Journal of Microbiology, Immunology and Infection 56 (2023) 695—704

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Original Article

A comprehensive microbial analysis of pediatric patients with acute appendicitis

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Received 23 June 2022; received in revised form 6 February 2023; accepted 12 March 2023
Available online 21 March 2023

KEYWORDS
Appendicitis;
Fusobacterium;
Microbiome;
Pediatric

Abstract *Background:* Pathogenesis of pediatric acute appendicitis (AA) is yet to be elucidated. Therefore, we performed a comprehensive microbial analysis of saliva, feces, and appendiceal lumen of AA patients using 16S ribosomal RNA (rRNA) gene amplicon sequencing to elucidate the pathogenesis of pediatric AA.
Methods: This study included 33 AA patients and 17 healthy controls (HCs) aged <15 y. Among the AA patients, 18 had simple appendicitis, and 15 had complicated appendicitis. Salivary and fecal samples were obtained from both groups. The contents of the appendiceal lumen were collected from the AA group. All samples were analyzed using 16S rRNA gene amplicon sequencing.
Results: The relative abundance of *Fusobacterium* was significantly higher in the saliva of AA patients as compared to that in HCs ($P = 0.011$). *Bacteroides*, *Escherichia*, *Fusobacterium*, *Coprobacillus*, and *Flavonifractor* were significantly increased in the feces of AA patients, as compared to that in HCs ($P = 0.020$, 0.010, 0.029, 0.031, and 0.002, respectively). In the appendiceal lumen, *Bacteroides*, *Parvimonas*, *Fusobacterium*, and *Alloprevotella* were the top bacterial genera with an average relative abundance >5% (16.0%, 9.1%, 7.9%, and 6.0%, respectively).
Conclusions: The relative abundance of *Fusobacterium* was high in the appendiceal lumen of pediatric AA patients. Moreover, the relative abundance of *Fusobacterium* was significantly higher in the saliva and feces of pediatric AA patients than in those of healthy children. These

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https://doi.org/10.1016/j.jmii.2023.03.006

results suggest that ectopic colonization of oral *Fusobacterium* in the appendix might play an important role in the pathogenesis of pediatric AA.

Introduction

Acute appendicitis (AA) is one of the most common abdominal emergencies in children; however, its pathogenesis has not yet been elucidated. Generally, luminal obstruction due to appendicolith or lymphoid hyperplasia is considered to drive AA pathogeneses.[1,2] However, an obstructed appendiceal lumen is not observed in all AA cases.[3] Additionally, it has been hypothesized that bacterial infection may be the main cause of AA.[4,5] Using the bacterial fluorescent *in situ* hybridization (FISH) technique, Swidsinski et al. revealed that *Fusobacterium* infiltrated the appendiceal submucosa in AA patients, thereby suggesting that most AA cases were triggered by local *Fusobacterium* infection.[6] Furthermore, studies employing next-generation sequencing have shown that oral bacteria, including *Fusobacterium*, are frequently observed in the appendiceal lumen of AA patients.[7–11]

A comprehensive analysis of oral and gut microbiomes is essential to understand the interaction between host and commensal bacteria. Particularly, the oral cavity has been considered to serve as a reservoir for potential intestinal pathobionts.[12,13] However, these analyses have not been investigated in pediatric AA patients. In this study, we elucidated the pathogenesis of pediatric AA through a comprehensive microbial analysis of the saliva, feces, and appendiceal lumens of AA patients.

Methods

Subject selection

This study included 33 AA patients aged <15 y who underwent appendectomy between April 2020 and September 2021 at a Japanese university hospital. Patients who had been previously diagnosed and treated for appendicitis, suffered exacerbations during conservative antibiotic treatment, and had undergone an interval appendicectomy were excluded. Additionally, 17 patients aged <15 y who had undergone elective surgery for inguinal hernia at the same hospital during the same period were included as healthy controls (HCs). To exclude the influence of intestinal inflammation on the gut microbiome, inguinal hernia patients with a history of incarcerated hernia and/or other gastrointestinal diseases were excluded.

The AA patients and their parents were informed about the study, and written consent was obtained from the parents at the time of admission. Written assent was also obtained from children aged >10 y, whenever possible. For the HCs, written consent and assent were obtained similarly. This study was approved by our local institutional review board.

Collection of clinical data

The height, weight, and body mass index of the participants, both HCs and AA patients, were measured at the time of admission to our hospital (0–1 d before surgery). Body temperatures were recorded at the time of the first visit to the emergency room before emergency surgery for AA patients and on the morning of elective surgery for HCs. Additionally, blood tests were performed at the time of the first visit to the emergency room before surgery for AA patients and during preoperative evaluation ≤3 m before elective surgery for HCs. Abdominal pain duration in each AA patient was calculated as the duration between the patient's first experience of pain and the beginning of the surgery. All resected appendices were subjected to histopathological examination, and according to its results, catarrhal and phlegmonous appendicitis were defined as simple appendicitis (SA), while gangrenous and perforated appendicitis were classified as complicated appendicitis (CA). The demographic characteristics of the participants are presented in Table 1.

