



# Fecal Microbiota, Lactic Acid and Short Chain Fatty Levels of Infants Following Rotavirus Infection Revealed by Illumina Miseq High-Throughput Sequencing and HPLC Method

Lin Li <sup>#1</sup>, Dongxu Huang <sup>#1</sup>, Austin Nevin <sup>2</sup>, Peng Fei <sup>3</sup> and Ling Guo <sup>1, \*</sup>

<sup>1</sup>Key Laboratory of Dairy Sciences, Ministry of Education, Food College, Northeast Agricultural University, Harbin, China

<sup>2</sup>Istituto Di Fotonica E Nanotecnologie, Consiglio Nazionale Delle Ricerche (IFN-CNR), Milano, Italy

<sup>3</sup>College of Food and Biological Engineering, Henan University of Science and Technology, Luoyang, China

\* Corresponding author: Key Laboratory of Dairy Sciences, College of Food Science and Engineering, Northeast Agricultural University, 600 Changjiang Road, Harbin 150030, Heilongjiang Province, China. Tel: +86-45155190459, Fax: +86-45155190577. Email: guoling@neau.edu.cn

# These authors are contributed equally as the first author.

Received 2018 March 07; Revised 2019 June 03; Accepted 2019 June 08.

## Abstract

**Background:** Rotavirus (RV) is one of the major causes of acute gastroenteritis in infants. It is indispensable to demonstrate the relationship between the diversity and richness of gut microbiota and RV infection using more accurate and effective technology.

**Objectives:** To investigate the differences in fecal microbiota, lactic acid, and short-chain fatty acids (SCFAs) levels between rotaviral-induced diarrhea (RD) infants and healthy (H) infants.

**Methods:** The infants comprised of 25 infants aged few days to six months, who were in good health (n = 12) or diagnosed with rotavirus (n = 13). Fecal matter was analyzed with Illumina Miseq high-throughput sequencing technique targeting the 16s rRNA gene V3-V4 region. Lactic acids and SCFAs were measured by the high-performance liquid chromatography (HPLC) technique.

**Results:** Compared to H infants, the fecal samples in RD infants had lower Shannon diversity index and the bacteria richness (P < 0.05). A higher proportion of *Proteobacteria*, *Enterobacteriaceae* and *Klebsiella*, and lower abundances of *Actinobacteria* and *Knoellia* (P < 0.05) were detected in fecal samples of RD infants. The total SCFAs content of fecal samples showed no distinction between RD and H infants, yet lower levels of lactic acid were observed in fecal samples of RD infants.

**Conclusions:** Rotaviral infection in infants led to an alteration of fecal microbiota and lactic acid concentration compared with healthy infants. Fecal microbiota and metabolite may advance the understanding and treatment of RD.

**Keywords:** Rotaviral-Induced Diarrhea (RD), Fecal, Microbiota, Infants, Lactic Acid, Short-Chain Fatty Acids

## 1. Background

To accomplish the establishment of the complex microbial ecosystem, the early neonatal period is composed of the foundation of a healthy intestinal microbiota (1, 2). For the normal development of the intestine, infant gut microbial colonization always connects with the homeostasis of the intestine and the barrier function of mucous (3). In the case of neonates, appropriate signals for intestine and immune maturation have been provided by healthy gut microbiota due to a reduced risk of infection (4). During this relatively sensitive and unstable initial stage, any variation in the microflora development process may rise up disease risk in later life. After weaning, the richness, complexity and diversity of the intestinal microflora increases quickly and the infant gut microbiota begins to resemble that of an adult and stabilizes after one year of

age (5).

Rotaviral-induced diarrhea (RD) is the leading cause of severe diarrhea in young children and infants (6). An estimated 48 to 64 million deaths in children aged < 5 years old are caused by rotavirus (RV) (6). Rotavirus infection also leads to the cost of approximately 21.3% to 39.5% of hospital admission spending for RD in Europe, even less the cost of rotavirus vaccines (7). The rotavirus can infect intestinal epithelium villi cells, increase intestinal motility, destroy gut microbial barrier, and lead to patients suffering from a watery diarrhea (8, 9). The etiology of typical intestinal diseases had proved that gut intestinal microflora had a close association and influence with the appearance of inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and even colon cancer (10). Highly elevated levels of *Proteobacteria* species and increased levels of *Enter-*

*obacteriaceae* species were observed in disrupted intestine bacteria communities, which were laid in those individuals with IBS (11).

