Whole metagenomic sequencing reveals different compositions of pathogenic bacteria in patients with appendicitis:Bacterial culture is not suitable as the only therapeutic guidance

Jie Yuan
Southern Medical University
Enming Qiu
Southern Medical University
Shuai Han
Southern Medical University
Zhou Li (gzlizhou@smu.edu.cn)
Southern Medical University https://orcid.org/0000-0002-4785-261X

Research

Keywords: Appendicitis, Microbiota, Metagenomics, Sequencing

Posted Date: July 27th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-42870/v1

Abstract

Background

Appendicitis is the result of a combination of factors, including bacterial infection, anatomy, blood supply, and intestinal contents. Previous diagnosis and treatment guidelines have suggested that bacteria are associated with the severity of appendicitis, and the use of postoperative antibiotics should be guided according to the results of intraoperative sample culture. However, this approach has many limitations. Therefore, in the present study, the relationship between pathogenic bacteria and appendicitis was assessed.

Result

We conducted a nonconsecutive case series analysis from January to July 2017. Nineteen patients were divided into two groups according to their intraoperative gross pathology and postoperative histological and bacterial culture results. During appendectomy, the diseased appendices were collected, and whole metagenomic sequencing was performed to identify the pathogenic bacteria in the specimens. We identified 361 species in the appendix samples. Six species in the appendix samples had relative abundances > 5%. No significant differences were observed in the bacterial composition of the three assayed groups. In particular, according to the grouping of culture results, the sequencing analysis results were completely different from those of the culture-based method.

Conclusion

Bacterial culture results are not suitable for exclusively guiding the use of antimicrobial agents after appendicitis. Next-generation sequencing has numerous advantages, such as in precisely characterizing the profiles of microbiota and their antibiotic resistance in appendicitis patients. Based on the above results, we propose that a combination of bacterial culture and next-generation sequencing should be used to improve the efficacy of guiding antibiotic therapy.

Introduction

Acute appendicitis (AA) is the most common surgical emergency, and after hundreds of years of research, surgeons have a considerable understanding of this disease. Appendectomy is recommended as the first choice for various types of appendicitis. However, it is difficult to explain why the severity and symptoms of AA patients vary despite exhibiting almost the same course of illness. A recent theory has suggested that the local microbiota is responsible for the occurrence and evolution of appendicitis(1–4). Matthew B. Rogers reported that the appendixes collected from children with AA harbored populations of fusobacteria, which are generally absent in fecal samples from healthy adults and children and likely contribute to the pathogenesis of appendicitis(5). In a 16S ribosomal RNA (rRNA) gene sequence

analysis-based study, by Diana Zhong observed that appendicitis samples contained an increased abundance of Fusobacterium spp. and other pathogens commonly detected in the oral cavity, while non-appendicitis samples contained a reduced abundance of Bacteroides spp.(6). Alexander Swidsinski's group applied rRNA-based fluorescence in situ hybridization to investigate sections of 70 appendixes, and their results supported the hypothesis that the local microbiota was responsible for the occurrence and development of appendicitis(7). However, some scholars believe that there is no correlation between local microbiota and the grade of inflammation(8). Moreover, it is difficult to explain why although the results of bacterial cultures were different, patients recovered similarly after treatment with appendectomy. At present, the relationship between the varying degrees of clinical symptoms and different intestinal microbial environments remains unclear, since the detection of appendicitis-causing pathogenic bacteria has been primarily based on bacterial culture methods. However, the development and use of next-generation sequencing approaches for the detection of pathogenic bacteria have obvious advantages and allows for the relationship between clinical manifestations and pathogenic bacteria to be studied in greater depth.

Currently, the methods used to detect pathogenic microorganisms include bacterial culture, 16S rRNA gene sequencing, fluorescence in situ hybridization (FISH) and metagenomic sequencing. All of these methods have certain limitations; specifically, culture-based approaches can only detect 1% of living bacteria, and 16S rRNA gene sequencing can identify pathogenic bacteria to the genus level but may not be able to identify species. Moreover, FISH cannot achieve 100% hybridization. As the cost of next-generation sequencing technology continues to decrease, metagenomic sequencing can be increasingly used to detect pathogenic microorganisms. Metagenomic sequencing can detect 99% of the studied microbes and provide results that are accurate at the species level. Due to its many advantages, metagenomic sequencing shows promise as a sensitive and rapid method to investigate host microbiomes.

To study the effects of pathogenic bacteria on appendicitis, we grouped patients according to postoperative pathology and postoperative routine bacterial culture results. First, the perforated and non-perforated appendicitis may be of different types and different pathological processes(9), and the current treatment strategies used for these two types of appendicitis are different. Thus, it is necessary to analyze the bacterial composition of appendicitis for these two types of pathology. Second, appendicitis with positive and negative bacterial culture results may have different bacterial compositions. The results of bacterial culture have long been used as a guide for antibiotic use. However, the bacterial culture method has limitations, and it is necessary to group these results and use next-generation sequencing methods to comprehensively compare the composition of bacteria. Therefore, in the present study, we analyzed the relationship between the classification of appendicitis and metagenome sequencing results to promote the use of metagenome sequencing to improve treatment strategies for appendicitis.

Results

Participants

Twenty-one patients diagnosed with appendicitis were included in the study, and appendix samples were collected from 19 subjects. Detailed information for the included patients is provided in Table 1 (grouped by postoperative histological results) and Table 2 (grouped by bacterial culture results).

