. In a recent trial in Finland, adults with uncomplicated appendicitis were randomized to receive either 10 days of antibiotics or early appendectomy [3]. Outcomes were generally good for antibiotic-treated patients, although 27% required appendectomy within 1 year of diagnosis. These results were comparable to those seen in other trials [4].

The success of antibiotics in treating most cases of appendicitis has prompted a reconsideration of appendicitis as an infectious disease, or at least a disorder of abnormal bacterial colonization. Until recently, it was widely accepted that appendicitis results from mechanical obstruction of the appendix by a fecalith or lymphatic hyperplasia, followed by secondary stasis, bacterial overgrowth, and occasionally appendiceal necrosis [5]. Careful studies have now demonstrated that most cases of appendicitis are not associated with either a fecalith or lymphatic hyperplasia [6–10]. In parallel, others have suggested that appendicitis represents a specific disorder of inflammation and immune activation distinguishable from other forms of intestinal inflammation [11].

Like other complex diseases, appendicitis may represent a convergence of host genetics and environmental exposures including diet and microbial colonization. Small studies have provided compelling evidence that bacterial community composition within the appendix differs between patients with and those without appendicitis [12–16]. Several of these studies identified an association between appendicitis and the presence of Fusobacteria in the appendix. Building upon prior studies of the appendicitis microbiome, this study was designed to determine if acute appendicitis (AA) is associated with a shift in the appendiceal microbiota, specifically an abundance of Fusobacteria and a depletion of Bacteroidetes.

METHODS

Subject Selection and Sample Collection

All study subjects were children <18 years of age hospitalized at Children's Hospital of Pittsburgh of the University of Pittsburgh

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Medical Center, Pennsylvania. All subjects underwent either (1) appendectomy for AA, (2) interval appendectomy performed 2–3 months after medical treatment for complicated appendicitis, (3) incidental appendectomy at the time of another procedure, (4) ileocolic resection for inflammatory bowel disease (IBD), or (5) cholecystectomy. Appendicitis specimens were classified as either simple or perforated appendicitis according to official surgical pathology reports. For data analysis, we also incorporated data from our prior report of 22 appendix swabs [14], for which the classification as simple or perforated appendicitis was a clinical judgment made by the operating surgeon without histologic confirmation.

Sample Collection and Processing

All sample collections were performed with informed consent under an approved institutional protocol. We collected appendix swabs from all appendectomy patients. Immediately following removal of the appendix, a sterile cotton swab was inserted to sample lumenal fluid. We also collected preoperative saliva samples in a sterile cup from all cholecystectomy patients and from a subset of appendectomy patients. Finally, we collected a rectal swab from another subset of patients undergoing appendectomy or ileocolic resection.

Samples were cryopreserved after collection and microbial DNA was later extracted from each swab using the PowerSoil DNA Isolation kit (MO BIO Laboratories, Inc, Carlsbad, California). Samples were added directly into bead tubes containing solution C1 (60 μL) and then incubated at 65°C for 10 minutes. Tubes were then shaken horizontally for 3–4 minutes at maximum speed using a vortex adaptor. All remaining steps followed the manufacturer's protocol.

Sequencing and Analysis of Bacterial 16S rRNA Genes

16S ribosomal RNA (rRNA) amplicons were produced utilizing fusion primers adapted for the Roche GS FLX Titanium pyrosequencing platform or the Illumina MiSeq. Detailed methods on primer design, library construction, and sequencing can be found in the Supplementary Methods.

Computational Analysis of 16S Amplicons

Computational analysis was done using the UPARSE, QIIME, LEfSe, and Corbata programs [17–20] for analysis of the microbiome, and PhyML [21] was used for phylogenetic analysis. Detailed bioinformatic methods can be found in the Supplementary Methods.

Data Deposition

Sequencing data from this study have been posted to the National Center for Biotechnology Information (biosample accessions SAMN04247151-SAMN04247331).

RESULTS

Study Cohort

A list of study subjects, samples collected, and sequencing results is provided in Supplementary Table 1. The mean age of study subjects was 11.5 years (range, 3–18 years). Eighty-five swabs of the appendiceal lumen were collected from 52 appendectomy patients with AA, 8 interval appendectomy patients, 18 incidental appendectomy patients without appendicitis, and 7 patients undergoing ileocolic resection for IBD. Of the 52 patients with AA, 37 had simple appendicitis and 15 had perforated appendicitis. Interval appendectomy patients generally received 10–14 days of broad-spectrum antibiotics, most commonly ertapenem, after being diagnosed with complicated appendicitis (in addition to drainage of associated abscesses if indicated). They subsequently underwent interval laparoscopic appendectomy within 3 months of initial diagnosis. The incidental appendectomy patients underwent their procedures for a variety of indications including chronic abdominal pain and intestinal malrotation, and the appendix was verified to be normal in each instance.