Tazume et al. firstly reported that the anaerobic bacteria in the intestines declined among RD patients and provided a new direction for understanding rotavirus infection (12). The author's previous study analyzed the dominant genus in RV infants and healthy infants by using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and indicated the dominant genus was *Escherichia coli*, *Bacteroidetes vulgatus*, *Enterococcus faecium*, and *Clostridium* in RV infants (13). Strains of *Bifidobacteria* have an improved effect on diarrhea infected rotavirus and *Clostridium difficile* (14, 15). Another researcher found a significant negative linkage between *Bifidobacterium* and abdominal pain in adults with acute viral gastroenteritis. However, the above correlation cannot be copied in fecal samples of children with acute viral gastroenteritis (16). The possible explanation may be that the species and amounts of *Bifidobacterium* in the infant microbiota is not stable and alternates swiftly in comparison with adult microbiota (17). Therefore, it is irreplaceable to demonstrate the relationship between the diversity and richness of gut microbiota and RV infection.

As the major end products of bacterial metabolism, short chain-fatty acids (SCFAs) are produced in human large intestine, which play a variety of physiological functions (18, 19). The changes in production levels of lactic acid and SCFAs, both as total levels that produced the pattern of individual acids can be used as markers of calculating the bacterial growth activity (20). Lactic was observed and there were higher levels in fecal samples of IBD compared with those in healthy subjects (21). Acetic acid also produced by *Bifidobacterica* can improve intestinal defense and protect against the contamination of *Escherichia coil* (22). Its concentration was decreased in Crohn's disease subjects (23). The differences in SCFAs in fecal samples between RD infants and H infants were analyzed to deepen the understanding of the relationship between RD and fecal metabolites profile.

## 2. Objectives

The study focused on the intestinal microecology through a study of intestinal microflora structure and changes of SCFAs of RD infants. It aimed to elucidate the effects on the establishment of the infant gut microbiome by RV infection and accompanying changes in bacteria metabolism products.

## 3. Methods

### 3.1. Sample Collection and Total DNA Extraction

The twenty-five infant (0 to 6 months) fecal samples used in this study were collected from November, 2016 to February, 2017. Thirteen samples were obtained from the infants diagnosed with RD patients by using specific enzyme immunoassay method in Harbin Children's Hospital. During the study enrollment period, the other 12 fecal samples were recruited from age- and gender-matched healthy infants (six females and six males) in Harbin city as control samples. All infants were provided with the same diet, including equal amounts of breast milk, fruit puree, and rice flour. All fecal samples after collection were transferred to sterile plastic tubes, immediately dispatched to the laboratory where the study was conducted, and then stored at -80°C before analysis. In the previous months, fecal samples were prepared for being collected, no infants in this study had taken antibiotics, probiotics, prebiotics or synbiotics. QIAamp DNA Stool Mini kit (Qiagen GmbH, Hilden, Germany) was used to extract DNA. The extraction method followed the manufacturer's specifications as the same as previously described (4). The extracted DNA was kept frozen (-80°C) before analysis.

### 3.2. 16S rRNA Gene Amplification and Sequencing

The V3-V4 region of the 16S rRNA gene as the target was amplified using the primer 338F (ACTCTAGGGAG-GCAGCAG) and 806R (GGACTACHVGGGTWCTAAT) (24). Amplicons of at least 450 bp were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction kit (Axygen Biosciences, Union city, CA, U.S.), according to the protocol. The concentrations of amplicon DNA were quantified using a QuantiFluor™ - ST Fluorometer (Promega, Madison, WI, U.S.). Purified amplicons were pooled and mixed in equimolar and sequenced (2 × 250) on an Illumina MiSeq platform at Maforbio Bio-Pharm Technology Co., Ltd., Shanghai, China (25). The PCR program was set by following the method described by Costa et al. (26).

