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Published in:
Pediatric infectious disease journal

DOI:
10.1097/INF.0000000000002434

Published: 01/01/2019

Citation for pulished version (APA):
The, S.-M. M. L., Bakx, R., Budding, A. E., de Meij, T. G. J., van der Lee, J. H., Bunders, M. J., Poort, L., Heij, H. A., van Heurn, L. W. E., & Gorter, R. R. (2019). Microbiota of Children With Complex Appendicitis: Different Composition and Diversity of The Microbiota in Children With Complex Compared With Simple Appendicitis. *Pediatric infectious disease journal*, *38*(10), 1054-1060. https://doi.org/10.1097/INF.0000000000002434

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Microbiota of Children With Complex Appendicitis

Different Composition and Diversity of The Microbiota in Children With Complex Compared With Simple Appendicitis

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Background: Two types of appendicitis are hypothesized, simple and complex, with potential different treatment strategies. To improve differentiation, underlying pathogeneses need to be further unraveled.

Aim: To determine if the microbial composition in the appendix differs between children with simple and complex appendicitis.

Methods: Two-center, prospective cohort study including 40 children (0–17 years old) undergoing appendectomy for suspected appendicitis. Appendix tissue was used for IS-pro analysis to identify bacterial species by their length of 16S-23S rDNA interspacer (IS) region. Cluster analysis, based on IS-profiles, and correspondence with type of appendicitis, using Fisher exact test, was performed. Simple and complex appendicitis were compared regarding bacterial presence, intensity and diversity, using Fisher exact test and Mann-Whitney *U* test, respectively.

Results: Appendicitis was confirmed in 36 of 40 patients (16 simple, 20 complex). Cluster analysis identified 2 clusters, encompassing 34 patients. Distribution of simple and complex appendicitis was 12 (80%) and 3 (20%) versus 3 (16%) and 16 (84%) patients for clusters 1 and 2, respectively ($P < 0.001$). Complex appendicitis was on phylum level characterized by an increased intensity (*Bacteroidetes* $P = 0.001$, *Firmicutes*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* (*FAFV*) $P = 0.005$ and *Proteobacteria* $P < 0.001$) and diversity (*Bacteroidetes* $P = 0.001$ and *Proteobacteria* $P = 0.016$) and an increased abundance of 5 species (*Alistipes finegoldii* $P = 0.009$, *Bacteroides fragilis* $P = 0.002$, *Escherichia coli* $P = 0.014$, *Parvimonas micra* $P = 0.022$ and *Sutterella* spp $P = 0.026$).

Conclusions: The microbial composition of the appendix differs between children with simple and complex appendicitis, regarding both composition and diversity. Future research should focus on the role of these bacteria in the pathogenesis of both types and its implications for preoperative diagnostics.

Key Words: complex appendicitis, simple appendicitis, intestinal microbiota, bacterial infection, pediatric surgery

(*Pediatr Infect Dis J* 2019;38:1054–1060)

Accepted for publication June 20, 2019.

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This work was supported by the foundation of research and management projects in paediatric surgery (KCHOMP). The KCHOMP did not have any influence in the design or conduct of this study. [Grant number €8.000]

A.E.B. is shareholder in IS-diagnostics (Amsterdam, The Netherlands). All remaining authors declare no conflict of interest.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (www.pidj.com).

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ISSN: 0891-3668/19/3810-1054
DOI: 10.1097/INF.0000000000002434

Appendicitis is a common gastrointestinal inflammatory disease in the Western world[1] with peak incidences among young children and adolescents. Until recently, it was considered to be an irreversible progressive disease provoked by intraluminal obstruction, eventually leading to a perforation. Novel insights question this progressive nature and rather support that 2 distinct types of appendicitis exist: simple (uncomplicated) and complex (complicated) appendicitis.[2–4] These types differ in terms of epidemiologic, clinical, biochemical and radiologic variables.[5–10] Moreover, safe and potentially effective results were demonstrated for a nonsurgical (initial antibiotics alone) treatment for simple appendicitis.[11,12] Accurate preoperative discrimination is thus important for both implementations of different treatment strategies as their comparison in research. Unfortunately, none of the previous developed clinical prediction rules have shown 100% accuracy and new diagnostic strategies need to be explored [13,14]. Recently, it has been reported that gut microbiota might play a role in the pathogenesis of a variety of gastrointestinal diseases [eg, inflammatory bowel disease (IBD)] and extra-intestinal (autoimmune) diseases. In this light, the microbiota of the appendix is worth mentioning, since its role in the pathogenesis of appendicitis has been previously considered.[15] In addition, novel, culture-independent techniques for microbiota analysis have shown compositional differences between noninflamed and inflamed appendices.[16–21] Nevertheless, the role of the microbiota in specifically each type is largely unknown. To contribute to the available literature, we aimed to elucidate the microbial composition of the appendix in children with simple and complex appendicitis, using a culture-independent DNA-based microbiota detection technique to detect differences and to identify specific disease-related bacteria.