Table 1
Study population based on intraoperative exploration combined with postoperative histological examination results.

	Appendicitis categorized by histological type	
Histological type	Non-perforated: phlegmonous (N = 9)	Perforated: gangrenous (N = 10)
Age	37 (14–64)	36 (7–62)

Table 2
Study population on the basis of bacterial culture results.

	Appendicitis categorized by bacterial culture	
Bacterial culture	Negative (N = 8)	Positive (N = 11)
Age	30 (7–64)	41 (14–63)

Table 2 *Escherichia coli* was detected in all bacterial culture samples, and *Klebsiella pneumoniae* was detected in one of them.

Values are presented as the median (min-max) or as the absolute number and percentage of patients; n (%)

Taxonomic profiles of appendix microbiomes

Genus level

One hundred ninety-four genera were identified in the appendix samples, with six genera exhibiting relative abundances > 5%, including *Bacteroides* (30.8%), *Odoribacter* (8.0%), *Escherichia* (6.4%), *Porphyromonas* (6.1%), *Tannerella* (5.3%) and *Shigella* (5.1%) (Fig. 1).

Figure 1 Genus-level taxonomic profiles of the microbiomes from the appendix samples.

The bar graph was generated a script developed by BGI in the R software environment. Each vertical bar represents a unique sample. Samples were ordered by different groups shown below the figure. The y-axis represents the relative abundance of each genus. Only the top 11 genera were plotted. After filtering, no bacteria were detected in one of the simple samples; therefore, these samples are not shown. (A) The vertical bar is grouped according to histological type. Perforated (case) and non-perforated (control) appendicitis, respectively. (B) The vertical bar of the graph is grouped according to bacterial culture results. Positive (case) and negative (control) for appendicitis, respectively.

Species level

Three hundred sixty-one species were identified in the appendix samples, six of which had relative abundances > 5%, including *Bacteroides fragilis* (17.1%), *Bacteroides thetaiotaomicron* (10.3%), *Odoribacter splanchnicus* (8.0%), *Porphyromonas gingivalis* (6.1%) and *Tannerella forsythia* (5.3%) (Figs. 2 and 3).

Figure 2 Alpha- and beta-diversity comparison of the samples grouped by histological type. Red and green represent patients with perforated (case) and non-perforated (control) appendicitis, respectively. Figure 3 Alpha- and beta-diversity comparison of the samples grouped by bacterial culture results. Red and green represent patients who were positive (case) and negative (control) for appendicitis, respectively. Figure 2(A) and Fig. 3(A) Alpha-diversity (Shannon-Wiener) indices in the different groups. Each p-value obtained by the Wilcoxon rank-sum test is reported in the. Boxes denote the interquartile range (IQR) between the 25th and the 75th percentile (first and third quartiles), and the central line represents the median. Figure 2(B) and Fig. 3(B) Euclidean distance principal coordinate analysis (PCoA) of species-level taxonomic profiles. The proportion of variance explained by each principal component is denoted in the corresponding axis label.

Table 3
Top 10 most abundant bacterial species detected by bacterial culture

Species	Culture positive	Culture negative
Shigella dysenteriae	0.00028	0.024359
Escherichia fergusonii	0.000362	0.024747
Escherichia coli	0.001924	0.037056
Streptococcus constellatus	0.02913	0.034143
Klebsiella pneumoniae	0	0.039488
Klebsiella variicola	0	0.024776
Morganella morganii	0.047511	0.047511
Bacteroides xylanisolvens	0.03085	0.067361
Tannerella forsythia	0.07353	0.078547
Odoribacter splanchnicus	0.077448	0.180031
Streptococcus anginosus	0.056816	0.058706
Bacteroides thetaiotaomicron	0.069636	0.18821
Faecalibacterium prausnitzii	0.054137	0.073353
Porphyromonas gingivalis	0.120998	0.257946
Bacteroides fragilis	0.135933	0.324893

Table 3 The top 10 most abundant bacterial species detected by the culture-based approach are shown. Since no significant difference was observed between the culture positive and negative groups, these data were combined. These data may allow pharmacists to make a better decisions.

Discussion

Appendicitis is an acute abdomen caused by bacterial infection. The treatment of appendicitis consists of surgical removal of the appendix and antibiotic administration. For non-perforated appendicitis, antibiotic treatment plays an important role in preventing postoperative wound infection and intraperitoneal abscess(10). It has been reported that patients who have received an emergency appendectomy should accept preventive antibiotic therapy(11, 12). A medical letter proposed guidelines for the selection of antibiotic therapy for appendicitis patients(13). Sandra I. Berríos-Torres et al. suggested that it is not necessary administer appendicitis patients antibiotic treatment postoperatively(14). However, for perforated appendicitis, antibiotic therapy is necessary. Empirical antibiotic therapy should treat gram-negative bacilli and anaerobic bacteria, and the antibiotics used should be adjusted according to the results of bacterial culture, which is in accord with most reports. However, due to the limitation of technology, only 1% bacteria can be successfully cultured. As shown in Table3, Escherichia coli could be cultured in all samples and was not the most abundant bacterium in either the culture positive or negative groups. Furthermore, bacterial requires a great deal of time, longer than is acceptable in some instances. Thus, simply using bacterial culture results will introduce bias in the selection of antibiotics. In the present study, we used next-generation sequencing to examine bacteria in the guts of patients to completely characterize appendicitis-associated pathogenic bacteria. Our results showed that the next-generation sequencing results were not in accord with those of bacterial culture, further suggesting that bacterial culture has limits in guiding the use of antibiotics in clinical practice.