### 3.3. Miseq Platform of Sequencing and Bioinformatic Analysis

Raw files after demultiplexed were quality-filtered using QIIME (version 1.17) with the criteria, according to Li et al. (27) described. The similar steps were followed and performed based on the description by Jia et al. (28) and Hao et al. (25). The chimeric sequences were removed using UPARS (version 7.1 <http://drive5.com/uparse/>), and operational units (OTUs) were clustered with the similarity cut-off set at 97%, while chimeric sequences were removed. The analysis of phylogenetic affiliation

of 16S rRNA gene sequence was performed by RDP Classifier (<http://rdp.cme.msu.edu/>) against the Silva (SSU115) 16S rRNA database using confidence threshold of 70% (28).

#### 3.4. Measurement of the Fecal Lactic Acid and Short-Chain Fatty Acids

The fecal samples placed in the refrigerator at  $-80^{\circ}\text{C}$  were taken out and put in the lyophilizer immediately. Then fecal samples were frozen and dried to the constant weight according to the method described by Alexandra Dostal et al. (29) with minor modifications. Fifty milligrams of freeze-dried samples were weight to tube of 10 mL accurately, and 1 mL of 0.15 mmol/L  $\text{H}_2\text{SO}_4$  and 1 mL pure water were added after blending and centrifugation (11000 r/minute,  $4^{\circ}\text{C}$ , 20 minutes). The supernatants were extracted and filtered via 0.22  $\mu\text{m}$  organic filter to 1.5 mL vials for HPLC, or sealed and placed at  $4^{\circ}\text{C}$  for the subsequent HPLC analysis.

The injection volume for HPLC analysis was 10  $\mu\text{L}$ . Separation was carried out on an Aminex HPX-87H column (300 mm  $\times$  7.8 mm, Bio-Rad, USA), with column temperature  $40^{\circ}\text{C}$ , flowing phase 10 mmol/L  $\text{H}_2\text{SO}_4$ , and flowing rate 0.6 mL/minute. The ultraviolet detector was operated at  $\lambda = 210$  nm, and the total running time was set at 28 minutes. The concentrations of lactic acid and SCFAs in the samples were calculated according to the standard curves of the corresponding standard for HPLC, under the same conditions (29).

#### 3.5. Statistical Analysis

All the test data were the averages of three parallel tests. The results of lactic acid and SCFAs were expressed as  $\mu\text{mol/g}$  dried feces. All data analyses were conducted by the SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). The "Mann-Whitney U" Test was used for difference analysis. P values less than 0.05 were considered statistically significant. Analysis of the structure of intestinal microflora diversity and phylogenetic analysis were carried out using the R language tool for plotting.

## 4. Results

#### 4.1. Assays of Diversity Indexes and Operational Taxonomic Units (OTUs) of fecal Microbiota

Nearly 1,100,000 gene sequences with an average length of 447 bp were adequately classified. After trimming the primers, 556,382 reads were remained from the fecal samples of RD infants and 537,733 reads were remained from the fecal samples of H infants. The bacterial

richness estimators (ace and chao) and the indexes of diversity (Shannon and Simpson) at a 3% sequence dissimilarity level were summarized in Table 1, using the MOTHUR program based on estimating the operational taxonomic units (OTUs). The bacterial richness in RD infants feces significantly decreased ( $P < 0.05$ ). Shannon diversity and Simpson indexes of fecal microbiota were lower in RD infants than in H infants.

#### 4.2. OTU Taxonomic Analysis

The overlapping areas of circles in Venn diagrams represented that RD and H infants apportioned a large number of OTUs, despite significant interpersonal variations (Figure 1). Based on the diagrams, the relatively unified and stable components were remained across intricate microbial accumulation or assemblages. The RD and H infants also apportioned a great deal of OTUs (167 OTUs), in term of male and female individuals. This result indicated that a core or "home" microbiome might be remained in the fecal microbiota of infants. Nine phyla identified including to *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Acidobacteria*, and *Saccharibacteria*. Overall, 99.86% of sequences were attached to the four dominant phyla (*Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*) of the infant intestine. However, the proportions of each group were different (Figure 2). Compared with H infants, infants infected RD had a greater abundance of *Proteobacteria* ( $P < 0.01$ ) and a decrease in the abundance of *Actinobacteria* ( $P < 0.05$ ).

