Small intestinal microbiota and prognosis of infants with ileostomy caused by different primary diseases

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Abstract

Background: Studies on microbiota characteristics of infants with small intestinal ostomy due to various etiologies are limited. Here, we investigated the intestinal microbiota of neonates with ileostomy due to different primary diseases.

Methods: Fifteen patients with necrotizing enterocolitis, eight patients with meconium peritonitis, and seven patients with Hirschsprung's disease were included in the study. The small intestinal microbiota composition in infants with ileostomy caused by different disease was investigated.

Results: The microbial diversity in neonatal ileostomy fluid was generally low, dominated by Proteobacteria and Firmicutes members. At the genus levels, the most abundant bacteria were *Klebsiella*, *Escherichia-Shigella*, *Streptococcus*, *Clostridium sensu stricto* 1, *Enterococcus*, and *Lactobacillus*. *Streptococcus* and *Veillonella* were related to carbohydrate metabolism and immunity, and breastfeeding could increase the proportion of these beneficial bacteria. The proportion of *Bifidobacterium* in the breastfeeding group was higher than that in the non-breastfeeding group, and the incidence of colitis and sepsis was significantly reduced in the breastfeeding group. The increase of body weight in the breastfeeding group was also higher than that in the non-breastfeeding group.

Conclusions: Excessive *Klebsiella* and *Escherichia-Shigella* and low abundance of *Streptococcus*, *Veillonella*, and *Faecalibacterium* indicated that the small intestinal microbiota was still in an unhealthy state. However, *Streptococcus*, *Faecalibacterium*, and *Veillonella* were commonly found, suggesting that these bacteria might promote the development of immune system after surgeries.

1 Background

Enterostomy formation is one of the important surgical treatment for infants with acute abdominal disease. It is commonly performed in variety of intestinal conditions, such as necrotizing enterocolitis (NEC), meconium ileus, focal intestinal perforation, intestinal atresia and volvulus. Extensive small intestine resection may lead to the experience of functional short bowel syndrome (SBS). SBS is a malabsorption disorder characterized by fluid and electrolyte imbalances that result from extensive bowel resection. These infants are placed on long-term parenteral nutrition (PN) and enteral nutrition (EN) to compensate for their nutritional deficiencies, which might have considerable effects on the microbiota structure in the small intestine of patients.

The small intestine is the main organ for digestion and absorption of nutrients, including almost all proteins, lipids, monosaccharides, and starch [1]. Although the abundance of microbiota in the small intestinal is relatively low, because of the rapid peristalsis of the intestine and the secretion of bactericidal substances [2], the small intestinal microbiota has functions of regulating immunity, metabolism, and endocrine system, whose effects on the host health cannot be ignored [3]. Hayashi reported that the majority of microorganisms in the jejunum and ileum was aerobic bacteria and facultative anaerobes, such as Streptococcus, Lactobacillus, Enterococcus, and γ-Pseudomonas [4]. Wang et al. reported that

Streptococcus accounted for more than 60% in the jejunal microbiota, and Clostridium clusters IVa and IV were the dominant bacteria in the distal ileum [5]. It has been shown that Streptococcus, Veillonella, and Lactobacillus are the main microorganisms in the small intestine and that they are involved in intestinal immune regulation [6]. Thus, changes in the small intestine microbiota composition might also affect the disease symptoms. Due to the location of the small intestine, the microbiota is difficult to be sampled. Therefore, only a few studies have focused on small intestinal microbiota.

Multiple primary diseases might result in ileostomy, including Hirschsprung's disease (HD), meconium peritonitis (MP), and NEC. During the infant stage, the composition of intestinal microbiota is relatively simple and can be influenced by a variety of factors. In this study, we investigated the intestinal microbiota of infants with ileostomy due to different primary diseases. Furthermore, the association between clinical symptoms and intestinal microbiota and therapeutic effects were also comprehensively analyzed. This study will provide valuable data for postoperative care and future clinical practice.