By using next-generation sequencing, we did not observe any significant difference between the bacterial culture negative and positive groups, nor between the phlegmonous and gangrenous groups. Hope T. Jackson et al. extracted DNA from the microbiota of non-perforated and perforated appendixes for use in 16S rRNA gene sequencing analysis and observed a significant difference between non-perforated and perforated samples(4). Martin Salö et al. conducted a study of 3 control samples, 11 phlegmonous samples, 4 gangrenous samples and 4 perforated samples. They also utilized 16S rRNA gene sequencing but did not observe a significant difference among the different groups(8). Our results demonstrated that there was not significant difference in the microbiota between the non-perforated and perforated appendicitis groups, suggesting that antibiotic selection does not need to be altered based on bacterial culture results. However, we advise that medical institutions should use next-generation sequencing to regularly reanalyze the microbiota spectrum of appendicitis patients and assess the presence drug resistance genes to allow for antibiotic treatments to be adjusted in a timely manner.

Specifically, completely excising lesions of the appendix and eliminating all the intraperitoneal effusion are the most important tasks in the treatment of appendicitis. Different acute abdomens harbor different bacterial species. Because of the similar bacterial species, appendicitis has similar pathogenesis among

individuals(15). In the light of all the above deficiencies, it is difficult to make an optimal decision om antibiotic usage based on bacterial culture results alone. Moreover, bacterial species and drug resistance genes change over time. Antibiotic resistance determinants are encoded by several genes, many of which can be transferred between bacteria(16). Thus, next-generation sequencing should be used to examine drug resistance genes at a regular intervals to adjust the choice of antibiotics. However, the cost of next-generation sequencing is high, making it difficult to use on all patients.

Based on the results of the present study and those of previous studies, we propose that a combination of next-generation sequencing and bacterial culture technology should be used guide treatment of appendicitis. Next-generation sequencing can be used to examine the bacterial species present in the appendix and analyze their of drug resistance profiles. Before empirical antibiotic treatment of appendicitis patients, bacterial culture should be performed to assess refractory infections and evaluate the species of bacteria present throughout the treatment period. The combination of next-generation sequencing and bacterial culture will improve the effects of treatments and the use of medical resources. From the perspective of pharmacists, we suggest that we can regularly monitor the top 10 abundant of bacteria to provide evidence that allows them to make antibiotic prescription recommendations.

Our research has several limitations. First, the sample size of our study was small. We collected samples from 22 patients, which may affects the reliability of our results. However, we utilize next generation sequencing in our research, and rather than 16S rRNA gene sequencing, we performed metagenomic sequencing to evaluate all the genes present to the fullest extent possible. The second limit of our research is the use of antibiotics. Before we collected the samples, all patients had already accepted antibiotic treatment, which may also have affected the results of our study. Thus, samples without antibiotic treatment should be evaluated to avoid alterations in the composition of the gut microbiota of appendicitis patients. However, performing such an analysis would be impossible, since antibiotic treatment is an important part of the standard treatment of appendicitis patients. Moreover, our analysis was based on DNA, which is stable enough to resist the influence of antibiotics. After bacteria are killed by antibiotics, their DNA can still can be examined, although its copy number would decrease over time. Since the time between antibiotics administration and surgery is short, it is unlikely that antibiotic administration would substantially altered our results.

Conclusion

In our present study, we divided appendicitis patients into non-perforated and perforated appendicitis groups. Surgical excision is still the gold standard in appendicitis treatment, and antibiotic administration plays an important role in preventing preoperative wound infection and intraperitoneal abscess. Traditionally, suitable antibiotics are typically chosen according to the results of bacterial culture. However, because bacterial culture has several disadvantages, such as being time-consuming and having a low positive rate, we believe that bacterial culture is not suitable as the sole guideline for antibiotic administration. Our findings demonstrated that compared with bacterial culture, next-generation sequencing can characterize the profiles of microbiota of appendicitis patients more precisely and also

analyze drug resistance. Furthermore, the results can be rationally applied based on current antibiotic administration analysis. Since the cost of bacterial culture is relatively low, we apply it to all the appendicitis patients to analyze the refractory infection and the retrospective analysis of empirical antibiotic treatment throughout the hospitalization. The combination of two technologies will optimize the treatment of appendicitis. Furthermore, in a future study, we should enlarge our sample size and verify our findings.

Methods

Study design and participants

We conducted an observational and nonconsecutive case series analysis from March 2017 to July 2017. Data were collected from the clinical database of the Fifth Affiliated Hospital of Southern Medical University. Preoperative diagnosis was based on medical history, physical examination, routine blood results and ultrasound. Postoperative pathology confirmed the preoperative diagnosis. After the surgeon suspected the diagnosis of AA, all patients received the same treatment, including second-generation cephalosporin therapy and a laparoscopic appendectomy. During the observation period, patients undergoing laparoscopic appendectomy for AA were preoperatively enrolled in the study. Patients with chronic diseases associated with other physiological systems, multiple infections, or low immune function were excluded from the study. We grouped the patients according to the results of postoperative pathology and routine bacterial culture. The details of the subjects are outlined in Tables 1 and 2. All laboratory tests were conducted in the BGI-Guangzhou Medical Laboratory. The Ethical Committee of the Fifth Affiliated Hospital of Southern Medical University reviewed the study details and confirmed that all methods were performed in accordance with the relevant guidelines and regulations (NYWY201804). Before any patient was enrolled in the study, we ensured that they sufficiently understood the study. All of the appendix specimens from our cases were examined by routine culture. According to the ethical approval guidelines, the subjects did not need to sign any written consent because all additional tests were conducted on the specimens leftover from routine microbiological examinations. The statistical analysts were blind to the identity information of the patients, and the sample information was grouped by specific identification codes.