Sample collection

Fecal samples were collected from AA patients trans-anally with a swab in the operating room after applying general anesthesia. Saliva samples were collected by oral cavity aspiration. Cefmetazole was administered 30 min before surgery as preoperative antibiotic prophylaxis. The patients underwent a trans-umbilical laparoscopic-assisted appendectomy. The lumen of each resected appendix was accessed through an incision on the contralateral mesenteric side under sterile manipulation. Swab specimens were obtained by scraping the appendiceal lumen. Similar to AA patients, fecal and salivary samples were collected from the HC group. Each sample was diluted with 4 mL saline, and 1 mL (final concentration 20%) of tissue RNA stabilization reagent was added. Subsequently, these samples were stored in clean reaction tubes at −80 °C.

Bacterial DNA extraction

At the laboratory, frozen samples were thawed, suspended in 1 mL phosphate-buffered saline, and centrifuged at 12,000×*g* for 10 min at 4 °C. The pellets were washed with TE20 [10 mM Tris–HCl + 20 mM ethylenediaminetetraacetic acid (EDTA)] buffer and further centrifuged at 12,000×*g* for 10 min at 4 °C. Subsequently, 800 μL TE20 was added to the pellets, followed by 15 mg lysozyme and 2000 units of purified achromopeptidase. The samples were incubated at 37 °C for 2 h with gentle mixing, followed by adding 1 mg

Table 1 Demographic characteristics of HCs and patients with AA.

	HC (n = 17)	SA (n = 18)	CA (n = 15)	AA (n = 33)	*p*-value (HC vs. AA)
Age (y)	8.7 ± 2.7	9.6 ± 2.1	9.8 ± 2.9	9.6 ± 2.5	0.164
Sex, male:female	7:10	10:8	8:7	18:15	0.551
Body weight (kg)	29.8 ± 9.2	36.0 ± 10.5	34.4 ± 11.9	35.3 ± 11.0	0.130
Height (cm)	130.8 ± 14.5	140.6 ± 13.9	138.8 ± 15.5	139.8 ± 14.4	0.060
BMI (kg/m^2)	16.9 ± 1.9	18.0 ± 3.6	17.2 ± 2.6	17.7 ± 3.1	0.610
Duration of abdominal pain (h)	–	26.3 ± 22.3	40.6 ± 37.7	32.8 ± 30.7	–
Antibiotic usage prior to visit, n (%)	0 (0)	4 (22.2)	1 (6.7)	5 (15.2)	0.044*
Body temperature (°C)	36.4 ± 0.3	37.0 ± 0.6	37.8 ± 0.7	37.4 ± 0.7	<0.001***
Leukocyte count (/μL)	6352 ± 1271	13,538 ± 4948	15,620 ± 2818	14,484 ± 4194	<0.001***
C-reactive protein (mg/dL)	–	1.23 ± 1.20	5.70 ± 5.43	3.39 ± 4.43	–
Hemoglobin (g/dL)	13.2 ± 1.1	13.0 ± 1.0	12.8 ± 1.5	12.9 ± 1.2	0.305
Albumin (g/dL)	4.5 ± 0.1	4.5 ± 0.2	4.2 ± 0.4	4.4 ± 0.3	0.063
Incidence of appendicolith, n (%)	–	6 (33.3)	11 (73.3)	17 (51.5)	–
Incidence of appendiceal perforation, n (%)	–	–	3 (20.0)	3 (9.1)	–

Data are presented as mean ± standard deviation.
SA: simple appendicitis, CA: complicated appendicitis, AA: acute appendicitis, HC: healthy control, BMI: body mass index.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

proteinase K and 100 μL 10% sodium dodecyl sulfate and further incubation at 55 °C for 1 h with gentle mixing. The DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1), precipitated with isopropanol and 3 M sodium acetate, washed with 75% ethanol, and re-suspended in 100 μL TE (10 mM Tris–HCl, 1 mM EDTA) buffer. Subsequently, 1 μg RNase A (final concentration 10 μg/mL) was added to each DNA sample and incubated for 30 min at 37 °C with gentle mixing. The DNA was purified using 20% polyethylene glycol solution, pelleted by centrifugation, rinsed with 75% ethanol, and dissolved in TE buffer.

16S rRNA gene amplicon sequencing

The V1–V2 region of the bacterial 16S rRNA gene was amplified using polymerase chain reaction (PCR) with the barcoded forward 27F-mod (5′-AGRGTTTGATYMTGGCTCAG-3′) and reverse 338R (5′-TGCTGCCTCCCGTAGGAGT-3′) primers.[14] The PCR mixture included 5 μL 10 × Ex *Taq* PCR buffer, 5 μL deoxynucleoside triphosphates (2.5 mM), 0.2 μL Ex Taq polymerase, 1 μL of each primer (10 mM), and 40 ng extracted DNA. The PCR conditions were as follows: 96 °C for 2 min; 20 cycles of 96 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. If 20 PCR cycles failed to yield sufficient DNA, 35 cycles were performed. Subsequently, PCR amplicons were purified using purification beads and quantified. Equal amounts of each PCR amplicon were mixed and subjected to multiplexed amplicon sequencing using MiSeq System (Illumina, San Diego, CA, USA), according to the manufacturer's instructions.