Microbial Diversity in the Human Appendix

To first characterize the bacterial communities of the normal uninflamed human appendix, we compared data from 18 incidental appendectomy specimens to publicly available datasets obtained from fecal samples of healthy adults and age-matched children [22, 23] (Figure 1). The α-diversity (local species diversity) was significantly lower in the normal appendix than in adult and pediatric stool samples (nonparametric Monte Carlo test, 999 permutations, P = .003 and .003, respectively).

To examine β-diversity, we constructed principal coordinate analysis (PCoA) plots of the taxonomic distances between the samples in each group. We observed that healthy appendix samples clustered together in PCoA space when the Jaccard and unweighted UniFrac distance metrics were used (Figure 1 and Supplementary Figure 1). Interestingly, however, this separation of samples was not observed when the analysis was performed using weighted UniFrac distances (Supplementary Figure 1).

LEfSE was used to identify taxonomic differences between the incidental appendectomy specimens and the pediatric and adult fecal samples (Figure 1*B*). We found that appendix swabs were enriched for sequences from the genera *Fusobacterium* and *Prevotella* ($P = 1.32 \times 10^{7}$ and .017, respectively). In contrast, appendix samples contained a lower abundance of the genus *Faecalibacterium* and genera belonging to the Ruminococcaceae and Clostridiales ($P < .05$ for each taxon). On balance, the microbiome of the normal appendix emerges as distinct from that of the fecal samples in these analyses, both at the level of ecologic diversity and taxonomic composition.

Bacterial Community Composition Within Inflamed and Noninflamed Appendixes

We next compared bacterial communities present within incidental appendectomy specimens and AA specimens (both simple and perforated). To assess whether microbiome changes in appendicitis reflect disease-specific alterations or simply nonspecific inflammation, we also included 7 appendix specimens