Sample acquisition

During the laparoscopic appendectomy, the appendix was removed through trocar holes, after which the appendix tissue was dissected into 3 subsamples and stored in aseptic tubes. One piece was used for bacterial culture, one piece was histopathologically examined, and the other piece was stored in a sterile tube at -80℃ for use in subsequent whole metagenomic sequencing analyses.

Sample processing and sequencing

DNA was extracted from the pus and appendix samples using a TIANamp Micro DNA kit (DP316, TIANGEN Biotech, Beijing, China) and a DNeasy® Blood & Tissue kit (69504, Qiagen, Hilden, Germany) according to the manufacturer's recommendations. DNA libraries were subsequently constructed through fragmentation, end repair, adapter ligation and PCR amplification. An Agilent 2100 bioanalyzer (Agilent, Santa Clara, USA) was used to assess the quality of the DNA libraries, and libraries of sufficient quality were sequenced using the BGISEQ-500 platform (BGI, Shenzhen, China).

Identification of bacterial sequences

An average of 46,439,586 sequence reads per sample were generated. High-quality sequencing data were obtained by removing low-quality and short (length <35 bp) reads, followed by the subtraction of human host sequences mapped to the indicated human reference genome (hg19) using the Burrows-Wheeler Aligner (BWA)(17). Reads with low complexity were identified using PRINSEQ(18) with the parameters "-derep 14 -derep_min 100 dust-threshold = 7" and then removed. The BWA was used to search for putative bacterial matches for the remaining reads against the bacterial and microbial genome databases, which were downloaded from the National Center for Biotechnology Information (NCBI) RefSeq database(19). This database contains 1,494 bacterial genomes or scaffolds associated with human diseases. Reads with unique bacterial hits were retained for the next comparative analysis.

Statistical analyses

To increase the reliability of the results, species with less than 10 reads per 20,000,000 reads within each sample were filtered. To account for potential variations in sequencing efficacy, we transformed read abundances into percentages based on the total number of high-quality mapped sequences within each sample at all taxonomic levels of classification, namely, the phylum, genus and species levels. These normalized percentages were used in all subsequent data and statistical analyses. All statistical analyses were performed using the R software environment. The evenness index was calculated using the formula $E = S/\log(R)$, where S is the Shannon diversity index and R is the number of operational taxonomic units (OTUs) in the sample (richness)(20). In the boxplots, the black central lines represent the median, and the box edges represent the first and third quartiles. Differences between groups were calculated with the Wilcoxon rank-sum test. Principal coordinate analyses (PCoAs) were conducted using species abundance profiles. These results were confirmed with a permutational multivariate analysis of variance (PERMANOVA) that resulted in increased pseudo-F and R2 values. These analyses were performed in R using the vegan library with 999 permutations and the Euclidean distance method. Significant differences between the simple and complex groups in genus and species abundances were assessed using the Wilcoxon rank-sum test with a significant p-value threshold of less than 0.05.

Abbreviations

Acute appendicitis AA

ribosomal RNA rRNA

fluorescence in situ hybridization FISH

Burrows-Wheeler Aligner BWA

National Center for Biotechnology Information NCBI

operational taxonomic units OTUs

permutational multivariate analysis of variance PERMANOVA

Declarations

Ethics approval and consent to participate

The Ethical Committee of the Fifth Affiliated Hospital of Southern Medical University reviewed the study details and confirmed that all methods were performed in accordance with the relevant guidelines and regulations (NYWY201804). Before any patient was enrolled in the study, it was confirmed that they had a sufficient understanding of the study. All of the appendix specimens from our cases were examined by routine culture. According to the ethical approval guidelines, the subjects did not need to sign a written consent form because all additional tests were run on specimens left over from routine microbiological examinations. Statistical analysts were blind to the identity information of the patients, and the sample information available to analysts was grouped by specific identification codes.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This research is funded by Guangdong Medical Research Foundation.

Authors' contributions

Z Li and S Han designed the research. J Yuan collected all the samples in this research and finished all the experiments such as sample processing, sequencing in this research. EM Qiu analyzed and interpreted the patient data. J Yuan and EM Qiu were major contributors in writing the manuscript.

Acknowledgements

Not applicable.