MATERIALS AND METHODS

Design and Participants

This pilot cohort study was performed between November 2015 and November 2016 in 2 hospitals in the Netherlands (VU Medical Centre, Amsterdam and Red Cross Hospital, Beverwijk). The medical ethics committee of the VU Medical Centre confirmed that the medical research involving human subjects act (WMO) did not apply to this study. All children (0–17 years old), presenting at the Emergency Department for the suspicion of appendicitis underwent routine diagnostic work-up as described in our national guideline.[22] In summary, in case of clinical suspicion of appendicitis, based upon medical history, physical examination and biochemical signs of inflammation (elevated white blood cell count and/or C-reactive protein), imaging studies (ultrasound and if required magnetic resonance imaging) were undertaken. In case appendicitis was highly suspected upon this (since confirmation is only possible on histopathologic examination), children were scheduled for appendectomy and eligible for inclusion. Prior to surgery, informed consent was obtained from parents and the child (if ≥12 years) for

data collection from their clinical files and to acquire a part of the removed appendix for further analysis. We excluded children with the suspicion of an alternative diagnosis or other pathology (intraoperative or at histopathology) than appendicitis, such as a malignancy. All patients were included in a consecutive manner.

Surgical Procedure and Sample Collection

Appendectomy was performed according to local/national protocol. All children received prophylactic antibiotics ~30 minutes before incision: (1) metronidazole (<12 years: 7.5 mg/kg/dose iv with a maximum of 500 mg/dose, ≥12 years: 500mg/dose iv) with a cephalosporin (cefazolin 100–150 mg/kg/day in 3 doses or cefuroxime 50 mg/kg/dose with maximum of 1.5 g/dose) or (2) Amoxicillin-clavulanic acid (<40 kg: 100/10 mg/kg/day in 3–4 doses, >40 kg: 1000/100 to 2000/200 mg/dose iv) with gentamycin (7 mg/kg/day). Approach was either by the gridiron incision (open procedure) or by laparoscopy based upon the surgeon's preference. Postoperative care (antibiotics) was according to the respective diagnosis and in line with national guidelines.[22] After resection of the appendix, 0.5 cm of the tip of the appendix was immediately frozen at −20°C until further microbiota analysis. The remainder of the appendix was sent for routine histopathologic examination. Additional data collection of patient files was performed according to a predefined data form.

Clinical and Pathologic Classification

Only children with histopathologically confirmed appendicitis were included in final microbiota analysis. Samples of these children were classified as either simple or complex based upon predefined criteria (for both hospitals in the same manner) by 2 of the authors (R.B. and R.R.G.) independently. Complex appendicitis was defined as a gangrenous or perforated appendix found intraoperatively with or without purulent intra-abdominal fluid or abscess formation or; histopathologic signs of extensive necrosis, ulceration or perforation of the appendix. Simple appendicitis was defined as inflammation of the appendix, with transmural invasion of neutrophils, in the absence of all of the abovementioned findings of complexity. In case of disagreement, discussion was held until consensus was reached. The researchers performing the microbial cluster analysis were blinded for the classified type of appendicitis (S.M.L.T., A.E.B., and L.P.).

Sample Work-up and DNA Isolation

All frozen samples were thawed on ice and 0.3 × 0.3cm of the center of the sample, including all available layers, was taken for analysis. Remainders were frozen at −80 for future research. A routine molecular diagnostic protocol was used to isolate DNA.[23] At first, 360 µl ATL buffer and 40 µl proteinase K were added to each sample. After vortexing and shaking, 400 µL of AL buffer was added. We used an easyMAG automated DNA isolation machine of Biomerieux for further DNA extraction. Samples were put in easyMAG containers and suspended in nucliSENS lysis buffers. After incubation silica beads were added. The specific "A" protocol of the machine was used and DNA was eluted in nuclisens easyMAG extraction buffer 3. After the full DNA isolation, it was stored at 2–8°C for PCR amplification. During every DNA extraction and PCR reaction, controls (empty samples) were used to evaluate reagent contamination for internal control.

IS-pro Profiling and Analysis

A PCR-based IS-pro assay was performed by IS-diagnostics, Amsterdam, The Netherlands.[23] DNA was isolated and amplified with a GeneAmp PCR system 9700 and used for 2 multiplex PCR reactions. The first consisted of *FAFV* and *Bacteroidetes*. The second consisted of *Proteobacteria.* Subsequently, the product was mixed with IS-pro eMix (IS-Diagnostics) and fragment analysis was performed on an ABI Prism 3500 Genetic Analyzer. If the PCR analysis was inhibited, the DNA was diluted 1:10 or 1:100 and the analysis was rerun. All IS profiles contained 3 levels of information. First, the fluorescent labels or peak-colors sorted species into the 3 main gastrointestinal phyla (*FAFV*, *Bacteroidetes* and *Proteobacteria*). Second, the length of 16S-23S rDNA IS regions, expressed by the number of nucleotides, were used to identify individual IS fragments. These individual IS fragments reflect bacterial operational taxonomic units and were linked to a database to identify bacteria at species level. This database was based upon all publicly available bacterial whole genome sequences (approximately ±1.2 million) in 2018. Third, the intensity of the PCR product is displayed by peak heights, visualized as (relative) abundance and expressed in relative fluorescence units (RFU).