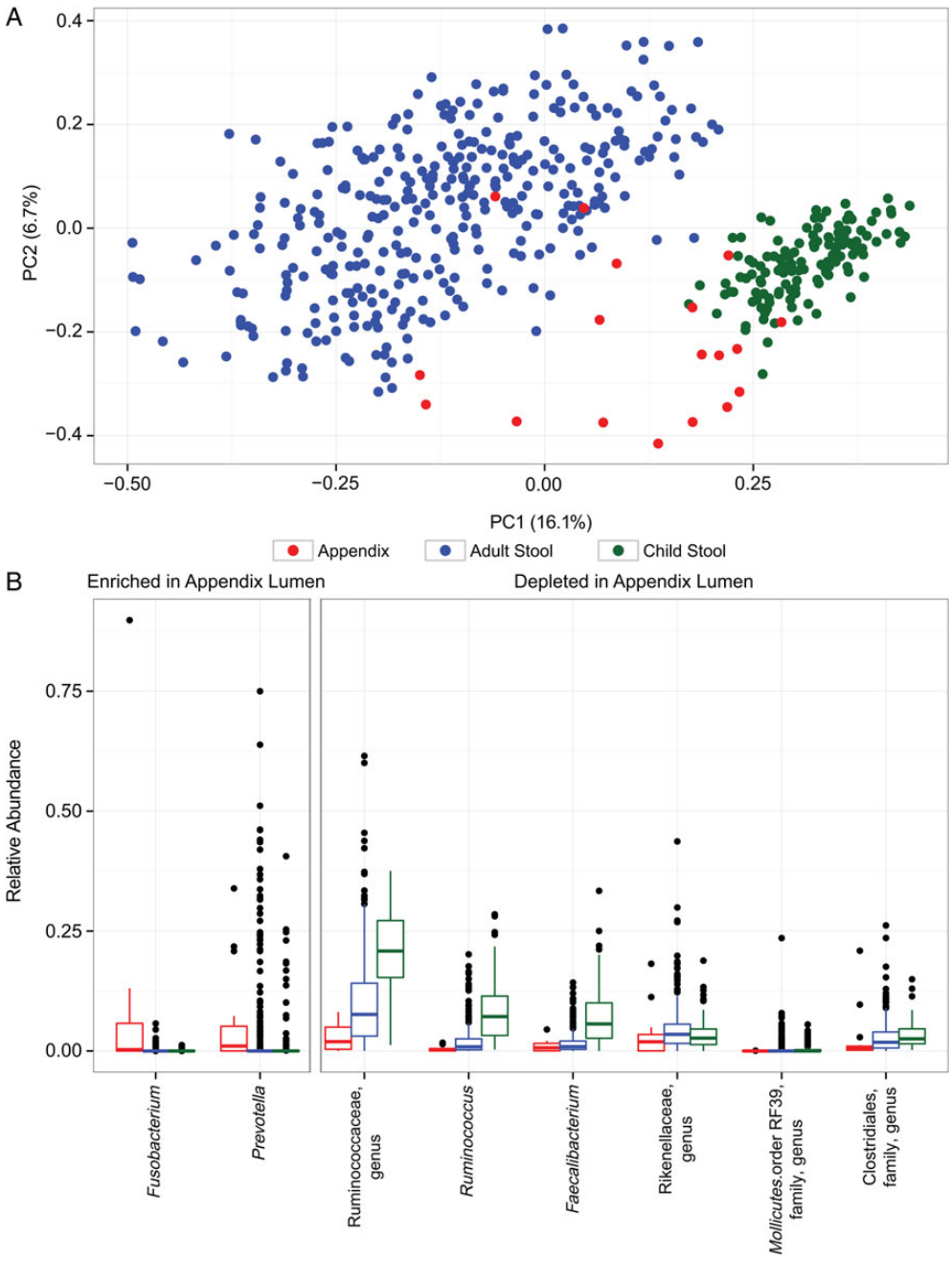

Figure 1. Microbial diversity within healthy appendixes, adult fecal samples, and pediatric fecal samples [27, 28]. *A*, Principal coordinate analysis plot of abundance Jaccard distances between samples from the 3 groups. *B*, Relative abundance of taxonomic groups predicted to be enriched (left panel) or depleted (right panel) in appendix samples relative to fecal samples.

removed at the time of ileocolic resection for IBD. We found no significant difference in α-diversity between incidental, ileocecectomy, and appendicitis samples (Supplementary Figure 2). The β-diversity analysis (Figure 2*A* and Supplementary Figure 3) indicated that many but not all normal appendixes grouped together within PCoA space. Similarly, AA samples were found to cluster together in a large group of samples, whereas the ileocecectomy samples were widely dispersed in PCoA space without clustering. To address the possible confounding effect of age differences, we performed β-diversity comparisons of samples from a narrow age range (12–17 years) to more closely reflect the age of ileocecectomy patients. In this limited analysis, we found that significant differences in β-diversity persisted between appendicitis-positive and -negative samples (permutational multivariate analysis of variance, $P = .007$; Supplementary Figure 4).

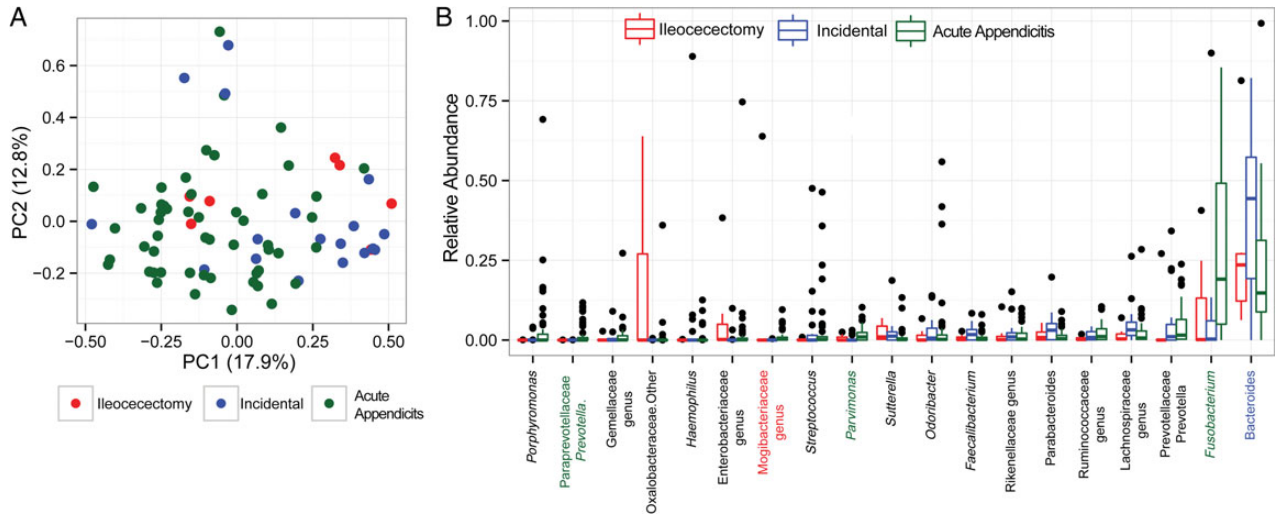

Figure 2. Microbial diversity within incidental appendectomy specimens, acute appendicitis (AA) specimens, and ileocecectomy specimens. *A*, Principal coordinate analysis plot of abundance Jaccard distances between samples from each group. *B*, Relative abundance box plots of taxa with high average relative abundances (>0.01) across sample groups. Labels of taxa predicted to be biomarkers ($P < .05$) are as follows: ileocecectomy (red), AA (green), incidental appendectomy (blue).