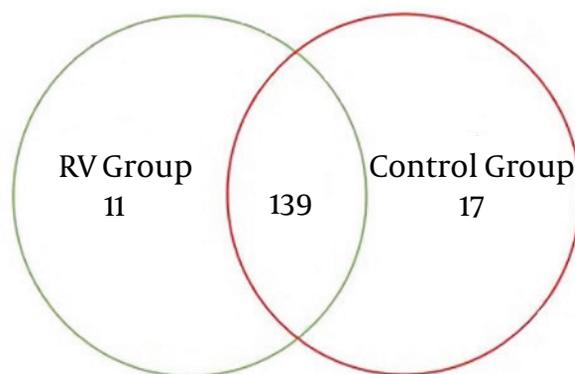


Figure 1. OTU Venn analysis of the two groups

The fecal microbial composition at the genus level in RD and H infants is shown in Figure 3. The most abundant bacterial genera in all fecal samples were *Escherichia-shigella*, *Bifidobacterium*, *Klebsiella*, *Veillonella*, *Streptococcus*, and *Lachnoclostridium*. These species represented more

**Table 1.** Comparison of Phylotype Coverage and Diversity Estimation of the 16S rRNA Gene Libraries at 3% Dissimilarity from the Pyrosequencing Analysis

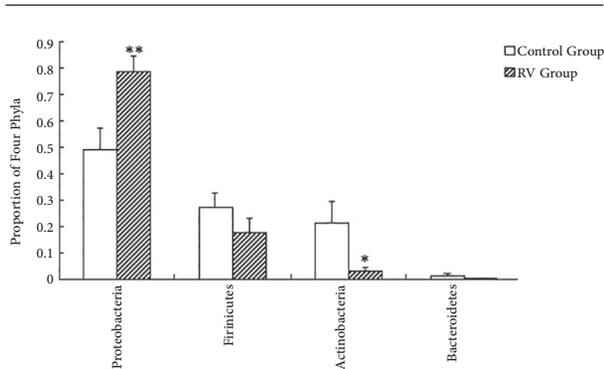
Group	Reads	OTUs <sup>a</sup>	Ace <sup>b</sup>	Chao	Coverage <sup>c</sup>	Shannon	Simpson
RD infants	556,382	1058	1396*	1333	0.999	16.85 <sup>d</sup>	7.604 <sup>d</sup>
H infants	537,733	1166	1720	1566	0.999	21.40	5.507

<sup>a</sup>The operational taxonomic units (OTUs) were defined with 3% dissimilarity level.

<sup>b</sup>Ace and Chao: richness estimators.

<sup>c</sup>Diversity indices (Shannon and Simpson) were calculated using coverage percentage's method and the MOTHER program.

<sup>d</sup>Significant differences vs. RD infants and H infants:  $P < 0.05$ .

**Figure 2.** Comparison of the four most abundant bacterial phylum in the two infants groups. The proportion of four phyla were respectively (RD vs. H) 78.32% vs. 47.53%, 18.42% vs. 27.41%, 3.03% vs. 23.43%, 0.20% vs. 1.36%.

than 80% of the total abundance. Other four genera (*Enterococcus*, *Clostridium-sensu-stricto-1*, *Lactobacillus*, and *raoultella*) were considered as the sub-dominant population, which represented more than 1% abundance each. *Enterococcus* and *Clostridium-sensu-stricto-1* were mainly found in the feces of RD infants while *Lactobacillus* and *Raoultella* were mainly found in the feces of H infants. Additionally, other 22 bacterial genera belonged to the non-dominant population (< 1% abundance). Fecal samples in RD infants had a greater abundance of *Klebsiella* than H infants ( $P < 0.05$ ). The abundance of *Knoellia* as the non-dominant genus was decreased in RV infants ( $P < 0.05$ ).