Microbiota and Statistical Analysis

The elementary analysis of this research was to determine if two or more types of appendicitis could be distinguished upon microbiota profiles. For this, a hierarchical cluster analysis was performed using the unweighted pair group method with arithmetic mean.[24] In short, the aim of a cluster analysis is to statistically, and objectively, classify samples into several categories or clusters. Subsequently, the Fisher exact can be used to analyze the association of the identified clusters with type of appendicitis (SPSS software version 22). Level of significance was determined as $P < 0.05$. The visualization of acquired IS-profiles was performed with TIBCO Spotfire software. Complementary to the cluster analysis, a comparison between simple and complex appendicitis was made at phylum and species level, the highest and lowest taxonomic rank within the domain of bacteria, respectively; presence of each phylum, based upon the previously mentioned allocation of peak-colors, was reported descriptively (numbers and percentages); the Shannon Diversity index was used to calculate diversity (number of different species) for each phylum; (relative) abundance in RFU's, intensity, of bacteria for each phylum was determined; presence of individual species was determined and reported descriptively. Mann-Whitney *U* test (diversity and abundance) and Fisher's exact test (presence) were used when appropriate. Association of presence was only calculated in case species were present in at least 5 samples of the same group. Visualization was performed with Prism Graphpad software, version 7.

RESULTS

In total, 40 children who underwent an appendectomy for suspected appendicitis were included in this study. The diagnosis of appendicitis (nor any other diagnosis) could not histopathologically be confirmed in 4 children. Of the 36 children with confirmed appendicitis, immediate agreement of classification was reached in 29 (81%). After discussion, the remaining samples were, classified as simple in 2 cases and complex in 5. Main topic of debate was the assessment and extent of necrosis and ulceration of these samples. Baseline characteristics of both groups, simple (n = 16) and complex (n = 20), are presented in Table 1.

Cluster Analysis and Characteristics

Figure 1 displays the cluster analysis of the 36 included samples. It shows 2 distinct high-order clusters encompassing 34 of 36 (94%) appendicitis samples. Two samples were not allocated to one of the two clusters based upon their microbiota profiles (1 simple and 1 complex, respectively). Cluster 1 encompassed 15 and Cluster 2 19 patients. Both clusters were closely related to the blinded classification of appendicitis phenotype: Distribution of simple and complex appendicitis was 12/15 (80%) and 3/15 (20%)

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TABLE 1. Baseline Characteristics

	All	Simple	Complex
Number of patients	36	16	20
Age	11 (1–17)	11 (6–17)	12 (1–16)
Male gender*	18 (50)	8 (50)	10 (50)
Days of abdominal pain	1 (1–8)	1 (1–4)	2 (1–8)
Temperature	37.4 (36.0–40.0)	36.9 (36.3–37.9)	37.7 (36.0–40.0)
C-reactive protein	40 (1–262)	25 (1–262)	59 (7–247)
Leukocyte count	16.1 (6.0–25.5)	12.3 (6.0–21.0)	17.2 (11.2–25.5)
Prophylactic antibiotics regimen*			
Metronidazole + cephalosporin	29 (81)	14 (88)	15 (75)
Amoxicillin-clavulanic acid + gentamycin	7 (19)	2 (12)	5 (25)

* Results presented as numbers of patients with (percentage).
Results are presented as median with (min–max) for the rest.