2 Methods

2.1 Study subjects and sample collection

In this study, we examined 30 infants with small intestinal ostomy caused by various primary diseases. All patients were recruited from the Children’s Hospital of Fudan University as shown in Tables 1 and S1. All the patients were divided into the following three groups according different primary diseases: HD (8 cases), MP (7 cases), and NEC (15 cases) groups. After the infants reached the full enteral feeding (daily enteral feeding > 120 ml/kg), samples of ileostomy fluid were collected and stored at -80 °C until the microbiota analyses. The children received oral formula or breast milk, and they were not fed any solid food. This study was approved by the Human Investigation Committee of Children’s Hospital of Fudan University. All the written informed consents were obtained from the parents.

Table 1

Clinical information for patients with different primary diseases (data were presented as median)

Infant characteristic	All patients with ileostomy (n = 30)	Primary diagnosis		
		Hirschsprung's disease (HD, n = 8, H01-H08)	Meconium peritonitis (MP, n = 7, M01-M07)	Necrotizing enterocolitis (NEC, n = 15, N01-N15)
Male sex, n	20	4	3	13
Gestational age (week)	34 (25–40)	36.8 (30–40)	36.3 (34–40)	31.4 (25–39)
The operation day age (day)	19.8 (0–77)	24.1 (1–50)	4.7 (0–17)	24.5 (3–77)
Operative weight (kg, range)	2.2 (1.0-3.5)	2.6 (1.3–3.5)	2.7 (1.6–3.3)	1.7 (1.0-3.5)
Remaining small bowel length (cm)	86.9 (40–160)	96.9 (80–110)	79.8 (40–160)	84.8 (72–100)
Breast feeding, n	8	1	1	6
Parenteral nutrition duration (day)	38.3 (11–91)	33.5 (11–91)	48.3 (17–85)	36.1 (20–74)
Colitis, n	12	6	3	3
Septicemia, n	22	7	4	11
Shannon index	1.17 (0.2–1.83)	1.37 (0.78–1.8)	1.14 (1.01–1.55)	1.07 (0.2–1.83)

2.2 16S rRNA gene sequencing

Bacterial genomic DNA was extracted from the ileostomy fluid samples using the E.Z.N.A. Stool DNA Isolation Kit (Omega Bio-Tek, Inc., GA, USA). The 16S rDNA V3-V4 region was amplified by PCR using barcoded Illumina adapter-containing primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') [7]. The final 16S rRNA gene amplicon library was sequenced on the MiSeq platform (Illumina) using the 2 × 300 bp paired-end protocol. Illumina MiSeq sequencing was performed by the Shanghai Mobio Biomedical Technology Co., Ltd. (China). Raw sequencing data were submitted to the NCBI Sequence Read Archive under accession number PRJNA553095.

Clean data was extracted from Raw data using USEARCH 8.0 with the following criteria: (i) Sequences of each sample were extracted using each index with zero mismatch, (ii) Sequences with overlap less than

50 bp and the error rate of the overlap greater than 0.1 were discarded, (iii) Sequences less than 400 bp after merge were also discarded. Quality-filtered sequences were stepwise clustered into operational taxonomic units (OTUs) at a similarity of 97% using UPARSE (version 7.1 http://drive5.com/uparse/) [8]. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the Silva (SSU123)16S rRNA database using confidence threshold of 70% [9].

2.3 Statistical analyses

The estimators of alpha diversity were calculated with standard methods using QIIME 1.9.0 [10]. The structure and characteristics of the microbial community in each treatment step were analyzed using R. Hierarchical clusters at the genus level were generated with Bray-Curtis average distance method using R. Genera with a relative abundance higher than 1% in at least one of the samples were included. A canonical correspondence analysis (CCA), which explained the relationships between the corresponding environmental parameters and relatively abundant genera in microbial communities, was performed using Canoco 4.5.

