




BRIEF REPORT

Ileocecal lavage fluid for gut microbiota investigation in children with gastrointestinal diseases

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Introduction

The ileocecal region is the end point of colonoscopy and comprises the junction of the ileum, cecum, and appendix. The ileocecal region has been reported as a common lesion site for various intestinal diseases (e.g. Crohn's disease [1], intestinal tuberculosis [2], and primary colorectal lymphoma [3]). However, to date, there have been few studies on the intestinal microbiota of the ileocecal region, because the sampling is relatively difficult and with only a local biopsy it is difficult to represent the whole region without deviation. Compared with ileocecal mucosa sampling, the collection of ileocecal lavage fluid is easier to be accepted by patients and can avoid the interference of human genomic DNA in the process of bacterial genome sequencing. This study aimed to reflect the bacterial composition of the terminal ileal microbiota by investigating that of ileocecal lavage fluid samples.

Patients and methods

A total of 33 children (10 female and 23 male) who were hospitalized for digestive-system diseases and underwent gastrointestinal

endoscopy in the Children's Hospital of Shanghai (Shanghai, China) were recruited ([Supplementary Table 1](#)). Ileocecal lavage fluid samples were collected during colonoscopy ([Supplementary Figure 1](#)). Bacterial pellets of these samples were used for bacterial genomic DNA extraction using Qiagen QIAamp Fast DNA Stool Mini Kits (QIAGEN, Hilden, North Rhine-Westphalia, Germany). High-throughput 16S rDNA sequencing was performed using primers 338 F and 806 R. The raw sequence data have been submitted to the NCBI database under accession number PRJNA610930. Culture media of peptone yeast extract glucose broth medium (PYG), reinforced Clostridium medium (RCM), brain heart infusion broth (BHI), BBL liquid medium (BBL) ordered from Qingdao Hope Bio-Technology Co., Ltd (Qingdao, Shandong, China), minimum essential medium (MEM; Thermo Fisher Scientific, Waltham, MA, USA), and *Bifidobacterium* medium (DSMZ medium No.58) were used for bacterial isolation. Vancomycin (Sangon Biotech Co., Ltd, Shanghai, China), potentially as the last line of defense against antibiotic-resistant gram-positive bacteria [4], was added in two usually used media (i.e. PYG, RCM) and one rarely used medium (i.e. MEM) for vancomycin-resistant bacteria isolation. Bacterial isolators were identified using 16S rRNA gene PCR amplification and sequencing using primers 27 F and 1492 R. All procedures

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used in studies involving human participants were in accordance with the ethical standards of the Children's Hospital of Shanghai ethics committee (ethical batch number: 2018R019-F01) and with the 1964 Helsinki Declaration and its later amendments, or comparable ethical standards.

Results

The basic information relating to the high-throughput 16S rRNA gene sequencing is listed in [Supplementary Table 2](#). A total of 2,935 bacterial amplicon sequence variants were obtained for microbe classification and identification. At the phylum level, 23 phyla were detected in the ileocecal lavage fluid samples and the dominant phyla were Bacteroidetes (42.20%), Firmicutes (27.00%), Proteobacteria (23.33%), Actinobacteria (5.30%), and Fusobacteria (1.65%). At the genus level, 353 bacterial genera were detected, among which the top 10 highly abundant bacterial genera were *Bacteroides* (29.64%), *Escherichia-Shigella* (16.67%), *Prevotella 9* (6.25%), *Faecalibacterium* (4.55%), *Parabacteroides* (3.61%), *Bifidobacterium* (3.31%), *Lachnospiraceae_unclassified* (3.00%), *[Ruminococcus] gnavus* group (2.56%), *Megamonas* (2.31%), and *Phascolarctobacterium* (2.19%) ([Figure 1](#)).

To investigate whether ileocecal lavage fluid is an alternative option for bacterial community analysis, we analysed the high-throughput 16S rRNA gene-sequencing data for normal terminal ileal mucosa obtained from age-matched non-inflammatory bowel disease patients [5] and compared them with our data. At the genus level, we found that the bacterial composition of the samples in our study was similar to that of mucosa samples in their study, with a similarity coefficient of 0.869. As illustrated in [Figure 1](#), *Bacteroides* and *Escherichia-Shigella* are predominant bacteria in this study and Alipour *et al.*'s study [5]. In addition, we constructed a Venn diagram involving 66 bacterial genera with >0.1% of relative abundance from these two studies. In total, 42 bacterial genera were intersected between the two studies.