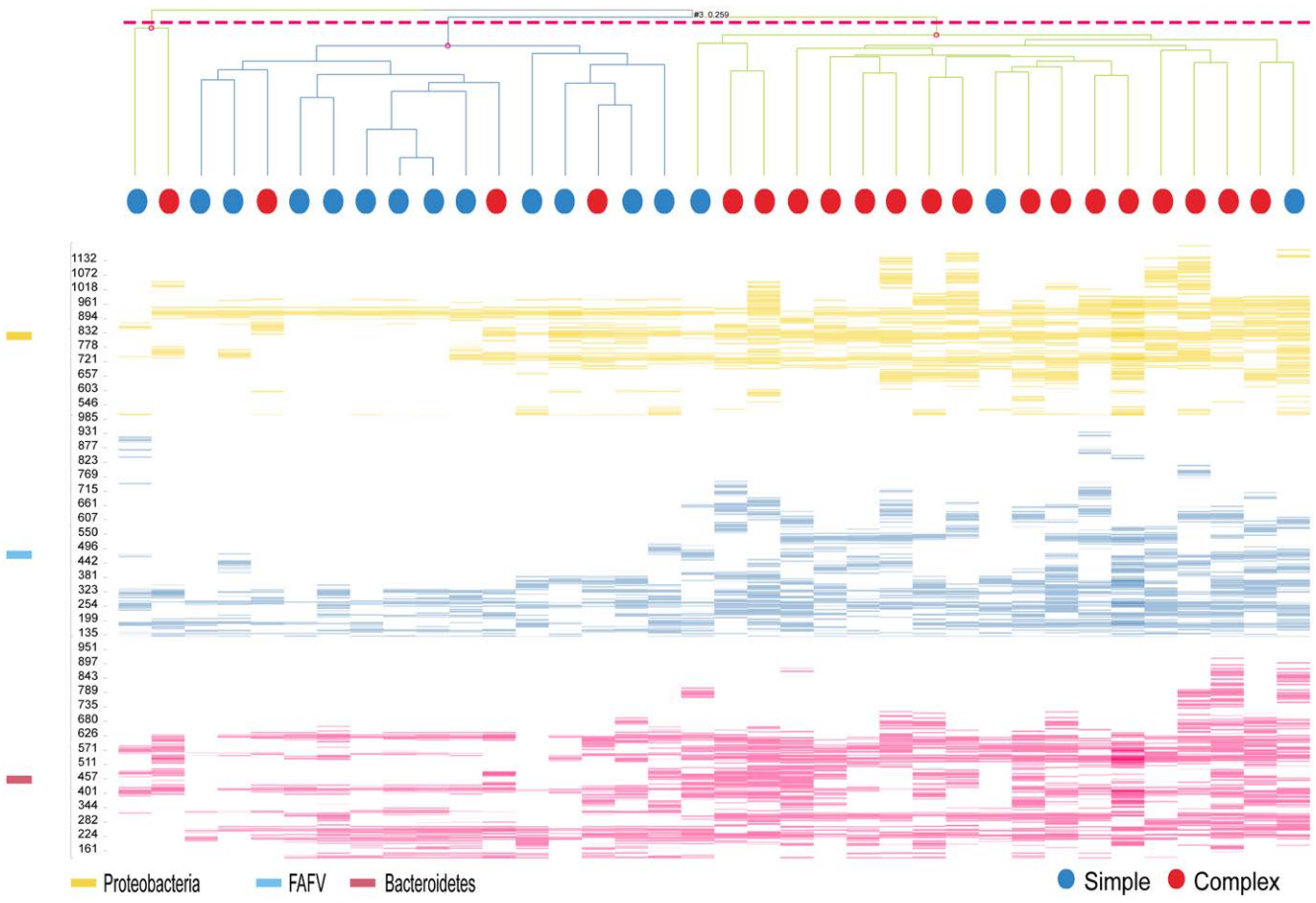

FIGURE 1. Cluster analysis. All 36 patients with histopathologically confirmed appendicitis are represented on the *x*-axis. Each dot represents an individual sample/patients, with color coding for simple and complex appendicitis. Nucleotide lengths of 3 main phyla Bacteroidetes, *FAFV* and Proteobacteria are represented on the *y*-axis. Cluster 1 (n = 15) is represented in blue on the left side of the *x*-axis. Cluster 2 is represented in green on the right side of the *x*-axis. full color online

versus 3/19 (16%) and 16/19 (84%) patients for Clusters 1 and 2, respectively ($P < 0.001$).

Phylum Analysis

Species within the phyla *Bacteroidetes*, *FAFV* and *Proteobacteria* were detected in all samples with complex appendicitis compared with 100%, 94% and 56% of the samples with simple appendicitis, respectively. In addition, complex appendicitis was associated with increased diversity of the phyla *Bacteroidetes* ($P = 0.001$) and *Proteobacteria* ($P = 0.016$), but not *FAFV* ($P = 0.062$) (Figure 2A). Moreover, a relative increased abundance, intensity (RFU), of all phyla was found in complex appendicitis: *Bacteroidetes* ($P = 0.001$), *FAFV* ($P = 0.005$) and *Proteobacteria* ($P < 0.001$) (Figure 2B).