Data processing

Two paired-end reads were merged using the fastq-join program. Reads lacking both forward and reverse primer sequences were removed using BLAST, and both primer sequences were trimmed off from the reads; reads with an average quality value of <25 were removed. Among the high-quality reads, we randomly selected 10,000 reads/samples and subjected them to operational taxonomic unit (OTU) clustering and UniFrac analyses. Incidentally, samples with <10,000 high-quality reads were excluded from the analysis. First, the selected reads were sorted according to the frequency of redundant sequences and then grouped into OTUs using UCLUST (https://www.drive5.com/) with a sequence identity threshold of 97%. The sequence with the highest redundancy in each OTU was selected as its representative. The representative sequences of all OTUs were subjected to a homology search among 16S databases using the GLSEARCH program for taxonomic assignments. Sequence similarity thresholds were set at 70% and 94% for taxonomic assignment at the phylum and genus levels, respectively. The taxonomic assignment of each OTU was determined based on similarity searching against the National Center for Biotechnology Information RefSeq database downloaded on January 8, 2020, using the GLSEARCH program. Microbial richness was estimated using the Chao1 index,[15] and microbial diversity was assessed using Shannon's index.[16] Principal coordinate analysis was performed based on UniFrac distances.

Statistical analyses

All statistical analyses were performed using the R software program (v4.0.5). The Wilcoxon rank-sum and Fisher's exact tests were used to compare patient demographic data. The Wilcoxon rank-sum test was used to compare alpha diversities and the proportion of bacteria at the phylum and genus levels. Permutational multivariate analysis of variance was used to compare intestinal microbiota structure among the groups based on both weighted and unweighted UniFrac distances.[17] *P*-values were corrected

for multiple testing using the Benjamini–Hochberg method, as appropriate. The significance level was set at $P < 0.05$.

Results

Salivary sample comparison between HC and AA groups

We obtained data for 16 HC and 33 AA (18 SA and 15 CA) salivary samples. The HC and AA groups did not significantly differ in alpha (Fig. 1A) or beta (Fig. 1B) diversities. Taxonomic analysis under the conditions of average relative abundance >0.1% did not reveal any significant differences between HC and AA at the phylum level. However, at the genus level, *Fusobacterium* abundance was significantly higher in AA patients than that in HCs ($P = 0.011$; Fig. 1C).

Subgroup analysis did not portray any significant differences in alpha diversity among HC, SA, and CA groups (Supplementary Fig. 1A). In contrast, unweighted UniFrac revealed significant dissimilarities between SA and CA in terms of beta diversity ($P = 0.036$; Supplementary Fig. 1B). Taxonomic analysis did not reveal any significant differences among the three groups at the phylum level. However, at the genus level, *Fusobacterium* and *Bifidobacterium* abundances were significantly higher in SA than those in HCs ($P = 0.018$ and 0.025, respectively; Supplementary Fig. 1C), while *Veillonella* and *Staphylococcus* abundances were significantly increased in CA than those in HCs ($P = 0.039$ and 0.026, respectively; Supplementary Fig. 1C). Additionally, *Bifidobacterium* abundance was significantly decreased in CA than that in HCs ($P = 0.031$; Supplementary Fig. 1C).

Fecal sample comparison between HC and AA groups

We obtained data for 16 HC and 33 AA (18 SA and 15 CA) fecal samples. The HC and AA groups did not differ significantly in alpha (Fig. 2A) or beta (Fig. 2B) diversities. Taxonomic analysis at the phylum level, under the conditions of average relative abundance >0.1%, revealed a significantly increased abundance in Bacteroidetes ($P = 0.011$) and a significantly lower Firmicutes abundance ($P = 0.003$) in AA patients than those in HCs. At the genus level, *Bacteroides*, *Escherichia*, *Fusobacterium*, *Coprobacillus*, and *Flavonifractor* abundances were significantly higher in AA patients ($P = 0.020$, 0.010, 0.029, 0.031, and 0.002, respectively; Fig. 2C) than those in HC.

Subgroup analysis did not reveal significant differences in alpha (Supplementary Fig. 2A) or beta (Supplementary Fig. 2B) diversities among HC, SA, and CA groups. At the phylum level, Bacteroidetes and Fusobacteria abundances were significantly greater in SA than those in HC ($P = 0.032$ and 0.040, respectively), while Firmicutes abundance was significantly lower in CA than that in HC ($P = 0.012$). At the genus level, *Fusobacterium*, *Flavonifractor*, and *Actinomyces* abundances were significantly higher in SA than those in HC ($P = 0.007$, 0.017, and 0.018, respectively; Supplementary Fig. 2C), while *Escherichia*, *Flavonifractor*, *Anaerotignum*, and *Citrobacter* abundances were significantly higher in CA than those in HC ($P = 0.004$, 0.032, 0.027, and 0.026, respectively; Supplementary Fig. 2C). Additionally, *Anaerotignum*, *Citrobacter*, and *Massilimicrobiota* abundances were significantly greater in CA than those in SA ($P = 0.031$, 0.016, and 0.043, respectively; Supplementary Fig. 2C).