3 Results

3.1 Low microbial diversity and chaotic microbial succession

To understand the relationship between various primary diseases and intestine microbiota shifts, the patients were classified into three groups according to the primary diseases, namely, the HD, MP, and NEC group. There were 8, 7, and 15 patients in the HD, MP, and NEC groups, respectively (Table 1). Shannon diversity index was relatively low in infants with ileostomy as shown in Table 1 and S1. Clinical profiles of patients are summarized in Table S1. Proteobacteria and Firmicutes were the most prevalent phyla, followed by Actinobacteria and Bacteroidetes (Figure S1). These four phyla accounted for more than 99.9% of the microbial communities in all samples. In these patients, Enterobacteriaceae (56.4%) was the dominant taxonomic family, followed by Streptococcaceae (11.8%), Clostridiaceae 1 (9.6%), Enterococcaceae (8.7%), Lactobacillaceae (5.5%), and Bifidobacteriaceae (1.6%). These six most common taxonomic families accounted for more than 93.6% of the microbial communities in all samples (Fig. 1A).

3.2 Structure of microbiotas under various conditions

To further understand the microbial composition of the three groups, the core genera were examined in detail (Fig. 1B and S2). One hundred and nineteen OTUs were obtained in all the samples. The most abundant bacteria were Klebsiella (26.0% on an average), followed by Escherichia–Shigella (24.6% on an average), Streptococcus (11.8% on an average), Clostridium sensu stricto 1 (9.6% on average), Enterococcus (8.7% on an average), and Lactobacillus (5.5% on an average). The cluster analysis showed that the relative abundance of Klebsiella was significantly higher in some patients (Fig. 2), and Klebsiella and Enterobacter always appeared simultaneously (indistinguishable based on the 16S V4 region).The

cluster analysis also showed that the patients were grouped according to the composition of genera (Fig. 2). The two large classes were Escherichia-Shigella and Klebsiella (Fig. 2). In addition, Clostridium sensu stricto 1 was the main species in samples M06 and N14, and Raoultella was the main species in sample N11 (Fig. 2). The microbiota composition analysis of ileostomy fluid revealed that individual subjects had a distinct microbiota structure. However, in addition to these distinct characteristics, these microbiotas had some common species. For instance, Streptococcus can be detected in almost every sample, albeit in variable relative abundance (Figs. 2 and S2).

3.3 Bifidobacterium and breastfeeding

Similar to Faecalibacterium, Bifidobacterium was also detected in some samples with low abundance. Eight of the 30 patients were breast-fed (Table S1). Streptococcus is associated with carbohydrate metabolism, and breastfeeding can increase the proportion of this beneficial bacterium in the small intestine. Breast milk usually contains Bifidobacterium, Lactobacillus, and Streptococcus, and another probiotic species Staphylococcus that usually settle on the areola skin; therefore, these species can be transferred directly from mother to the baby via breastfeeding [11, 12]. In addition, the incidence of colitis and sepsis in the breast-feeding group was significantly lower than that in the non-breast-feeding group (Table 2), while the increase in body weight of the breastfeeding group was significantly higher than that of non-breast-feeding group (Table 2). The CCA analysis also revealed that Bifidobacteria, Streptococcus, and Veillonella were closely associated with breastfeeding (Fig. 3).

Table 2
Influence of breastfeeding (BF) on ileostomy patients

Breast feeding	Growth rate of body mass (g/d)	Colitis	Sepsis	Bifidobacterium	Streptococcus
No BF (22/30)	21.2	0.5%	0.82%	0.34%	9.3%
BF (8/30)	27.4	0.125%	0.5%	4.9%	18.4%

3.4 Influence of different factors on the intestinal microbiota compositions

To investigate the effects of various external factors on the structure of intestinal microbiotas, the CCA analysis was performed. As shown in Fig. 3, more than 60% of the variations in microbiotas were explained by both the axes. According to the results, different etiologies have a significant influence on the microbial community structure. Under the combined influence of external factors, infants with ileostomy with different primary diseases can be grouped (Fig. 3). In addition to the etiologies, these intestinal microbiotas were also affected by several other factors, including duration of antibiotic withdrawal, breastfeeding, PN duration, and residual intestinal length.