References

1. Swidsinski A, Dörffel Y, Loening-Baucke V, Tertychnyy A, Biche-Ool S, Stonogin S, et al. Mucosal invasion by fusobacteria is a common feature of acute appendicitis in Germany, Russia, and China. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association. 2012;18(1):55–8.
2. Guinane CM, Tadrous A, Fouhy F, Ryan CA, Dempsey EM, Murphy B, et al. Microbial composition of human appendices from patients following appendectomy. mBio. 2013;4(1).
3. Arlt A, Bharti R, Ilves I, Häsler R, Miettinen P, Paajanen H, et al. Characteristic changes in microbial community composition and expression of innate immune genes in acute appendicitis. Innate immunity. 2015;21(1):30–41.
4. 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(4):e95414.
5. Rogers MB, Brower-Sinning R, Firek B, Zhong D, Morowitz MJ. Acute Appendicitis in Children Is Associated With a Local Expansion of Fusobacteria. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2016;63(1):71–8.
6. Zhong D, Brower-Sinning R, Firek B, Morowitz MJ. Acute appendicitis in children is associated with an abundance of bacteria from the phylum Fusobacteria. Journal of pediatric surgery. 2014;49(3):441–6.
7. Swidsinski A, Dörffel Y, Loening-Baucke V, Theissig F, Rückert JC, Ismail M, et al. Acute appendicitis is characterised by local invasion with Fusobacterium nucleatum/necrophorum. Gut. 2011;60(1):34–40.
8. Salö M, Marungruang N, Roth B, Sundberg T, Stenström P, Arnbjörnsson E, et al. Evaluation of the microbiome in children's appendicitis. Int J Colorectal Dis. 2017;32(1):19–28.
9. Andersson R, Hugander A, Thulin A, Nyström PO, Olaison G. Indications for operation in suspected appendicitis and incidence of perforation. BMJ. 1994;308(6921):107–10.

10. Andersen BR, Kallehave FL, Andersen HK. Antibiotics versus placebo for prevention of postoperative infection after appendicectomy. The Cochrane database of systematic reviews. 2003(2):Cd001439.
11. Fry DE. Surgical site infections and the surgical care improvement project (SCIP): evolution of national quality measures. Surg Infect. 2008;9(6):579–84.
12. Bratzler DW, Houck PM. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2004;38(12):1706–15.
13. Bratzler DW, Dellinger EP, Olsen KM, Perl TM, Auwaerter PG, Bolon MK, et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. American journal of health-system pharmacy: AJHP : official journal of the American Society of Health-System Pharmacists. 2013;70(3):195–283.
14. Berríos-Torres SI, Umscheid CA, Bratzler DW, Leas B, Stone EC, Kelz RR, et al. Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection, 2017. JAMA surgery. 2017;152(8):784–91.
15. Wu SC, Rau CS, Liu HT, Kuo PJ, Chien PC, Hsieh TM, et al. Metagenome Analysis as a Tool to Study Bacterial Infection Associated with Acute Surgical Abdomen. Journal of clinical medicine. 2018;7(10).
16. Wright GDJCC. Molecular mechanisms of antibiotic resistance. 2011;47(14):p. 4055–61.
17. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26(5):589–95.
18. Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. Bioinformatics. 2011;27(6):863–4.
19. Pruitt KD, Tatusova T, Maglott DR. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic acids research. 2007;35(Database issue):D61-5.
20. Bacci G, Paganin P, Lopez L, Vanni C, Dalmastri C, Cantale C, et al. Pyrosequencing Unveils Cystic Fibrosis Lung Microbiome Differences Associated with a Severe Lung Function Decline. PloS one. 2016;11(6):e0156807.

Figures

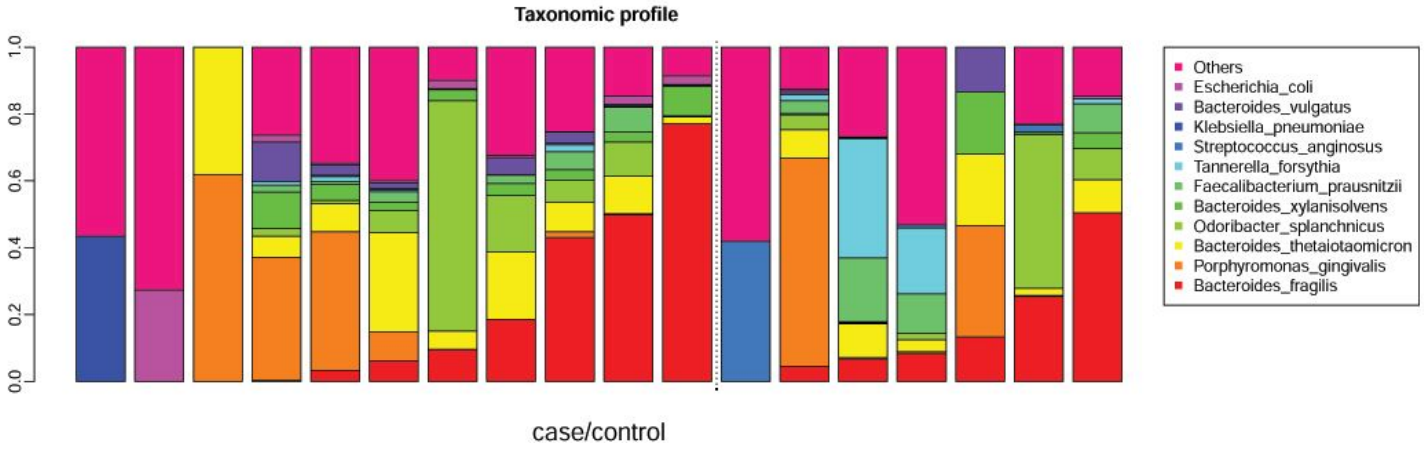

Figure 1

Fig. 1 Genus-level taxonomic profiles of the microbiomes from the appendix samples. The bar graph was generated a script developed by BGI in the R software environment. Each vertical bar represents a unique sample. Samples were ordered by different groups shown below the figure. The y-axis represents the relative abundance of each genus. Only the top 11 genera were plotted. After filtering, no bacteria were detected in one of the simple samples; therefore, these samples are not shown. (A) The vertical bar is grouped according to histological type. Perforated (case) and non-perforated (control) appendicitis, respectively.