Microbial profiling of appendiceal lumen in the AA group

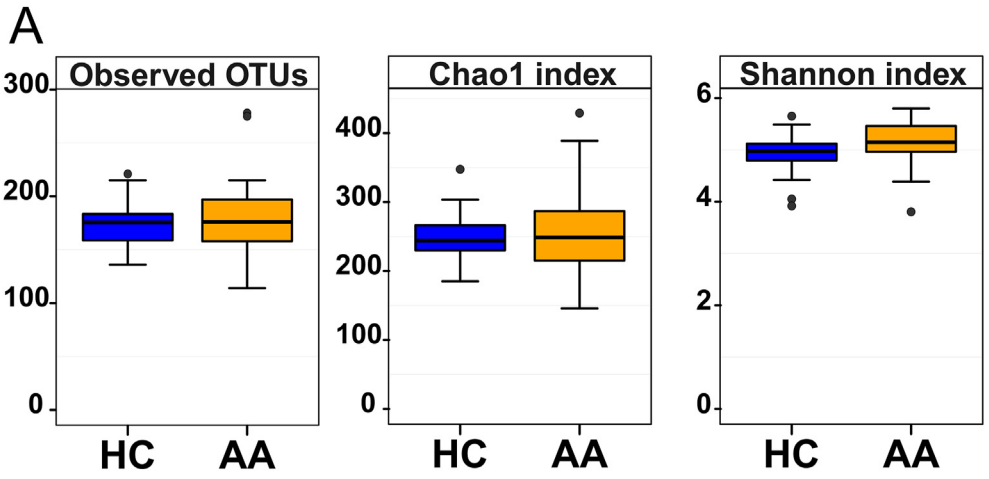
We obtained data for 29 appendiceal lumen samples of AA patients (15 SA and 14 CA). Taxonomic analyses revealed average relative abundance of bacteria at the phylum and genus levels (Fig. 3). Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria were the top bacterial phyla with respect to an average relative abundance >5% [38.7 ± 2.0%, 34.8 ± 2.2%, 12.4 ± 2.1%, 8.1 ± 1.0%, and 5.1 ± 1.0% (± standard error), respectively; Fig. 3A]. *Bacteroides*, *Parvimonas*, *Fusobacterium*, and *Alloprevotella* were the top bacterial genera with respect to an average relative abundance >5% [16.0 ± 1.8%, 9.1 ± 1.7%, 7.9 ± 1.0%, and 6.0 ± 1.4% (± standard error), respectively; Fig. 3B].

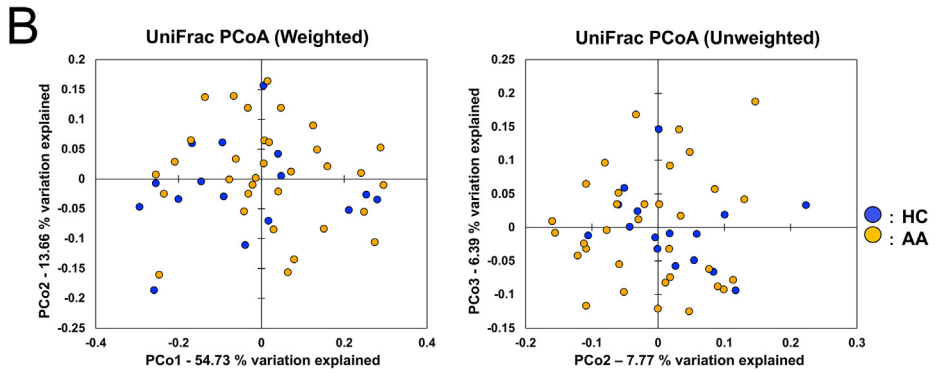
Difference in bacterial composition in the appendiceal lumen by AA severity

To clarify the differences in the appendiceal lumen with AA severity, appendiceal lumen samples of SA and CA were compared. We collected data for 15 SA and 14 CA samples. The SA and CA samples did not differ significantly in their alpha diversities (Fig. 4A). In contrast, weighted and unweighted UniFrac revealed significant dissimilarities in beta diversities of SA and CA ($P = 0.044$ and 0.002, respectively; Fig. 4B). Taxonomic analyses at the phylum level under conditions of average relative abundance >0.1% demonstrated a significantly increased Spirochaetes abundance in CA than that in SA ($P = 0.048$). At the genus level, *Rothia*, *Moraxella*, *Finegoldia*, *Cutibacterium*, *Fusicatenibacter*, *Legionella*, *Corynebacterium*, *Capnocytophaga*, *Granulicatella*, and *Robinsoniella* abundances were significantly higher in SA than that in CA ($P = 0.005$, 0.016, 0.016, 0.022, 0.032, 0.013, 0.001, 0.042, 0.020, and 0.033, respectively; Fig. 4C). Additionally, *Bacteroides* abundance was lower in CA than that in SA, although the difference was not statistically significant ($P = 0.051$). *Treponema*, *Anaerotignum*, *Frisingicoccus*, *Extibacter*, and *Ralstonia* abundances were significantly greater in CA than that in SA ($P = 0.048$, 0.005, 0.010, 0.037, and 0.010, respectively; Fig. 4D).