Species Analysis

In all samples combined, a total of 57 species could be identified within 31 genera (Supplemental Table 1, http://links.lww.com/INF/D571). Fifteen species were detected in 5 or more samples

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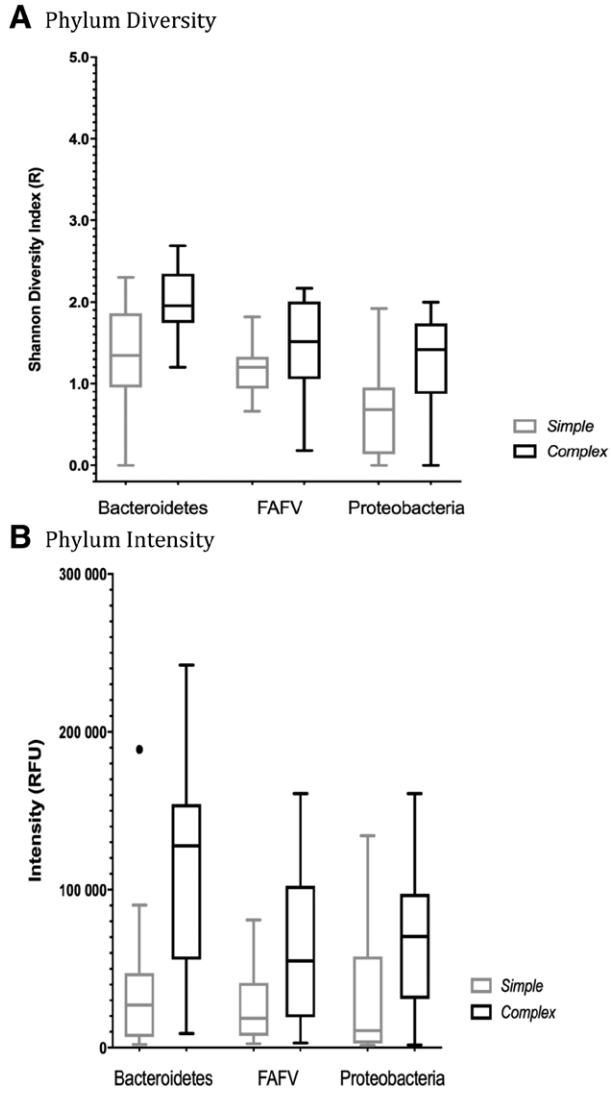

FIGURE 2. Phylum diversity analysis. **A**: On the *x*-axis, type of appendicitis is provided per phylum: Bacteroidetes, *FAFV* and Proteobacteria. On the *y*-axis, diversity is represented as Shannon Diversity index. Complex appendicitis is displayed in black, simple appendicitis in gray. Increased diversity was found in complex appendicitis for *Bacteroidetes* ($P = 0.001$) and *Proteobacteria* ($P = 0.016$), but not for *FAFV* ($P = 0.062$). **B**: On the *x*-axis, each of 3 phyla is provided: Bacteroidetes, *FAFV* and Proteobacteria. On the *y*-axis, bacterial load is represented as intensity in RFU. Complex appendicitis is displayed in black, simple appendicitis in gray. Increased intensity of all phyla was found in complex appendicitis: *Bacteroidetes* ($P = 0.001$), *FAFV* ($P = 0.005$) and *Proteobacteria* ($P < 0.001$)

of the same group, with their relative abundance presented in Figure 3. Results of these 15 species in terms of presence and relative abundance are listed in Table 2. Of these species, 5 were significantly more often present in complex appendicitis: *Alistipes finegoldii* (6% vs. 50%, $P = 0.009$), *Bacteroides fragilis* (25% vs. 80%, $P = 0.002$), *Escherichia coli* (44% vs. 85%, $P = 0.014$), *Parvimonas micra* (6% vs. 45%, $P = 0.022$) and *Sutterella* spp (6% vs. 40%, $P = 0.026$).

DISCUSSION

To our knowledge, this study is the first to show differences in microbial composition in the appendix of children with simple and complex appendicitis (not only perforated), using a culture-independent DNA-based microbiota detection technique, IS-pro. We observed 2 microbial clusters, closely related to the intraoperative and histopathologic classification, strengthening the theory of 2 different phenotypes of appendicitis. In this study, complex appendicitis showed an increased diversity of species within the phyla *Bacteroidetes* and *Proteobacteria* and an increased intensity of the phyla *Bacteroidetes*, *FAFV* and *Proteobacteria* compared with simple appendicitis. More specifically, 5 different species (*A. finegoldii*, *B. fragilis*, *E. coli*, *P. micra* and *Sutterella* spp) were found more often present in the appendix of children with complex appendicitis. Results from this study are of importance as they form the basis of future studies, investigating whether or not these found microbial differences reflect underlying different pathogeneses and if they can be useful in accurate preoperative (noninvasive) discrimination.