#### 4.3. Assays of Intestinal Microbiota Structure and Similarity

A clear clustering was observed using unweighted pair group method with arithmetic mean in Figure 4. The results of dendrograms showed similarity between fecal microbiota in RD and H infants, despite significant inter-individual variability (for example, sample c, d and e). The samples were clustered together because of the similarity of both infected rotavirus. In PCA analysis, fecal samples in RD and H infants were separated in the direction of PC2 (19.2%) and were clustered together due to RV infection (Figure 5). Enterobacteriaceae as a dominant family in all samples is demonstrated in the heatmap (Figure 6).

It was obviously significant that an unidentified strain of *Bacteroidales* S24-7 group differed in fecal samples of RD and H infants ( $P < 0.05$ ). It is noted that clustering in the heatmap was similar with Hierarchical clustering (Hcluster).

#### 4.4. Assays of fecal Lactic Acid and SCFAs

Total SCFAs content of all feces varied from 1.37 mmol/kg to 1.83 mmol/kg (Table 2). Only lactic acid content in fecal samples of RD infants was lower than that of H infants ( $P < 0.05$ ). In this study, acetic acid was the dominant SCFA, followed by butyric and propionic acids. However, the content of total SCFAs had no significant difference in all samples.

**Table 2.** Comparison of the Concentrations of Fecal SCFAs in the Two Infants Groups

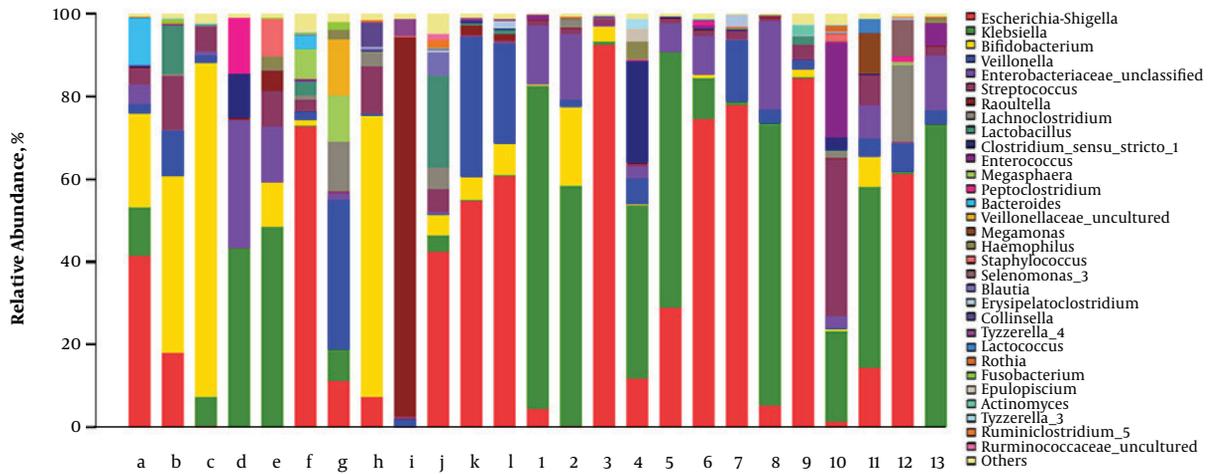
Organic Acids	RD Infants, $\mu\text{mol/g}$	H Infants, $\mu\text{mol/g}$
Lactic acid	191.36 $\pm$ 134.91 <sup>a</sup>	222.63 $\pm$ 49.48
Formic acid	5.07 $\pm$ 2.82	13.04 $\pm$ 7.35
Acetic acid	1321.04 $\pm$ 681.99	1783.34 $\pm$ 488.39
Propionic acid	19.77 $\pm$ 9.84	10.50 $\pm$ 5.17
Butyric acid	65.04 $\pm$ 55.57	33.64 $\pm$ 29.13
Total SCFAs	1373.00 $\pm$ 690.06	1829.30 $\pm$ 494.37

Abbreviation: H, healthy; RD, rotaviral-induced diarrhea; SCFAs, short-chain fatty acids.