We found that AA samples were strongly enriched with the genus *Fusobacterium* (mean abundance, 28.4% vs 7.8% and 9.6% in incidental and ileocecectomy samples; $P = .0001$) (Figure 2*B*). Appendicitis samples were also enriched with sequences from *Parvimonas* (mean abundance, 1.8% vs 0.4% and 0.6% in incidental and ileocecectomy samples; $P = .005$). In contrast, we found that incidental samples were enriched with the genus *Bacteroides* (mean abundance, 38.9% vs 27.1% and 20.2% in ileocecectomy and appendicitis samples; $P = .02$). To discover taxa that differed in ubiquity, we compared appendicitis and nonappendicitis samples with a ubiquity-ubiquity plot constructed in Corbata (Figure 3). *Fusobacterium* exhibited the highest ubiquity of all genera in AA samples in contrast to negative samples (present in 85% of all positive samples and 33% of incidental and ileocecectomy samples). *Campylobacter*, *Parvimonas*, and another oral taxon, *Dialister*, were also frequently identified in appendicitis samples. These results indicate that these taxa, including *Fusobacterium*, are more commonly observed in appendicitis samples even when they are not present at high abundance.

Subgroup Analysis of Appendicitis Specimens

We sought to test the hypothesis that microbial diversity within perforated appendixes is distinct from cases of simple appendicitis. We compared microbial diversity among 3 groups of patients with appendicitis: patients with simple appendicitis, patients with perforated appendicitis, and patients with complicated appendicitis undergoing interval appendectomy 2–3 months after initial diagnosis and treatment of appendicitis. As expected, α-diversity was significantly reduced in samples from interval appendectomy subjects when compared to both simple and perforated appendicitis samples (nonparametric Monte Carlo test, $P = .05$ and .006 for simple and perforated samples, respectively). The β-diversity analysis indicated that samples from these groups overlap considerably within PCoA space (Figure 4*A*). Importantly, a high abundance of *Fusobacterium* and relatively low abundance of *Bacteroides* was observed in all 3 groups of appendicitis specimens (Figure 4*B*). This was surprising because the interval appendectomy patients received prolonged courses of broad-spectrum antibiotics with <3 months for the microbiome to recover prior to surgery. Despite overall similarities in community structure, we observed several taxa that were enriched within 1 of the 3 appendicitis subgroups (LEfSe $P < .05$) (Figure 4*B*). Notably, perforated samples were predicted to be enriched in *Parvimonas*, *Prevotella*, and unidentified genera from the family Ruminococcaceae, Mogibacteriaceae, and Rikenellaceae. Simple appendicitis samples were enriched with *Odoribacter*, and interval samples were enriched with *Veillonella* and an unclassified genus of the Lachnospiraceae.

Microbial Communities in Rectal Swabs and Saliva Samples From Patients With and Without Appendicitis

It was recently reported that rectal swabs can be informative in identifying appendicitis-associated changes in the microbiome [16]. In a subset of patients with appendicitis (n = 11) and without appendicitis (n = 4), we profiled microbial diversity within rectal swabs and compared these results to data from corresponding appendix swabs. To identify relationships between the microbiota of the appendix and the rectum, we compared β-diversity of appendix samples and rectal swabs. A dendrogram was generated from weighted UniFrac distances with samples jackknifed to 100 sequences (minimum coverage) or 1000 sequences (excluding 2 appendix samples with <1000 reads). We found that paired samples (ie, appendix and rectal swabs