4 Discussion

The gut microbiota is an important factor affecting human health, having evolutionarily conserved roles in the metabolism, immunity, development, and behavior of the host [13, 14]. It has been reported that the fecal microbiota of caesarean newborns is rich in Enterobacter, Streptococcus australis, and Veillonella, which are mainly obtained from the skin, oral cavity, and the surrounding environment during birth. The intestinal microbiota of vaginal delivery neonates is rich in Escherichia-Shigella, Bacteroides, and Bifidobacterium, among which Escherichia-Shigella is the most abundant genus [15]. It is reported that the enriched genes involved in vitamin K2 synthesis in newborns were correlated with the high abundance of Escherichia-Shigella having the ability to produce vitamin K2 [15, 16].

Because Streptococcus and Veillonella might interact in the metabolic process and often coexist in this ecosystem [17, 18], their combined immunoregulatory properties have been reported [6]. The source of these bacteria in newborns is their mothers, and therefore, it can be inferred that these bacteria might play a role in the recovery and immune development of patients with ileostomy after surgery. These findings suggest that microorganisms in the small intestine are mainly associated with simple carbohydrate metabolism, a task predominantly performed by some specific bacteria, such as Streptococcus and Veillonella. This is consistent with the findings of previous studies, that is, enterobacterial microbiota always include Streptococcus and Veillonella spp. in the ecosystem, and they are the most active members of ileal microbiota [3]. Streptococcus and Veillonella often function together. Streptococcus can metabolize a variety of carbohydrates, while Veillonella can use lactic acid as a carbon source and energy source, and was speculated to metabolize lactic acid produced by Streptococcus [19, 20]. A metatranscriptome analysis of ileostomy fluids also revealed that Streptococcus in the small intestine was mainly related to the transportation and metabolism of carbohydrate substrates [17]. Escherichia or other microorganisms can play the same role when the number of Streptococcus is insufficient [17]. Further studies on the underlying mechanisms are needed to explain the contribution of Streptococcus and Veillonella to immunity and homeostasis in pediatric patients with ileostomy, which might help guide clinical practice. Faecalibacterium, a major member of Firmicutes, has been reported to have immunoregulatory and anti-inflammatory functions [21]. Faecalibacterium prausnitzii can produce anti-inflammatory proteins. The ecological disorder associated with Crohn's disease (CD) is characterized by the decrease in F. prausnitzii. Moreover, the decrease in F. prausnitzii is associated with increased risk of recurrence after ileal CD surgery [21, 22]. The disadvantage of this article is that the sample size is too small to link specific bacterial populations to the 3 primary diseases. In addition, many of the clinical parameters collected having an influence on the dataset, including antibiotic removal duration, breastfeeding, PN duration, residual intestinal length.

Nutritional conditions have significant effects on the composition of the intestinal microbiota. Breast milk is an excellent source of nutrition for infants, providing proteins, carbohydrates, lipids, fats, and some micronutrients essential for infant growth. In addition, breast milk contains several biologically active components such as immunoglobulins and oligosaccharides, and microbiota plays critical roles in infant intestinal homeostasis and immune development [23]. Thus, breastfeeding might play an important role in maintaining intestinal balance and postoperative recovery on the patients.

Excessive Klebsiella and Escherichia-Shigella and low abundance of Streptococcus, Veillonella, and Faecalibacterium indicated that the small intestinal microbiota was still in an unhealthy state. It has been reported that the relative abundance of Enterobacter or Klebsiella is significantly higher in the breast milk of mothers of infants infected with rotavirus and in the intestine of infected neonates, whereas the abundance of Streptococcus and Staphylococcus was significantly lower [24]. These data indicates the correlation between the presence of Klebsiella/Enterobacter and neonatal gastrointestinal diseases, as well as the potential protective effects of Staphylococcus and Streptococcus. A high proportion of Klebsiella species has also been reported in the pathogenic genera of SBS II patients [25]. Therefore, it is necessary to further study the effects of Klebsiella/Enterobacter on ileostomy and its prognosis, in order to provide theoretical support for clinical practice.