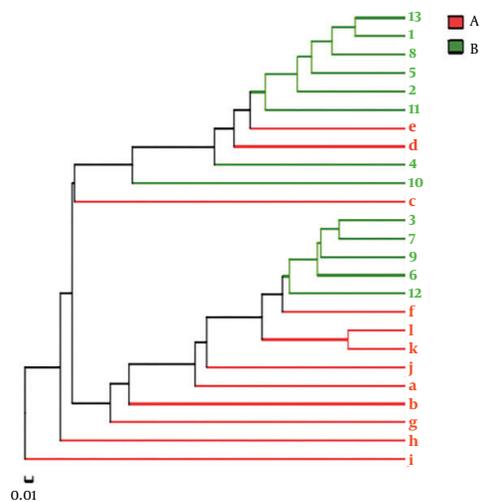
<sup>a</sup>Significant differences vs. RD and H infants:  $P < 0.05$ .

## 5. Discussion

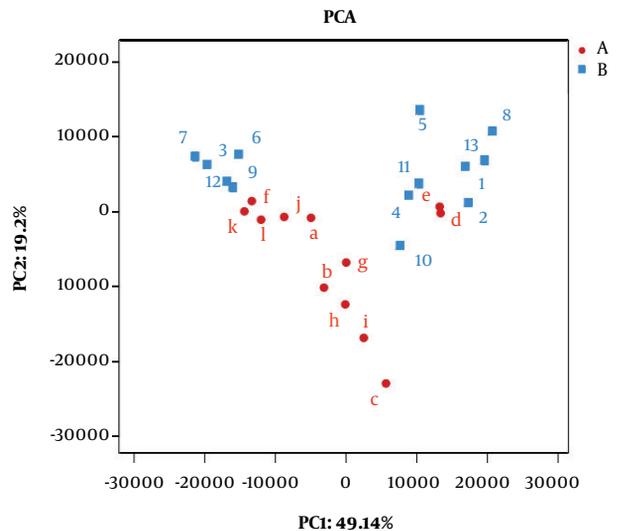
Microbial colonization of the intestine can be beatification for infants to develop a normal and healthy intestine, establish the intestinal homeostasis and prevent of infections by pathogenic bacteria and virus (3). To some extent, the levels of later health were mainly influenced by controlling or minimizing the invasion of postnatal gut diseases. However, the cognition about the process of microbiome establishment and development in the neonatal and infants gut is still limited. This research showed that some RD infants reflected an altered intestinal microbiota



**Figure 3.** Relative abundance of fecal flora at the genus level. The predominant genera in RD and H infants feces were respectively (RD: 1-13 vs. H: a-l), *Escherichia-shigella* (37.06% vs. 26.13%), *Bifidobacterium* (2.78% vs. 23.55%), *Klebsiella* (38.73% vs. 10.49%), *Veillonella* (3.78% vs. 9.92%), *Streptococcus* (5.14% vs. 5.01%), *Lachnoclostridium* (2.02% vs. 1.95%).



**Figure 4.** Hcluster tree of fecal microbiota in RD infants (A: 1-13) and H infants (B: a-l).



**Figure 5.** PCA analysis of fecal flora in all samples. RD infants (A: 1-13) and H infants (B: a-l).

based on the results of fecal microbiota. This may lead to these infants developing elevated risk of health problems in the future, because the matagenomic analyses of the gut microbiota of RD infants taking part in the study propose that rotavirus infection has necessarily influenced on the establishing gut microbiome, even though drawing the firm conclusions need large numbers of RD infants participated in the study.