Microbiota in Children With Appendicitis

In 2000, Carr[15] stated that multiple factors could cause appendicitis, eventually resulting in the invasion of the appendix by intraluminal bacteria. Preceding studies investigating intraluminal bacteria during appendicitis used conventional in vitro culture techniques. Most common findings were the presence of *E. coli, Bacteroides* spp or *B. fragilis*, *Pseudomonas aeruginosa* and *P. micra* in the inflamed appendix.[25–28] Relevance of this conventional, culture-dependent technique lies in the determination of antibiotic resistance and thus proper antibiotic choice. Unfortunately, the majority of gastrointestinal microbial species is not cultivable. In our opinion, this technique is therefore not suitable to describe the complex microbial composition in the appendix. Luckily, possibilities expanded with the emergence of DNA-based detection techniques, like PCR, providing a culture-independent analysis and several studies have been published using these techniques in the field of appendicitis.[16–21]

Of interest in these studies is the reported increased abundance of *Fusobacteria*, especially of *Fusobacterium nucleatum* (an oral pathogen) in appendicitis, since its increase was also linked to severity.[16–19] We found *Fusobacteria* in about a third of all patients in our study, and an increased trend was found in complex appendicitis (25% simple vs. 50% complex), but the difference was not statistically significant and we could therefore not confirm its association with complex appendicitis. Although *Fusobacteria* was most often mentioned, it was not the only found oral pathogen. Consistent with previous literature, we reported an increased presence of *P. micra* in complex appendicitis.[18,19] Taking abovementioned findings together, it may indicate a potential role for oral pathogens in the pathogenesis of particularly complex appendicitis.

Both *B. fragilis* and *E. coli* were commonly found with conventional culture techniques in patients with appendicitis. While most studies reported an increase of *E.*coli in appendicitis, and especially complex appendicitis, the findings of *Bacteroides* and *B. fragilis* in appendicitis are inconsistent. Our study supports the association of an increased presence of *B. fragilis* with complex appendicitis, but the interpretation of this finding is challenging. A review into the beneficial effects of *B. fragilis* noted that the species is considered commensal in the healthy gut with immune-modulatory functions.[29] While on the other hand, the specific strain of *Enterotoxigenic B. fragilis (ETBF)* is associated with IBD, especially in active disease.[30] Could these specific *Enterotoxigenic B. fragilis* be present in complex appendicitis?

The presence of *Sutterella* spp in predominantly complex appendicitis is to our knowledge not previously described. The

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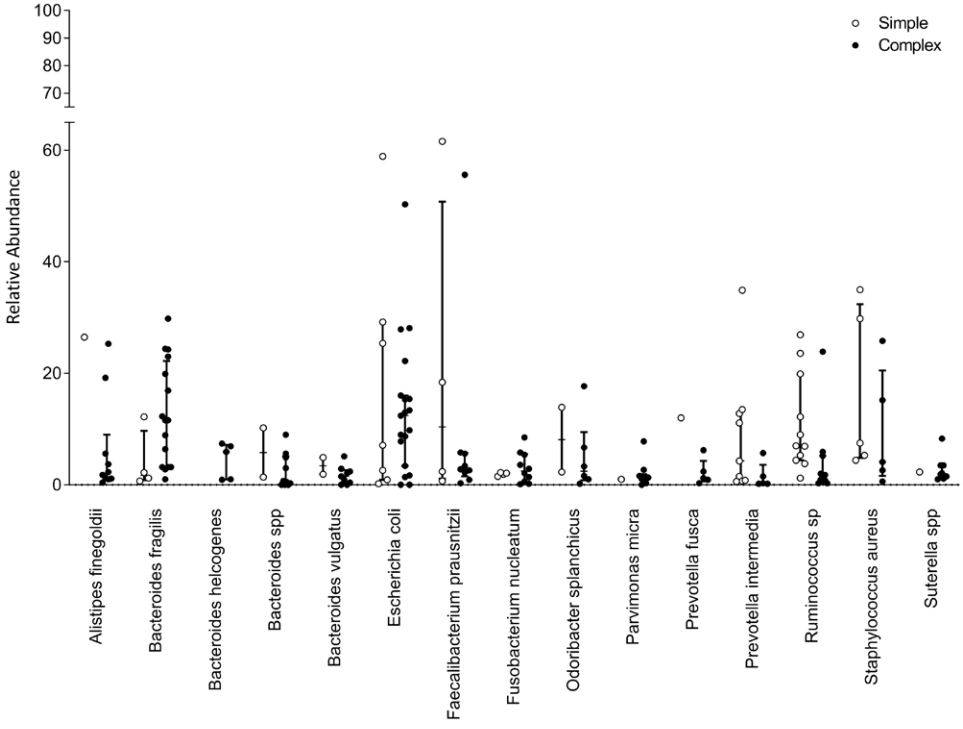

FIGURE 3. Relative abundance analysis. On the *x*-axis is each species identified. Relative abundance is presented on the *y*-axis of all samples in which the unique species was found present). Data are displayed as relative distribution (0–1.0). Complex appendicitis is displayed in black, simple appendicitis in gray. No statistical analysis was performed.