5 Conclusions

This is the first report describing the characteristics of intestinal microbiota of neonates with ileostomy caused by different etiologies. The results revealed that patients with small intestinal ostomy due to different etiologies have different microbial characteristics. Furthermore, inter-individual differences were observed, which indicated a relatively individualized small intestinal microbiota profile. The diversity of bacteria in the small intestinal fluid of neonates with ostomy was low, which was also reflected by the low Shannon index. They were mainly distributed in the phyla Proteobacteria and Firmicutes, whereas less in the phyla Actinobacteria and Bacteroidetes. More research will be essential to understanding the relationship between intestinal dysbiosis and the primary disease, as well as the influence of microbiota on the prognosis of infants with ileostomy.

Abbreviations

NEC: necrotizing enterocolitis; SBS: short bowel syndrome; PN: parenteral nutrition; EN: enteral nutrition; HD: Hirschsprung's disease; MP: meconium peritonitis; CCA: canonical correspondence analysis; CD: Crohn's disease

Declarations

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Availability of data and materials

All data supporting our findings are included in this published article and its Additional files 1 and 2.

Authors' contributions

TQ, RZ and CS contributed to conceptualization. TQ and RZ contributed to methodology. TQ, HZ and LZ contributed to investigation. TQ contributed to writing the original draft. HZ, LZ and CC contributed to writing the review and to editing. RZ and CS contributed to funding acquisition and supervision.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This study was approved by the Human Investigation Committee of Children's Hospital of Fudan University. All the consents were obtained from the parents.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Figures