Influence of prehospital antibiotics

The five AA patients listed in Table 1 received antibiotics before being transferred to our department. We performed a MaAsLin2[18] analysis, adjusting for the effect of prehospital antibiotic administration. Consequently, the relative abundance of *Fusobacterium* in the saliva and feces of the AA group was significantly higher than in those of the HC





Category	No. subject	Weighted Unifrac		Unweighted Unifrac	
		R^2	adjusted *p*-value	R^2	adjusted *p*-value
HC vs AA	HC: 16 AA: 33	0.030	0.18	0.027	0.08

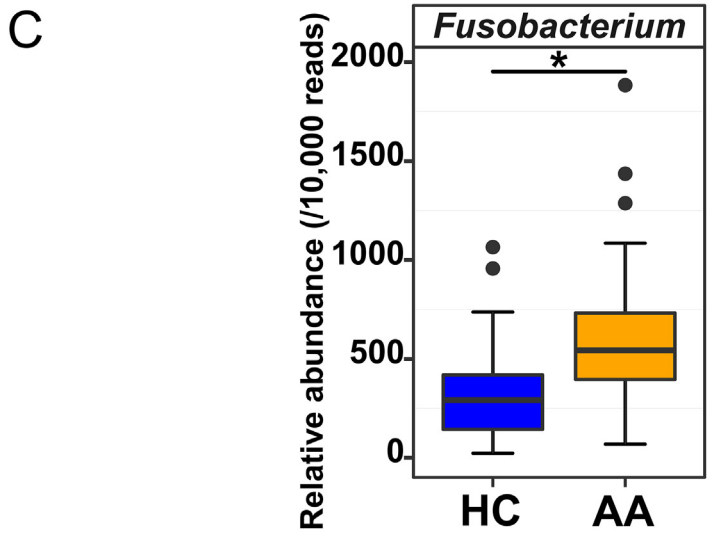
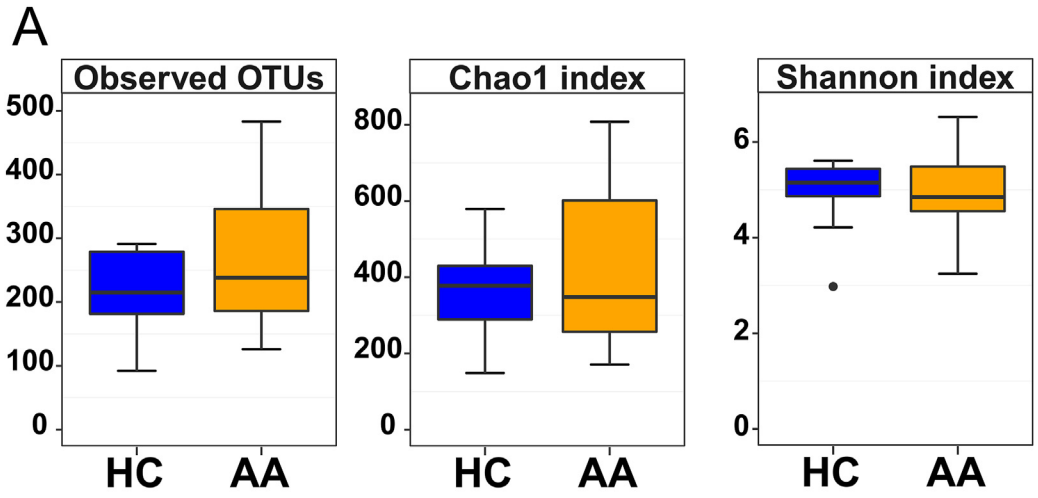
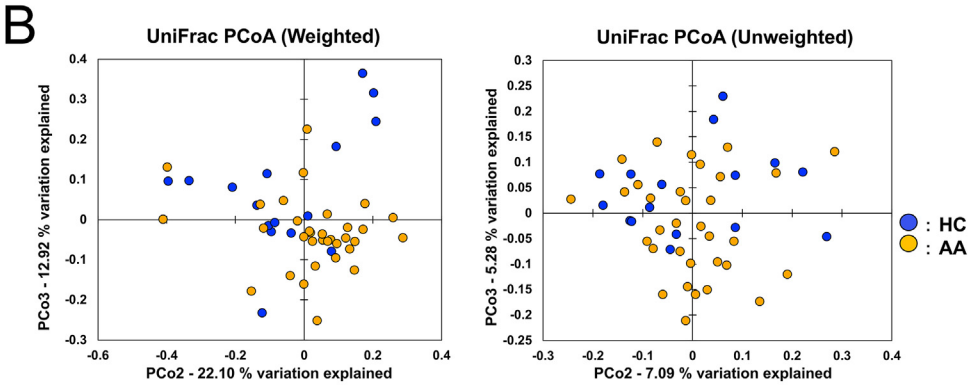


Fig. 1. Comparison of salivary microbiomes between HC and AA. (A) Alpha-diversity indices. (B) Beta-diversity indices. (C) Significantly increased or decreased microbial genera between the two groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. AA: acute appendicitis, HC: healthy control.