TABLE 2. Species Analysis

	Presence Number (%)				Relative Abundance†	
	All	Simple	Complex	*P*	Simple	Complex
Number of patients	36	16	20	–	16	20
Alistipes finegoldii	11 (31)	1 (6)	10 (50)	0.009	0.265	0.062
*Bacteroides fragilis**	20 (56)	4 (25)	16 (80)	0.002	0.041	0.127
Bacteroides helcogenes	5 (14)	0 (0)	5 (25)	0.053	–	0.044
Bacteroides spp	9 (25)	2 (13)	7 (35)	0.245	0.058	0.034
Bacteroides vulgatus	9 (25)	2 (13)	7 (35)	0.245	0.034	0.019
Escherichia coli	24 (67)	7 (44)	17 (85)	0.014	0.206	0.150
Faecalibacterium prausnitzii	13 (36)	4 (25)	10 (50)	0.176	0.208	0.088
Fusobacterium nucleatum	14 (39)	4 (25)	10 (50)	0.176	0.019	0.030
Odoribacter splanchnicus	8 (22)	2 (13)	6 (30)	0.257	0.081	0.051
Parvimonas micra	10 (28)	1 (6)	9 (45)	0.022	0.010	0.020
Prevotella fusca	6 (17)	1 (6)	5 (25)	0.196	0.120	0.022
Prevotella intermedia	14 (39)	9 (56)	5 (25)	0.087	0.089	0.016
Ruminococcus sp	22 (61)	11 (69)	11 (55)	0.501	0.109	0.040
Staphylococcus aureus	10 (28)	5 (31)	5 (25)	0.723	0.164	0.096
Sutterella spp	9 (25)	1 (6)	8 (40)	0.026	0.023	0.029

Alfa<0.05 was considered as significant. In case of Bonferroni correction (0.05/15 = 0.003), species with "*" reached significance.

† Relative abundance is presented as mean relative abundance among all patients in which the species was reported. Data shown as relative distribution (0–1.0).

finding is notable since it has been suggested that *Sutterella* spp are able to interact via intra epithelial cells that are capable of directing antigen-presenting cells in the lamina propria into a pro-inflammatory, Th17, response.[31–34] Although, the pro-inflammatory functions of *Sutterella* spp were found to be mild, it is specifically this pro-inflammatory Th17 response that is also mentioned in association with complex appendicitis.[9,10]

Bacterial Diversity in Appendicitis

Bacterial diversity remains a common topic in microbiota research and in inflammatory diseases, such as IBD, often a decreased diversity is found.[35] On the other hand, an increased diversity, as found in complex appendicitis, was demonstrated in patients with *Clostridium difficile* and patients with diverticulitis.[36,37] The consequence or role of this increased bacterial diversity is however still unknown. It must be noted that an increased bacterial diversity in complex appendicitis, or decreased diversity in simple appendicitis may also reflected in the calculated mean relative abundance of all species in Table 2.

Strengths and Limitations

As opposed to earlier research, we used a culture-independent DNA-based microbiota detection technique, the IS-pro technique, which is a nonselective detection method, able to unravel the highly complex intestinal microbiota composition.[23] In a recent validation study, comparable results of intestinal microbiota characterization

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were shown for IS-pro and 454-pyrosequencing.[38] An advantage of the IS-pro technique is the relative fast determination of bacteria (several hours compared with days of culture techniques) with potential benefits for clinical implementation. In addition of this novel technique, a nonsupervised cluster analysis was performed, to objectively determine, instead of subjectively classify, if 2 phenotypes of appendicitis exist. This method was previously and successfully demonstrated in diverticulitis and necrotizing enterocolitis (A.E.B., T.G.J.d.M.]. Moreover, since bacterial infiltration, especially of *Fusobacterium*, was demonstrated in the submucosa of samples of appendicitis, we chose to use appendiceal tissue, instead of swabs of the lumen for analysis (Swidsinksi, 2011).

Firstly, a limitation of this study could be the use of the tip of the appendix since it was therefore not available for histopathologic confirmation of appendicitis. Since inflammation of the appendix tends to start in the tip, it might have led to sampling bias. Secondly, we could not investigate previously described difference between the proximal and distal (tip) parts, since usage of the base of the appendix was reviewed as nonethical (histopathologic assessment is essential for detection and indicated treatment of carcinoid of the appendix).[21] Thirdly, a limitation of this study is the relatively small sample size, which is also reflected in the comparison between species after Bonferroni correction for multiple comparisons. Results of species analysis should therefore be interpreted with caution. Fourthly, in this study, we could not answer a potential correlation between the presence of a fecalith and the microbiome in appendicitis. It is however of interest for future research. At last a limitation of this study is the use of prophylactic antibiotics. However, the beneficial effects of antibiotics have been widely accepted and since they were only administered a short time before operation we believe that this does not explain the found differences.