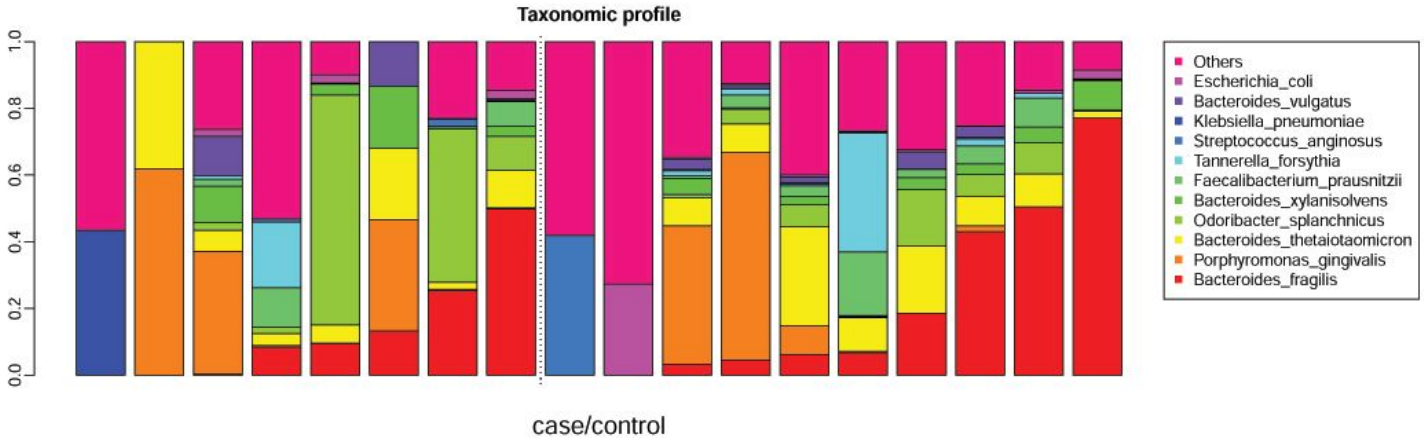

Figure 2

(B) The vertical bar of the graph is grouped according to bacterial culture results. Positive (case) and negative (control) for appendicitis, respectively.

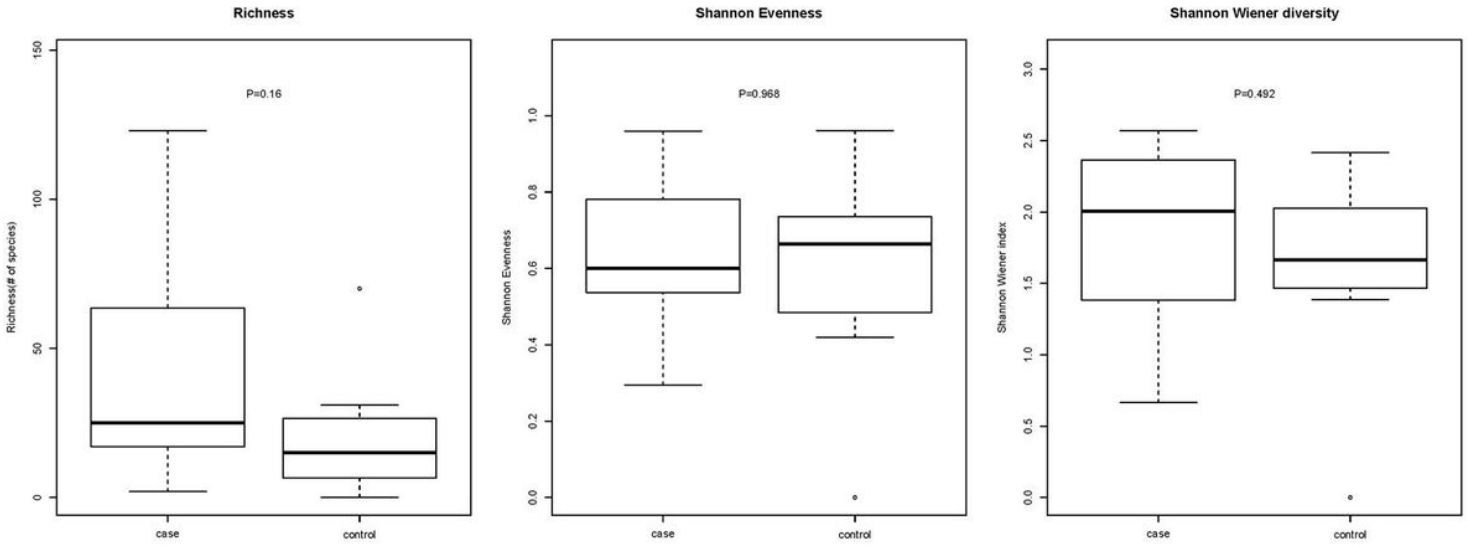

Figure 3

Fig. 2 Alpha- and beta-diversity comparison of the samples grouped by histological type. Red and green represent patients with perforated (case) and non-perforated (control) appendicitis, respectively. Fig. 3 Alpha- and beta-diversity comparison of the samples grouped by bacterial culture results. Red and green represent patients who were positive (case) and negative (control) for appendicitis, respectively. Fig. 2(A) and Fig. 3(A) Alpha-diversity (Shannon-Wiener) indices in the different groups. Each p-value obtained by the Wilcoxon rank-sum test is reported in the. Boxes denote the interquartile range (IQR) between the 25th and the 75th percentile (first and third quartiles), and the central line represents the median.