Category	No. subject	Weighted Unifrac		Unweighted Unifrac	
		R^2	adjusted *p*-value	R^2	adjusted *p*-value
HC vs AA	HC: 16 AA: 33	0.017	0.55	0.025	0.15

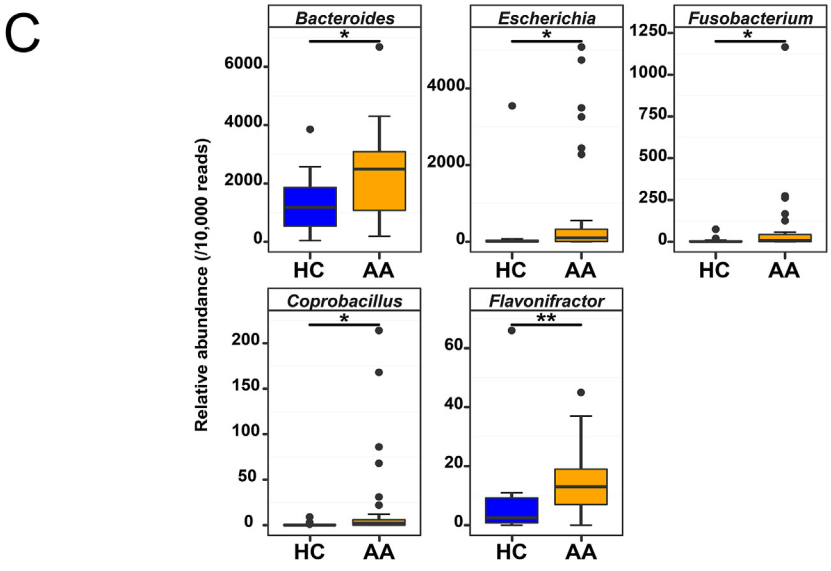


Fig. 2. Comparison of fecal microbiomes between HC and AA. (A) Alpha-diversity indices. (B) Beta-diversity indices. (C) Significantly increased or decreased microbial genera between the two groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. AA: acute appendicitis, HC: healthy control.