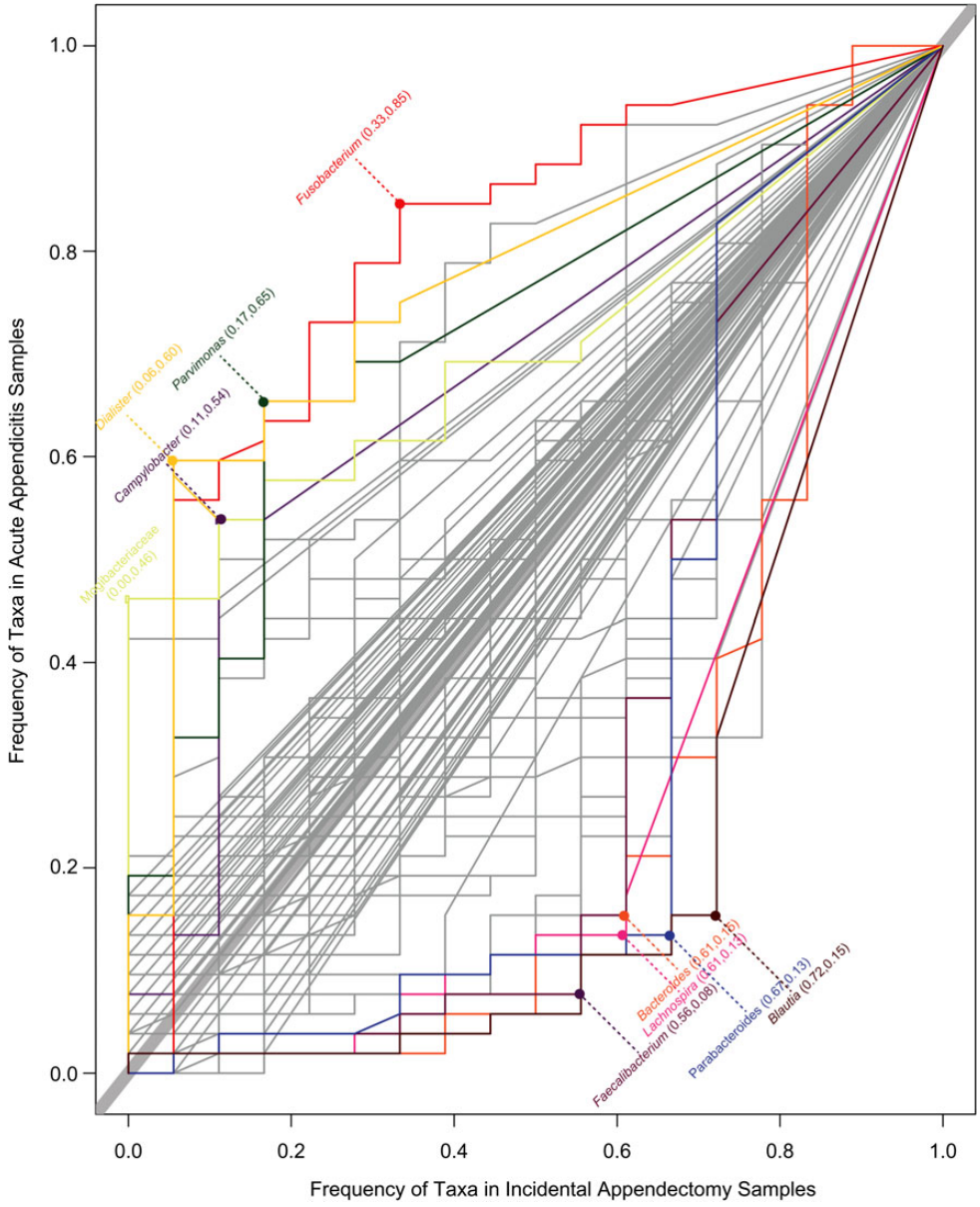

Figure 3. Ubiquity of taxa observed in appendectomy specimens in acute appendicitis (AA) samples minus interval appendectomies, compared to ubiquity of taxa in incidental appendectomies. Shown is a Corbata ubiquity-ubiquity (U-U) plot of genera observed in AA samples and genera observed in incidental appendectomies. Numbers in parentheses indicate ubiquity of genera in both incidental and AA samples.

from the same subject) did not branch together. Rather, all supported nodes within the dendrogram grouped samples according to body site rather than study subject.

We compared rectal swabs and found no significant difference in α-diversity within samples from appendicitis and nonappendicitis patients (Supplementary Figure 6). Similarly, we did not see significant community-wide differences in β-diversity within rectal swabs from patients with appendicitis and without appendicitis, or at the level of taxonomic differences between these groups.

Having observed that the appendicitis microbiome is enriched with *Fusobacterium*, a genus commonly associated with periodontal disease [24], we hypothesized that the oral microbiota of children with appendicitis might differ from that of children without appendicitis. To test this hypothesis, we collected saliva from 22 AA patients, 7 patients undergoing incidental appendectomy, and 3 patients undergoing ileocolic resection. We also collected saliva samples from 12 patients undergoing cholecystectomy without appendectomy. The α-diversity was similar in all sample groups with the exception of interval appendectomy patients, who exhibited reduced salivary diversity (Supplementary Figure 7). Overall, we did not observe different community composition in saliva samples from