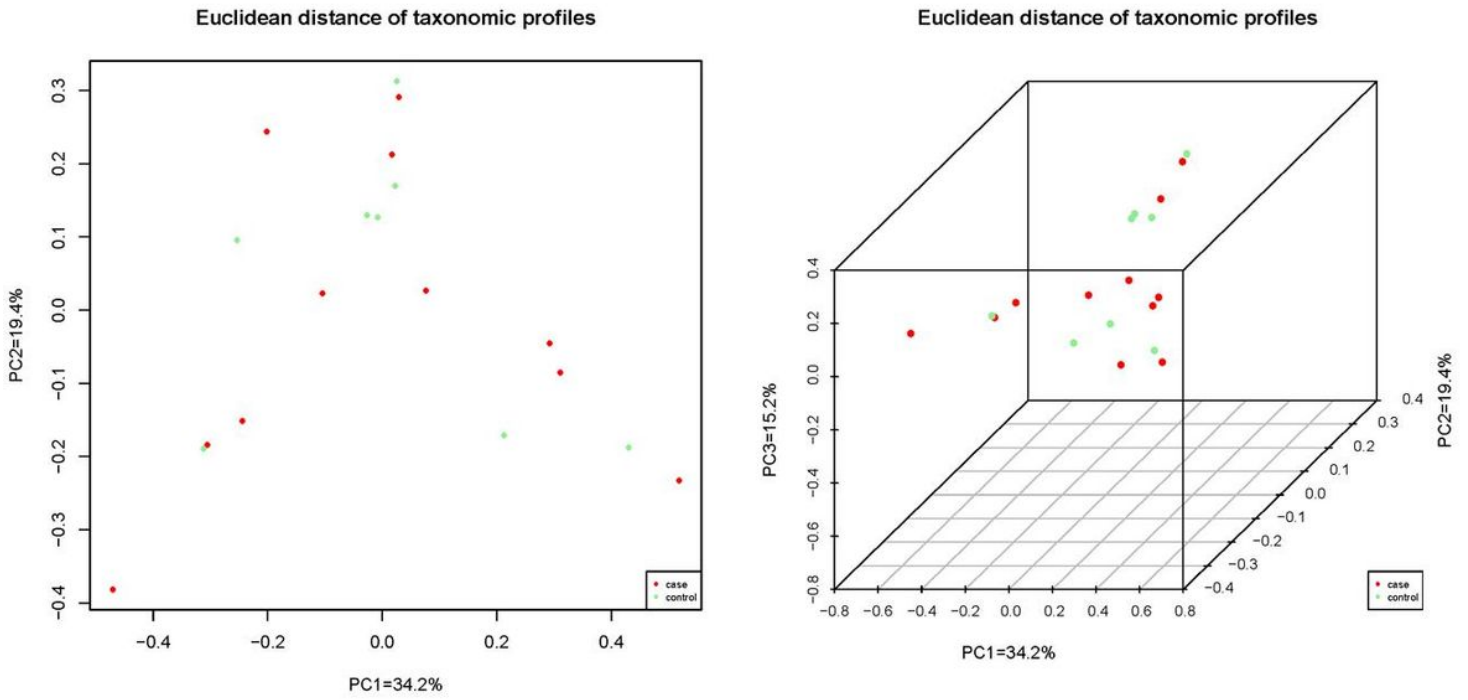

Figure 4

Fig. 2(B) and Fig. 3(B) Euclidean distance principal coordinate analysis (PCoA) of species-level taxonomic profiles. The proportion of variance explained by each principal component is denoted in the corresponding axis label.