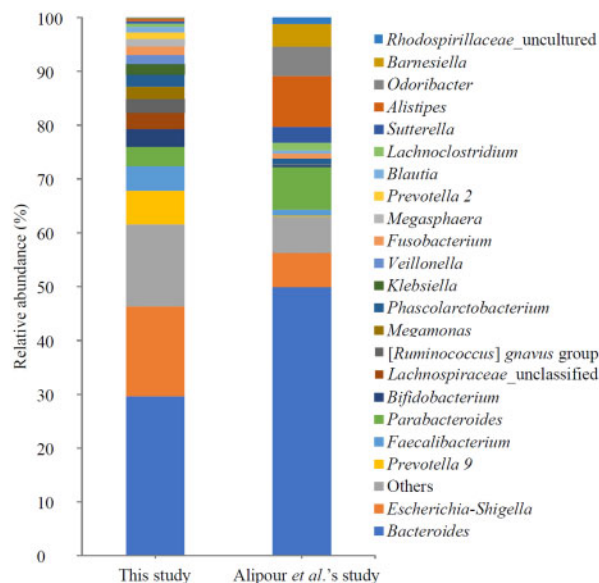


Figure 1. Comparison of samples obtained by different sampling methods for bacterial community analysis at the genus level. In this study, we studied the bacterial community composition of ileocecal lavage fluid samples (left). The bacterial community composition of ileum mucosa samples (right) was reported by Alipour *et al.*

Although alpha diversity analysis showed that there was no significant difference in Ace, Chao, Shannon, and Simpson indices between different gender groups and between different age groups, LEfSe (linear discriminant analysis effect size) results showed that the bacterial abundances of 5 families and 14 genera in school-age children (aged >7 and ≤13 years) were significantly higher than those in preschool children (aged >3 and ≤7 years), whereas the bacterial abundances of two orders, three families, and five genera in the preschool-children group was significantly higher than those in the school-age-children group ([Supplementary Figure 2A](#)). Between different gender groups, the bacterial abundances of 3 classes, 6 orders, 6 families, and 10 genera were significantly higher in the female group, and those of 1 phylum, 2 orders, 1 family, and 5 genera were significantly higher in the male group ([Supplementary Figure 2B](#)).

We obtained 139 and 58 effective sequences without antibiotics and with vancomycin, respectively. BLAST (basic local alignment search tool) analysis at the National Center for Biotechnology Information with percent identity higher than 97% was used for bacterial isolates identification. BLAST results showed that bacteria isolated without antibiotics were distributed in 20 bacterial species, of which *Escherichia coli*, *Enterococcus faecium*, and *Enterococcus casseliflavus* are the predominant bacteria. Vancomycin-resistant bacteria were isolated and divided into 12 species, of which *Escherichia coli* and *Enterobacter cloacae* are the predominant bacteria ([Supplementary Figure 3](#)).

Discussion

Intestinal microbiota has become one of the important targets of disease prevention and treatment, and understanding their composition is one of the important premises [6, 7]. In this study, we found that *Bacteroides* and *Escherichia-Shigella* are the predominant bacteria in the ileocecal lavage fluid samples. Bacterial isolation and sequencing results showed that the abundance of *Escherichia-Shigella* was high in these samples. We also found that the composition pattern of isolated bacteria with or without vancomycin is similar. That indicated that most of the *E. coli* may have resistance to vancomycin. Dogan *et al.* [8] indicated that 29% and 47% of *E. coli* strains isolated from the ileal mucosa of patients with ileal Crohn's disease showed resistance to ciprofloxacin and ciprofloxacin/rifaximin, respectively. These results indicated that in the clinical treatment of these digestive-tract diseases, only some drugs with a good killing effect on antibiotic-resistant *E. coli* can achieve better efficacy.

The microbiota from one specific location selectively colonizes its homologous gut region and own different physiology functions [9]. For microbiota composition and related diseases of the ileocecal region, it would be better to study samples from the ileocecal region than to study fecal samples. As biopsy is relatively difficult and aggressive for subjects, ileocecal lavage fluid may be a great option to replace ileum mucosa for microbiota research. According to the results, *Bacteroides* and *Escherichia-Shigella* are the predominant bacteria in ileocecal lavage fluid and ileum mucosa samples, and the similarity coefficient of bacterial composition is >85%.

However, some limitations of this study were as follows: (i) the volunteers had gastrointestinal symptoms before coming to perform the examination; (ii) shotgun metagenomic sequencing may be a better way to clarify the microbial composition and functions; (iii) additional culture media and improved conditions are required for more adequate results; (iv) the mucosa

samples and ileocecal lavage fluid samples were collected from patients in different countries.

Supplementary Data

Supplementary data is available at *Gastroenterology Report* online.

Authors' Contributions

Y.S.Y. and H.F.L. conceived of and designed the project. H.H.C., X.W., and L.W. collected the data. H.H.C., X.W., L.W., H.F.L., and Y.S.Y. analysed and interpreted the data. H.H.C., Y.S.Y., and H.F.L. drafted the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

None declared.

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