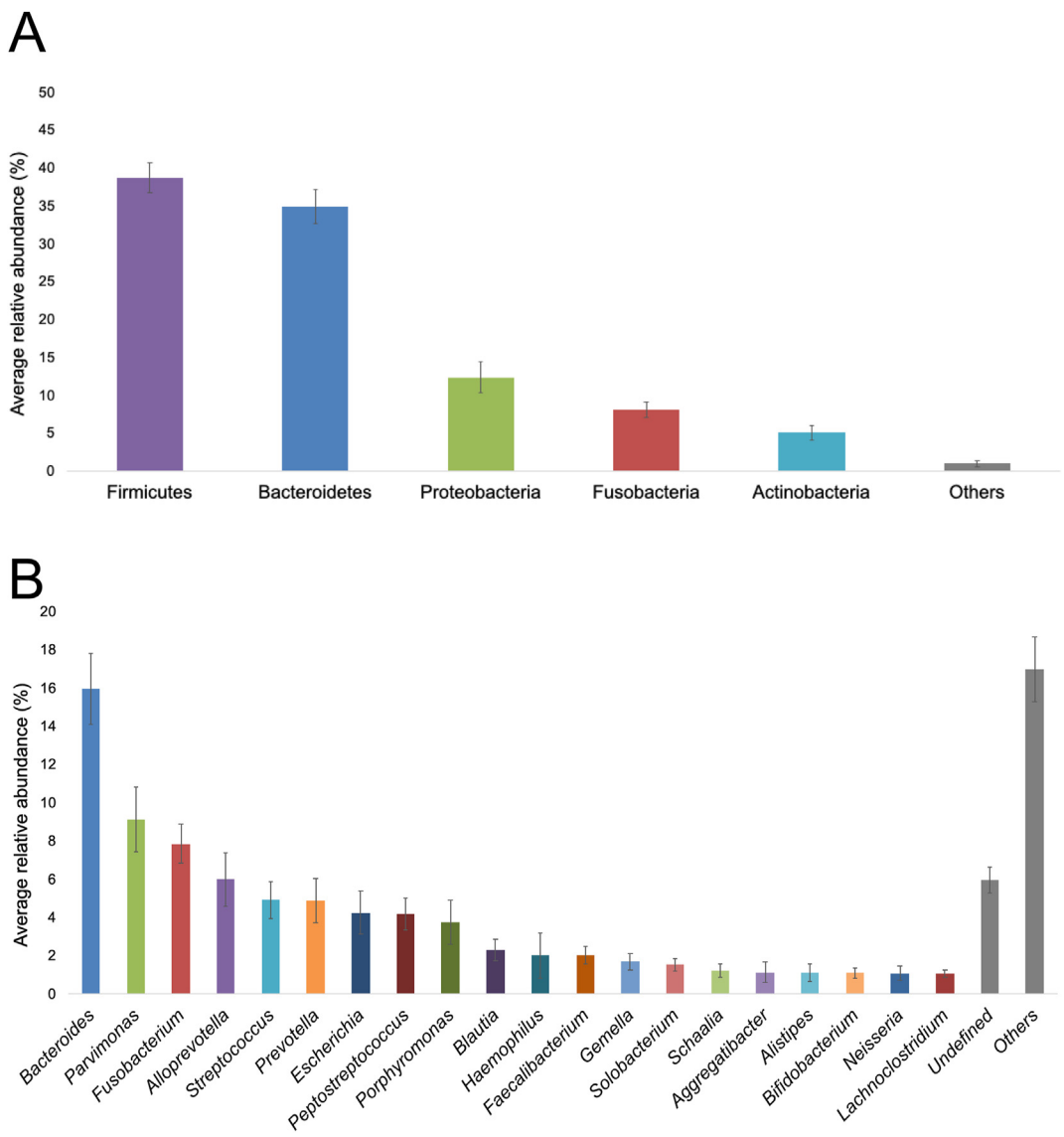


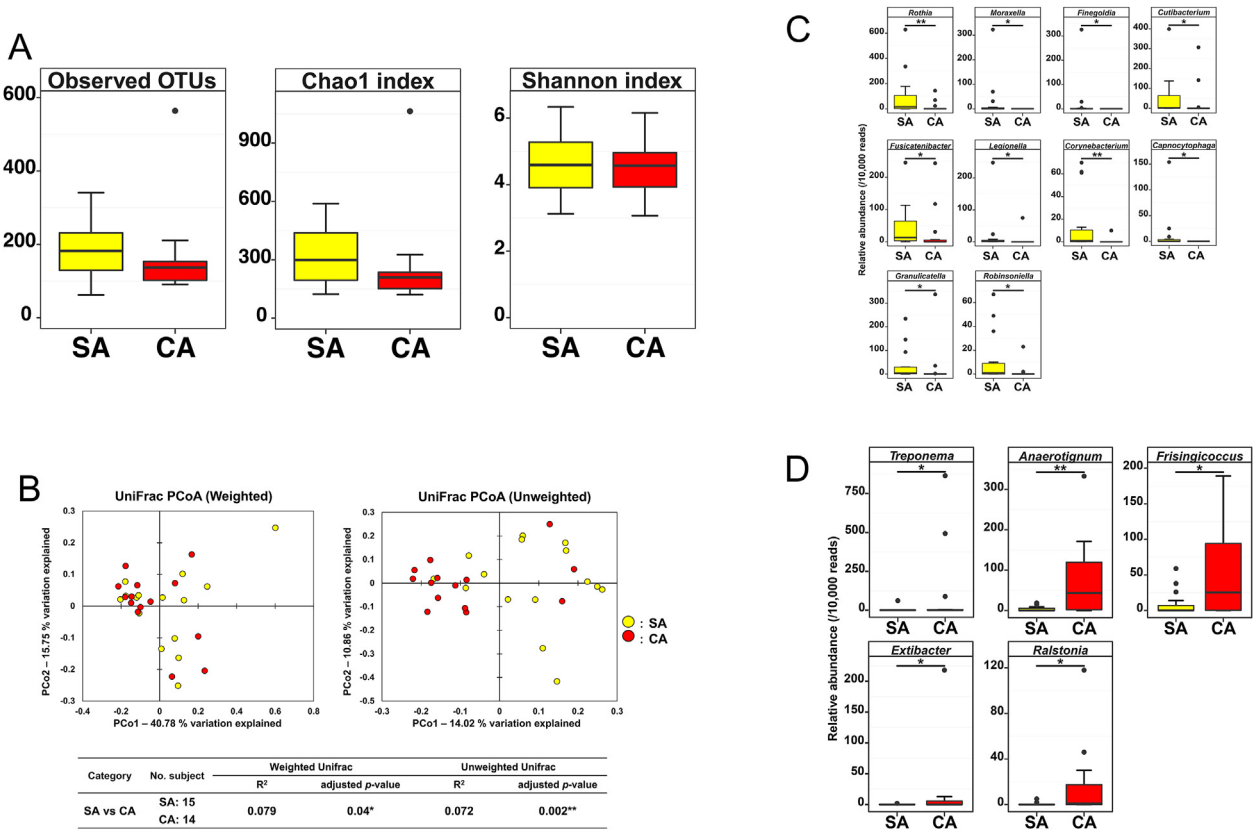
Fig. 3. Microbial profiling of appendiceal lumen in AA patients. (A) Average relative microbial abundance (± standard error) in the appendiceal lumen at the phylum level. (B) Average relative microbial abundance (± standard error) in the appendiceal lumen at the genus level. AA: acute appendicitis.

group, even after adjustment (saliva, $P = 0.006$; feces, $P = 0.028$).

Discussion

In this study, we comprehensively analyzed the microbiomes in salivary, fecal, and appendiceal lumen samples of pediatric AA patients to elucidate the pathogenesis of AA in children. Our results showed that the relative abundance of *Fusobacterium* was high in the appendiceal lumen of pediatric AA patients, and its abundance in the saliva and feces was significantly higher in pediatric AA patients than that in pediatric HCs.

Fusobacterium is a commensal bacterium residing in the human oral cavity causing periodontal disease.[19] Additionally, it is associated with gastrointestinal diseases, including colorectal cancer (CRC) and inflammatory bowel disease.[20,21] Interestingly, Swidsinski et al. performed a bacterial FISH analysis in AA patients and discovered that *Fusobacterium* sp., mainly *Fusobacterium nucleatum/necrophorum*, infiltrated the appendiceal submucosa.[6] Furthermore, they revealed a positive correlation between the presence of *Fusobacterium* sp. in the appendiceal mucosa and appendicitis severity.[6] Several studies have revealed that the relative abundance of *Fusobacterium* in the appendiceal lumen of AA patients is higher than that in individuals without appendicitis using 16S rRNA gene amplicon sequencing.[8—10] In this study, *Fusobacterium* was the third most abundant genus in the appendiceal lumen of AA patients, with an average relative abundance of approximately 8%. Moreover, consistent with previous studies, the relative abundance of oral bacteria, such as *Parvimonas*, *Alloprevotella*, *Streptococcus*, *Prevotella*,



Category	No. subject	Weighted Unifrac		Unweighted Unifrac	
		R^2	adjusted *p*-value	R^2	adjusted *p*-value
SA vs CA	SA: 15 CA: 14	0.079	0.04*	0.072	0.002**

Fig. 4. Comparison of microbiomes in the appendiceal lumen by AA severities. (A) Alpha-diversity indices. (B) Beta-diversity indices. (C) Significantly increased microbial genera in SA in comparison to that in CA. (D) Significantly increased microbial genera in CA in comparison to that in SA. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. SA: simple appendicitis, CA: complicated appendicitis, AA: acute appendicitis.