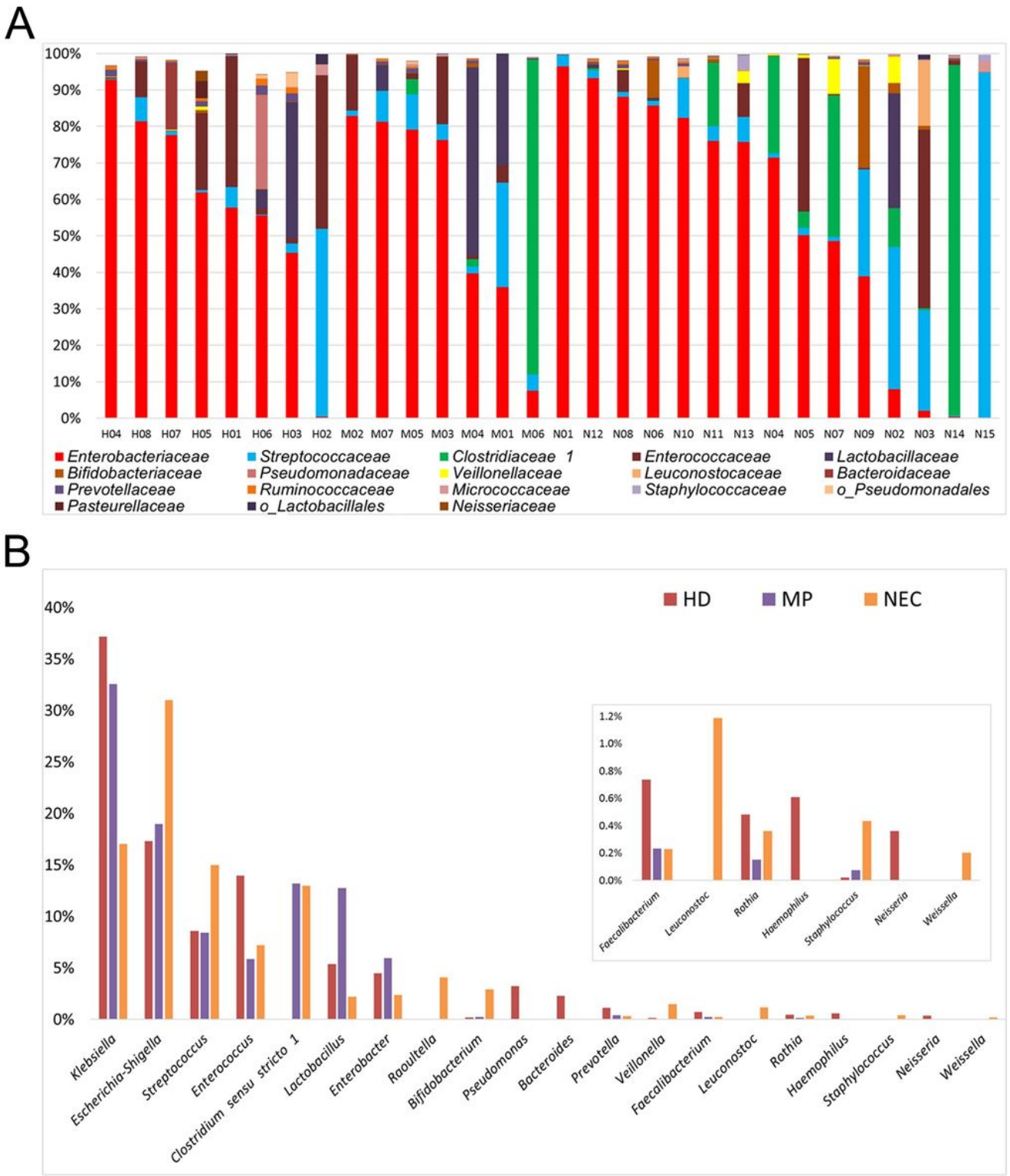

Figure 1

Microbial communities in infants with ileostomy. The figure shows the relative abundance of the 16 taxonomic families (A) and the 20 taxonomic genera (B).