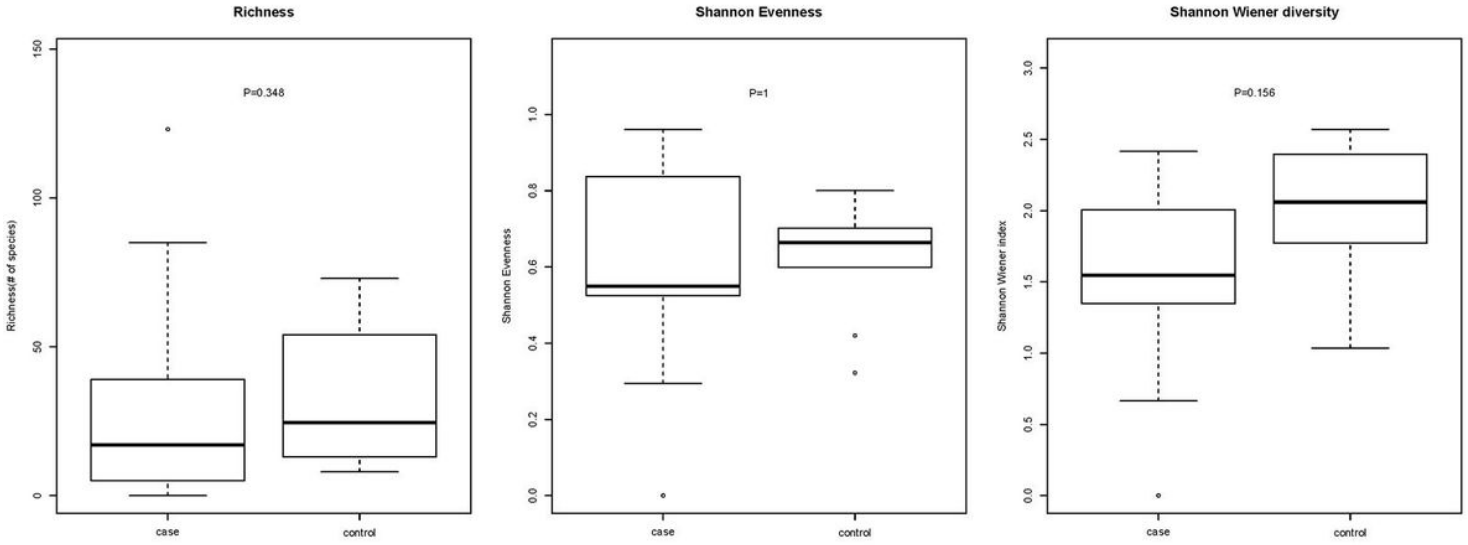

Figure 5

Fig. 2 Alpha- and beta-diversity comparison of the samples grouped by histological type. Red and green represent patients with perforated (case) and non-perforated (control) appendicitis, respectively. Fig. 3 Alpha- and beta-diversity comparison of the samples grouped by bacterial culture results. Red and green represent patients who were positive (case) and negative (control) for appendicitis, respectively. Fig. 2(A) and Fig. 3(A) Alpha-diversity (Shannon-Wiener) indices in the different groups. Each p-value obtained by the Wilcoxon rank-sum test is reported in the. Boxes denote the interquartile range (IQR) between the 25th and the 75th percentile (first and third quartiles), and the central line represents the median.

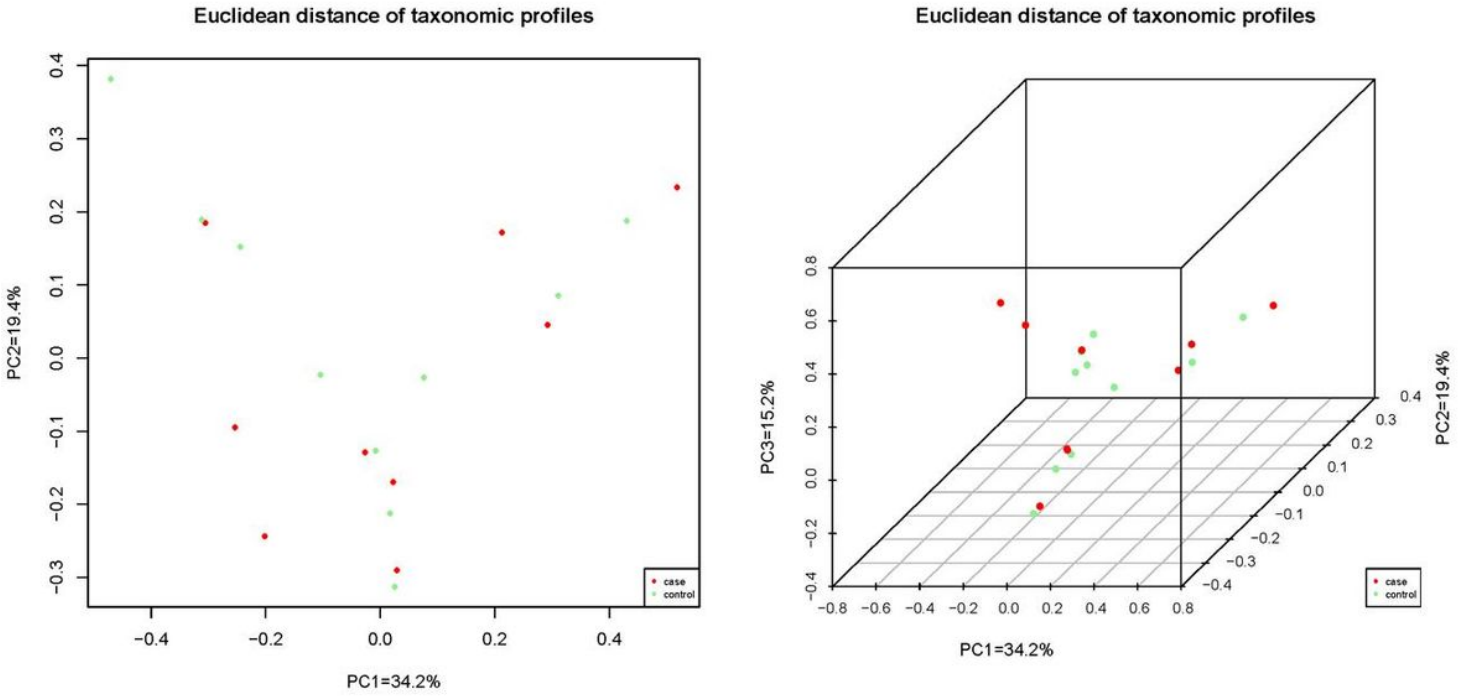

Figure 6

Fig. 2(B) and Fig. 3(B) Euclidean distance principal coordinate analysis (PCoA) of species-level taxonomic profiles. The proportion of variance explained by each principal component is denoted in the corresponding axis label.