Peptostreptococcus, and *Porphyromonas*, was high in the appendiceal lumen of AA patients.[7–9] Among these, *Parvimonas* had the highest relative abundance. Incidentally, *Parvimonas* is among the major pathogens causing periodontal disease.[22] Additionally, several studies have reported that the relative abundance of *Parvimonas* is higher in the feces and intestinal mucosa of CRC patients than that in the feces and intestinal mucosa of controls.[23–25] Interestingly, Horiuchi et al. reported that *Parvimonas micra* and *F. nucleatum* have synergistic effects on biofilm formation.[26] Therefore, oral bacteria, such as *Fusobacterium* and *Parvimonas*, may potentially form biofilms in the appendiceal lumen of pediatric AA patients, contributing to mucosal inflammation.

Recent studies indicate that the migration of oral bacteria to the gastrointestinal tract induces intestinal inflammation. First, Atarashi et al., using gnotobiotic techniques, showed that *Klebsiella* strains isolated from the saliva of patients with Crohn's disease strongly induced T helper 1 cells when colonizing the gut.[12] Second, Kitamoto et al. demonstrated that oral bacteria, including *Klebsiella* and *Enterobacter*, migrate to the intestine, activate the inflammasome in colonic mononuclear phagocytes, and ultimately trigger inflammation in a periodontal disease mouse model.[13] They also discovered that oral pathobiont-reactive T helper 17 cells, which can be activated by translocated oral pathobionts, migrate to the inflamed gut and cause the development of colitis.[13] The mechanism of *Fusobacterium* migration from the oral cavity to the lower intestine and the subsequent gut inflammation has not been elucidated. However, these findings support the hypothesis that oral *Fusobacterium* might migrate and colonize the gastrointestinal tract, triggering inflammation of the appendix.

We compared the microbiome of the appendiceal lumen among patients with different AA severities. Interestingly, the relative abundance of Spirochaetes and *Treponema* was significantly higher in CA than that in SA. *Treponema* causes periodontal disease and can induce abscesses, causing necrotic and ulcerative lesions.[27] *Extibacter*, whose abundance was also increased in the appendiceal lumen of CA patients, produces secondary bile acids, including deoxycholic acid, in mouse intestines.[28] Incidentally, elevation in the intestinal levels of secondary bile acids is cytotoxic.[29] Therefore, the differences between SA and CA may not only be due to a simple difference in duration since disease onset but may also involve differences in bacterial composition within the appendiceal lumen.

Since all study participants were treated at a single hospital in Japan, we have the advantage of uniformity in diagnosis and sample handling and preparation. Nevertheless, there were certain limitations. First, the number of participants was relatively small. However, compared with previous studies that analyzed salivary and/or fecal

samples of AA patients, this study analyzed the largest number of samples.[9—11] Second, the HC group did not comprise completely healthy participants. However, the control patients with inguinal hernia did not have a history of incarceration or other gastrointestinal diseases. Therefore, its influence on gut microbiota can be considered minimal compared with that of healthy children without inguinal hernias. Third is the influence of antibiotics. As preoperative antibiotic prophylaxis, cefmetazole was administered after salivary and fecal collection and before the appendectomy. Therefore, it would not have affected the microbiomes of the saliva and fecal specimens. Regarding the appendiceal lumen specimens, some bacteria may have been killed; however, 16S rRNA gene amplicon sequencing can analyze dead bacterial DNA. Therefore, it is unlikely that the antibiotics affected our assessment. Prehospital antibiotic administration did not affect the main findings of the study, as described in the Results section.

In conclusion, this is the first comprehensive analysis of the microbiomes of saliva, feces, and appendiceal lumen in pediatric AA patients. We revealed that *Fusobacterium* was more abundant in the saliva and feces of pediatric AA patients than in those of healthy children. Hence, *Fusobacterium* might migrate from the oral cavity and colonize ectopically in the appendix leading to the pathogenesis of pediatric AA.

Financial support

This work was supported by JSPS KAKENHI (to JF, Grant Number: 19H05561) and a RIKEN Integrated Symbiology Grant (to WS).

Declaration of competing interest

The authors have no conflicts of interest to disclose.

Acknowledgments

We would like to thank the entire staff of the Department of Pediatric Surgery, Faculty of Medicine, University of Tsukuba, for obtaining informed consent from the participants and collecting necessary samples. We would also like to thank C. Shindo, K. Kaida, M. Tanokura, and M. Takagi for their technical support in the Laboratory for Microbiome Sciences, RIKEN Center for Integrative Medical Sciences.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.03.006.