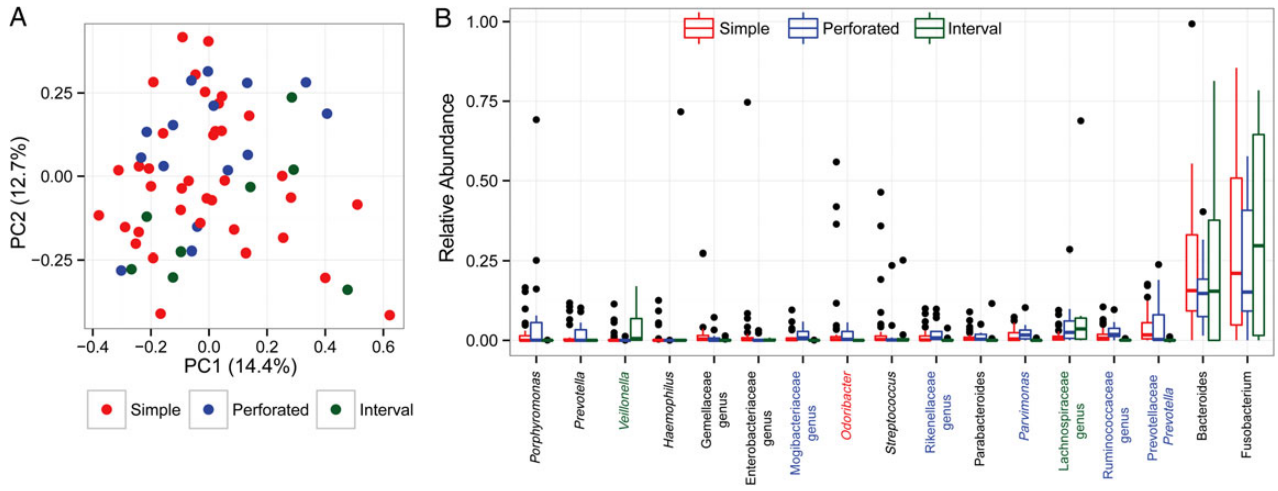

Figure 4. Microbial diversity within simple, perforated, and interval appendectomy samples. *A*, principal coordinate analysis (PCoA) plot of abundance Jaccard distances between samples from each group. No separation of groups is apparent in PCoA space. *B*, Relative abundance box plots of taxa with high average relative abundances (>0.01) across sample groups. Labels of taxa predicted to be biomarkers ($P < .05$) are as follows: simple appendicitis (red), perforated appendix (blue), interval appendectomy (green).

appendicitis-positive subjects when compared to appendicitis-negative subjects (Supplementary Figure 8). Analysis of bacterial abundance patterns with LEfSe did not reveal any taxa enriched specifically within the saliva of patients with and without appendicitis.

Phylogenetic Analysis of Fusobacterium Sequences in Samples From Patients With Appendicitis

To learn more about which *Fusobacterium* species are associated with appendicitis, we constructed a phylogenetic tree based on 16S sequences from the SILVA database (version 119.1) [25] to resolve operational taxonomic units (OTUs) classified as *Fusobacterium* at the genus level (Supplementary Figure 9). The most abundant OTU (by total read counts) assigned to the *Fusobacterium* genus (labeled OTU_2), clearly branches within the *F. nucleatum* clade of the phylogenetic tree and is principally found in appendix samples (vs saliva and rectal samples). The second most abundant OTU (labeled OTU_434) also branches within the *F. nucleatum* clade. These 2 OTUs are clearly distinct but are often found concomitantly.

The third and fourth most common *Fusobacterium* OTUs in our dataset (OTU_19 and OTU_22) branch in the phylogenetic tree with species of *F. necrophorum* and *F. varium*, respectively, and are almost exclusively found in the appendix. Three appendix samples harbor abundant populations of these 2 species: *F. necrophorum* in subjects 11 (simple) and 52 (perforated), and *F. varium* in subject 67 (interval). Along with *F. nucleatum*, *F. necrophorum* has been previously associated with acute appendicitis [12]. There are currently no reports of *F. varium* being associated with appendicitis, though it has been previously shown to induce a proinflammatory response in colonic cells contributing to ulcerative colitis [26].

OTU_788 branches within the *F. periodonticum* clade, which itself branches paraphyletically within the *F. nucleatum* clade. Although it is also present in appendix samples at low abundance, OTU_788 is the most abundant species of *Fusobacterium* found in saliva samples from this study.

DISCUSSION

Recent evidence that uncomplicated appendicitis can be treated with antibiotics and without surgery is changing our understanding of the disease. Although it is premature to conclude whether appendectomy should be abandoned in favor of antibiotic therapy, our results provide compelling evidence that appendicitis can be considered a disorder of the host–microbe relationship, at least in some cases. As with other inflammatory disorders (eg, Crohn disease), there are several plausible mechanisms by which microbes might contribute to the pathogenesis of appendicitis. It is possible that a single causative pathogen is responsible, but a more realistic explanation may be that the disease represents an inappropriate immune response to changes in the composition of the microbiome. Appendiceal dysbiosis could be mediated by environmental factors (eg, low fiber content in the Western diet).

Prior culture-based studies of the bacteriology of appendicitis have been inconclusive. They have identified a wide range of organisms but have not identified clear differences in the appendiceal microbiota of patients with and without appendicitis [27–31]. Organisms isolated from inflamed appendices include *Bacteroides*, *Peptostreptococcus*, *Fusobacterium*, and *Bilophila*. Recent culture-independent studies have expanded our knowledge in this area. In 2 companion papers, Swidsinski et al used a FISH technique and observed a striking preponderance of *Fusobacteria* in appendix tissue specimens from patients with appendicitis [12, 13]. More recently, 3 small studies used 16S rRNA

gene sequencing to demonstrate differences in the microbial communities of patients with and without appendicitis [14–16].