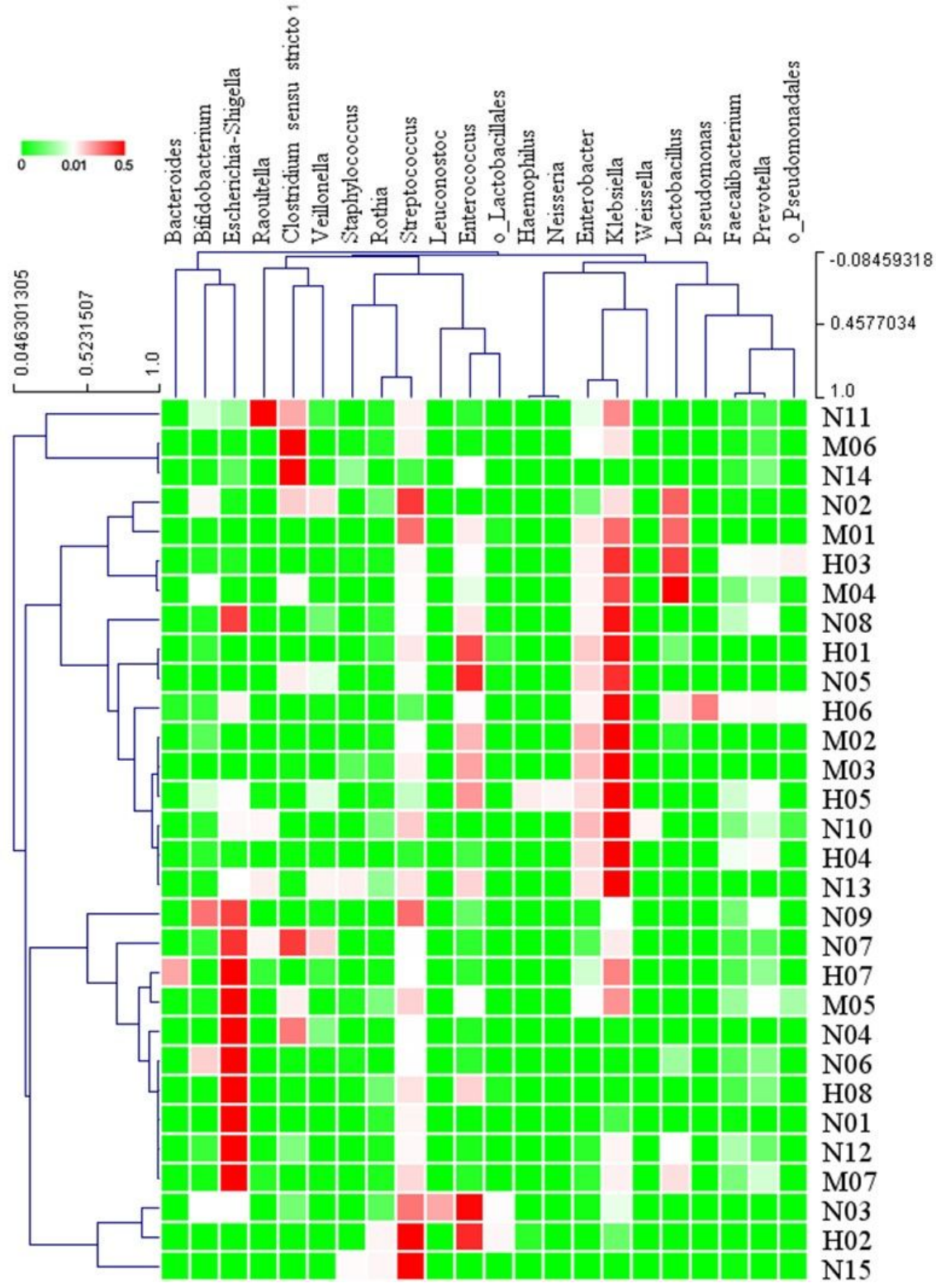

Figure 2

Heatmap and hierarchical clustering analysis using Bray-Curtis dissimilarity. Relative abundance percentage of each genus in the corresponding whole community is indicated by the color scale.

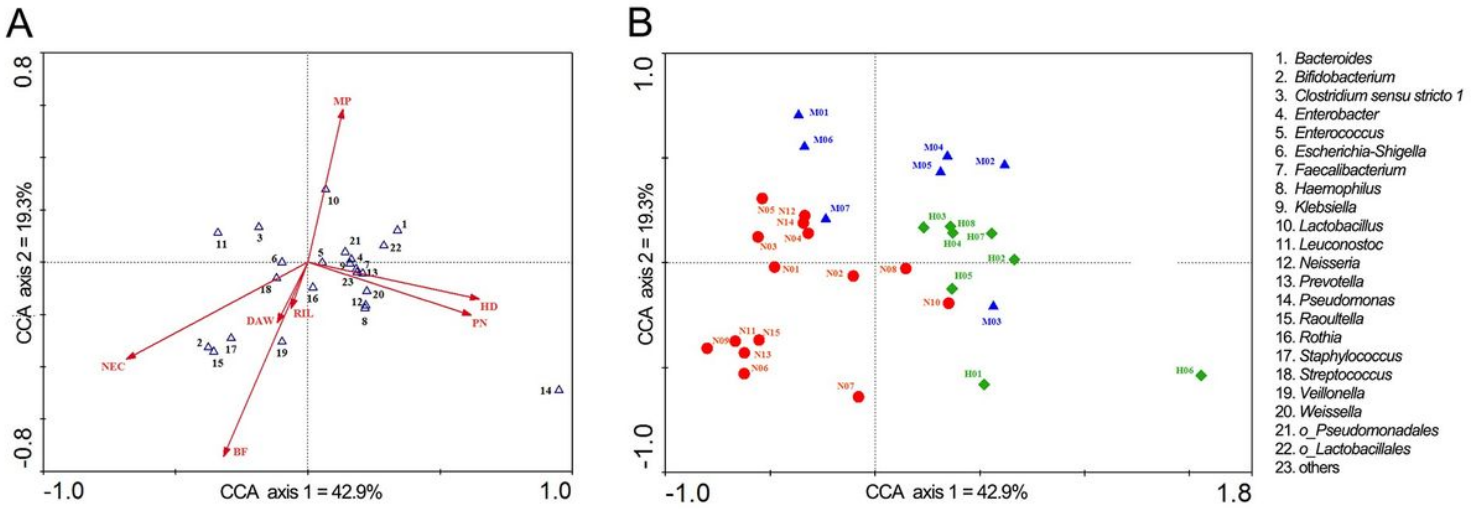

Figure 3

Canonical correspondence analysis of the effects of different factors. A, Influence of environmental variables on bacterial species; B, Influence of environmental variables on samples.

Supplementary Files

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- SupplementaryMaterialFigureS1andS2.docx
- SupplementaryMaterialTableS1PatientsInformation.xls