Future Implications

Results of this study will lead to future studies from our group. Firstly, we hypothesize that the microbial differences are not only present in the appendix itself, but will also be reflected in stool, since previous studies already describe differences in rectal swabs of children with and without appendicitis.[18] Potential future findings may provide us with a novel noninvasive diagnostic modality, such as oral and rectal swabs and analysis of volatile organic compound (VOC). The latter has shown promising results discriminating between children with and without necrotizing enterocolitis, based upon fecal VOC profiling by an electronic nose device.[39] Secondly, we will further investigate the role of specific bacteria in the pathogenesis of simple and complex appendicitis. It must be noted that to date we cannot certainly assess whether the reported findings of this study, or previous studies, are cause or consequence. It would therefore be of great interest to investigate the duration of abdominal pain, as well as other factor such as dietary influences or days of fasting, on the microbiome. We would suggest either a larger study into the topic with stratification of days of abdominal pain or an animal model on appendicitis. Thirdly, it would be of interest to investigate whether or not the reported bacteria in children with complex appendicitis are able to initiate the earlier mentioned Th17-response.

Conclusion

In conclusion, we demonstrate the presence of 2 clusters, based upon the microbial composition of the inflamed appendix, that are closely related to simple and complex appendicitis in the pediatric population, respectively. Complex appendicitis was associated with an increased diversity and intensity, different relative abundance of bacteria and with the increased presence of 5 species compared with simple appendicitis in the appendix itself. Whether these findings and specific bacteria play an etiologic role in either simple or complex appendicitis or are rather a consequence of an inflamed state remains still unclear. Further studies should focus on the exact role of these bacteria and in their potential for use in preoperative discrimination between simple and complex appendicitis.

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Current Abstracts

Edited by Robert J. Leggiadro, MD

Insights From the Geographic Spread of the Lyme Disease Epidemic

Eddens T, Kaplan DJ, Anderson AJM, et al. *Clin Infect Dis*. 2019;68:426–434

Lyme disease is the most common reportable vector-borne infection in the United States. Over the past 4 decades, Lyme disease has become increasingly common throughout the Northeastern and Mid-Atlantic states, as well as regions of Minnesota and Wisconsin. In the Mid-Atlantic region, Lyme disease has been largely restricted to areas east of the Appalachian Mountains. Within western Pennsylvania, populations of the tick vector, *Ixodes scapularis*, have expanded in number and become increasingly infected with *Borrelia burgdorferi*, the infectious agent. Ticks infected with *B. burgdorferi* are now detectable in every county in Pennsylvania, with infection rates similar to that of endemic Northeastern states. Clinical presentations and healthcare use patterns for pediatric Lyme disease in western Pennsylvania were determined to better understand the changing characteristics of pediatric Lyme disease and the implications for healthcare use.

The electronic medical records of all patients with an International Classification of Diseases, 9th revision diagnosis of Lyme disease between 2003 and 2013 at Children's Hospital of Pittsburgh were individually reviewed to identify confirmed cases of Lyme disease. The records of 773 patients meeting Centers for Disease Control and Prevention (CDC) 2011 criteria for Lyme disease were retrospectively analyzed for patient demographics, disease manifestations, and healthcare use. Of these patients, 262 had Lyme disease diagnosed clinically based on erythema migrans rash alone. Serologic diagnosis using CDC 2-tiered testing was performed in 511 patients, with 193 IgM+/IgG−, 121 IgG+/IgM− and 197 IgM+/IgG+ positive Western blots.

Forty-five percent of patients were between 5 and 9 years of age, consistent with previously published CDC surveillance. The patient population was predominantly male (59%) and white (93%). Twenty-nine percent of patients reported a history of tick bite. Lyme disease was more commonly diagnosed in May through August, accounting for 62% of cases. The most common symptom reported or observed in the total study population was erythema migrans rash (56%), followed by joint pain (47%) and fever (45%). Fatigue and headache were present in 30% of cases, and joint swelling and difficulty walking were present in 31% and 22%, respectively. Neurologic symptoms, such as cranial nerve palsy (12%) and neck stiffness (11%), were less common.

There was an exponential increase in the number of cases of pediatric Lyme disease over the study period, with a calculated doubling time of 1.6 years. A southwestward migration of Lyme disease cases occurred in western Pennsylvania, with a shift from rural to nonrural zip codes. As the incidence of Lyme disease increased over time, the type of provider changed. Healthcare provider involvement evolved from subspecialists to primary care pediatricians and emergency departments (EDs). Patients from nonrural zip codes more commonly presented to the ED, while patients from rural zip codes used primary care pediatricians and EDs equally.

Comment: In this study, patients from nonrural zip codes presented to the ED more frequently than patients from rural zip codes, often with manifestations of early Lyme disease (eg, rash). Conversely, patients from rural zip codes were more likely to seek care from a primary care provider with symptoms of late Lyme disease (eg, arthritis). This may reflect limited access to care in rural communities or may represent differing referral patterns of rural and nonrural pediatricians. Targeted provider education and public health awareness based on community urbanization may be an effective strategy to enhance care as the geographic expansion of Lyme disease progresses. An understanding of epidemic changes over time with regard to disease manifestation and healthcare provider use in western Pennsylvania could serve as a model for both rural and nonrural communities that may see an increase in Lyme disease cases.

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