To our knowledge, this study represents the largest published dataset of 16S rRNA gene sequences obtained by sampling the human appendix. We analyzed lumenal swabs, which we acknowledge may provide information that differs from analysis of appendiceal tissue. Consistent with prior reports, we observed a striking abundance of Fusobacteria in appendicitis samples. In addition to *F. nucleatum*, we identified other Fusobacterium species, including *F. varium and F. necrophorum. Fusobacterium* species (particularly *F. nucleatum*) are normal colonizers of the oral cavity and lower gastrointestinal tract that are well known for their contribution to periodontal disease. Unlike most gram-negative strict anaerobes, Fusobacteria possess significant pathogenic potential owing to their well-documented capacity to invade human mucosal surfaces and to activate an immune response [32]. The most well-studied *Fusobacterium* infection is Lemierre syndrome, but Fusobacteria isolates have additionally been identified as the cause of ear infections, pneumonia, brain or liver abscesses, and, as noted, periodontal disease. Associations have also been made recently between *Fusobacterium* colonization and colorectal cancer [32], IBD [33], and the onset of Crohn disease [34]. It is not known whether these associations reflect the "escape" of oral pathogens [35] or whether Fusobacteria can appear independently and thrive at these body sites (including the appendix) .

An unexpected finding in our study was the high abundance of *Fusobacterium* in the appendix of 4 of 8 interval appendectomy patients for which the swabs were collected 2–3 months later. Surprisingly, the appendiceal microbiota in these cases strongly resembled the samples of appendicitis patients undergoing urgent surgery. This finding supports the notion of the appendix as a "reservoir" for gut microbes during acute environmental disturbances such as antibiotic therapy [36, 37]. The clinical relevance of this observation is unclear, but the possibility exists that patients harboring persistent *Fusobacterium* populations after first-line antibiotic therapy may be at risk for subsequent disease recurrence.

The abundance of Fusobacteria in our cohort was also associated with an abundance of *Parvimonas* and a depletion of the genus *Bacteroides*. These results validate findings from prior studies. Specifically, the abundance of *Fusobacterium* within appendicitis specimens was identified in 5 prior studies [12–16]. The enrichment of *Parvimonas* was also observed in each of the 3 prior sequencing-based studies [14–16]. Strikingly, Swidsinksi et al similarly observed a depletion of *Bacteroides* in appendicitis in their FISH-based investigations [12, 13]. Together, these studies provide compelling evidence that the appendiceal dysbiosis is not a "nonspecific" set of microbial changes related to gut inflammation, but rather that these changes are likely unique to the appendix itself. This concept aligns with the report that the mucosal inflammatory changes seen in appendicitis are distinct from changes observed elsewhere in the gastrointestinal tract [11].

A notable finding in this study was that histologically normal appendectomy specimens contained a low abundance of *Fusobacterium* populations that were generally not seen in healthy fecal samples. This indicates that the appendix represents a unique microbial niche distinct from the large intestine, which supports the growth of *Fusobacterium*. We speculate that, in the presence of specific genetic and environmental factors, *Fusobacterium* populations expand and contribute to the pathogenesis of AA. Such a paradigm represents a marked departure from classical teachings about the disease, but it would be in line with the apparent efficacy of first-line antibiotic therapy as treatment for appendicitis. An unresolved dilemma is why the disease is so common in developed nations and rarely seen in developing countries [38]. Further research may allow for advances in understanding the complex relationship between host genetics, microbiome, environment, and disease phenotype.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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References

1. Williams GR. Presidential address: a history of appendicitis. With anecdotes illustrating its importance. Ann Surg **1983**; 197:495–506.
2. Flum DR. Acute appendicitis—appendectomy or the 'antibiotics first' strategy. N Engl J Med **2015**; 372:1937–43.
3. Salminen P, Paajanen H, Rautio T, et al. Antibiotic therapy vs appendectomy for treatment of uncomplicated acute appendicitis: the APPAC randomized clinical trial. JAMA **2015**; 313:2340–8.
4. Varadhan KK, Neal KR, Lobo DN. Safety and efficacy of antibiotics compared with appendicectomy for treatment of uncomplicated acute appendicitis: meta-analysis of randomised controlled trials. BMJ **2012**; 344:e2156.
5. Sullins VF, Lee SL. Appendicitis. In: Ashcraft's Pediatric Surgery. Philadelphia: Elsevier Inc, **2014**.
6. Carr NJ. The pathology of acute appendicitis. Ann Diagn Pathol **2000**; 4:46–58.
7. Singh JP, Mariadason JG. Role of the faecolith in modern-day appendicitis. Ann R Coll Surg Engl **2013**; 95:48–51.
8. Chandrasegaram MD, Rothwell LA, An EI, Miller RJ. Pathologies of the appendix: a 10-year review of 4670 appendicectomy specimens. ANZ J Surg **2012**; 82:844–7.
9. Chang AR. An analysis of the pathology of 3003 appendices. Aust N Z J Surg **1981**; 51:169–78.
10. Arnbjörnsson E, Bengmark S. Role of obstruction in the pathogenesis of acute appendicitis. Am J Surg **1984**; 147:390–2.
11. Murphy CG, Glickman JN, Tomczak K, et al. Acute appendicitis is characterized by a uniform and highly selective pattern of inflammatory gene expression. Mucosal Immunol **2008**; 1:297–308.
12. Swidsinski A, Dorffel Y, Loening-Baucke V, et al. Acute appendicitis is characterised by local invasion with *Fusobacterium nucleatum/necrophorum*. Gut **2010**; 60:34–40.

13. Swidsinski A, Loening-Baucke V, Biche-Ool S, et al. Mucosal invasion by Fusobacteria is a common feature of acute appendicitis in Germany, Russia, and China. Saudi J Gastroenterol **2012**; 18:55.
14. Zhong D, Brower-Sinning R, Firek B, Morowitz MJ. Acute appendicitis in children is associated with an abundance of bacteria from the phylum Fusobacteria. J Pediatr Surg **2014**; 49:441–6.
15. Guinane CM, Tadrous A, Fouhy F, et al. Microbial composition of human appendices from patients following appendectomy. mBio **2013**; doi:10.1128/mBio.00366-12.
16. Jackson HT, Mongodin EF, Davenport KP, Fraser CM, Sandler AD, Zeichner SL. Culture-independent evaluation of the appendix and rectum microbiomes in children with and without appendicitis. PLoS One **2014**; 9: e95414.
17. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods **2010**; 5:335–6.
18. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods **2013**; 10:996–8.
19. Li K, Bihan M, Methé BA. Analyses of the stability and core taxonomic memberships of the human microbiome. PLoS One **2013**; 5:e63139.
20. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol **2011**; 6:R60.
21. Guindon S, Dufayard JF, Lefort V, et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol **2010**; 3:307–21.
22. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature **2012**; 486:207–14.
23. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature **2012**; 486:222–7.
24. Han YW. *Fusobacterium* nucleatum: a commensal-turned pathogen. Curr Opin Microbiol **2015**; 23:141–7.
25. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and Web-based tools. Nucleic Acids Res **2013**; 41: D590–6.
26. Ohkusa T, Okayasu I, Ogihara T, Morita K, Ogawa M, Sato N. Induction of experimental ulcerative colitis by *Fusobacterium varium* isolated from colonic mucosa of patients with ulcerative colitis. Gut **2003**; 52:79–83.
27. Lamps LW. Infectious causes of appendicitis. Infect Dis Clin North Am **2010**; 24:995. –1018., ix–x.
28. Rautio M, Saxén H, Siitonen A, Nikku R, Jousimies-Somer H. Bacteriology of histopathologically defined appendicitis in children. Pediatr Infect Dis J **2000**; 19:1078–83.
29. Chen C-Y, Chen Y-C, Pu H-N, Tsai C-H, Chen W-T, Lin C-H. Bacteriology of acute appendicitis and its implication for the use of prophylactic antibiotics. Surg Infect **2012**; 13:383–90.
30. Guillet-Caruba C, Cheikhelard A, Guillet M, et al. Bacteriologic epidemiology and empirical treatment of pediatric complicated appendicitis. Diagn Microbiol Infect Dis **2011**; 69:376–81.
31. Roberts JP. Quantitative bacterial flora of acute appendicitis. Arch Dis Child **1988**; 63:536.
32. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe **2013**; 14:195–206.
33. Strauss J, Kaplan GG, Beck PL, et al. Invasive potential of gut mucosa-derived *Fusobacterium nucleatum* positively correlates with IBD status of the host. Inflamm Bowel Dis **2011**; 17:1971–8.
34. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe **2014**; 15:382–92.
35. Han YW, Wang X. Mobile microbiome: oral bacteria in extra-oral infections and inflammation. J Dent Res **2013**; 92:485–91.
36. Laurin M, Everett ML, Parker W. The cecal appendix: one more immune component with a function disturbed by post-industrial culture. Anat Rec Adv Integr Anat Evol Biol **2011**; 294:567–79.
37. Randal Bollinger R, Barbas AS, Bush EL, Lin SS, Parker W. Biofilms in the large bowel suggest an apparent function of the human vermiform appendix. J Theor Biol **2007**; 249:826–31.
38. Bickler SW, DeMaio A. Western diseases: current concepts and implications for pediatric surgery research and practice. Pediatr Surg Int **2007**; 24:251–5.