*Proteobacteria* and *Actinobacteria* have a major impact on human health (30, 31). *Proteobacteria* was elevated and *Actinobacteria* was reduced in the study, and the fact could be viewed as a marker for disruption of gut microbiota. In Crohn's disease, Norovirus infection and ulcerative colitis, increased *Proteobacteria* had been found to be in lineage to intestinal inflammation or without inflammation (10). The current study found multiple mechanisms may con-

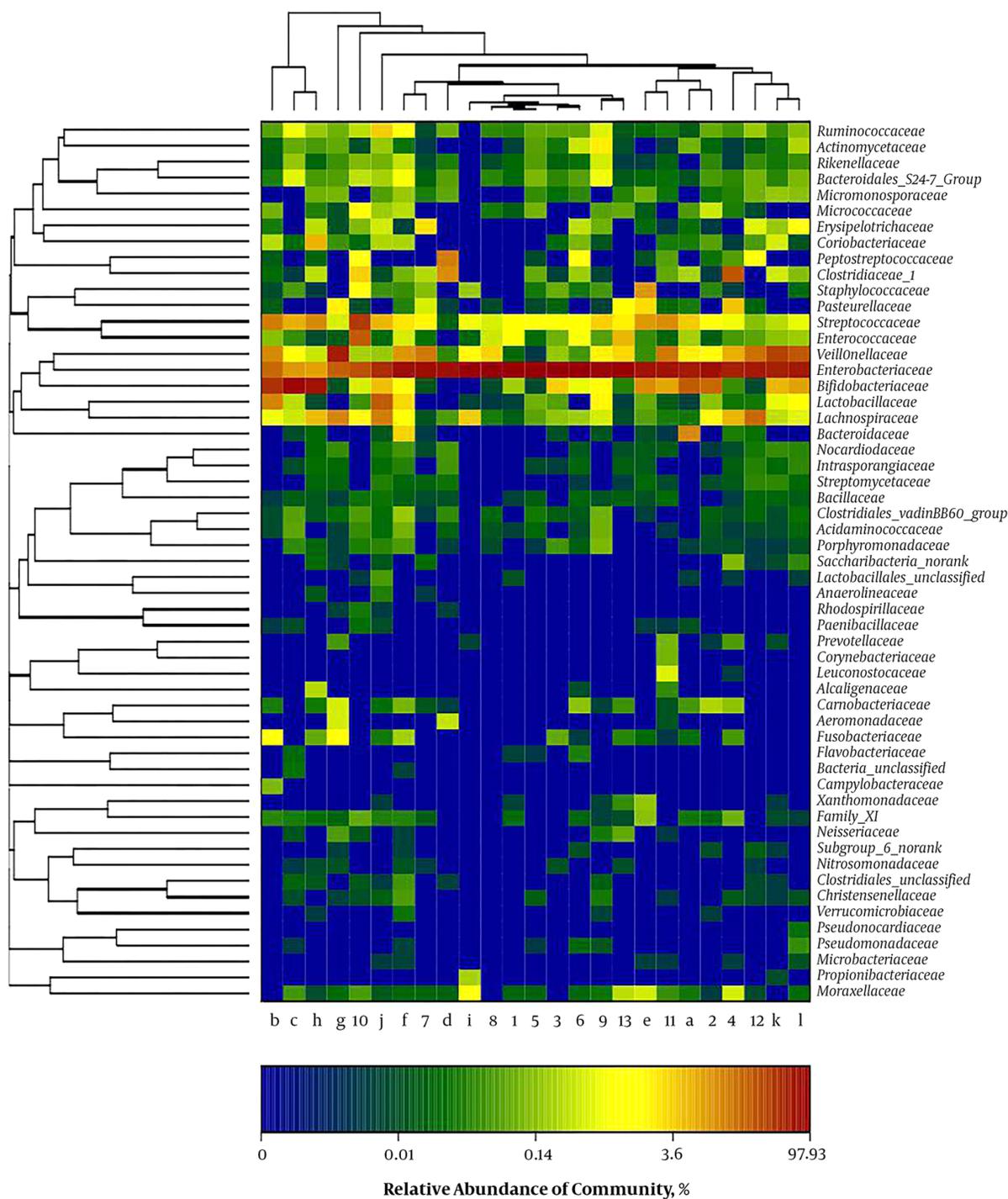


Figure 6. Heatmap analysis at the family level on fecal flora. RD infants (A:1-13) and H infants (B: a-l).

tribute to the explanation for increased *Proteobacteria* levels, particularly elevated *E. coli* levels in fecal samples (32).

It is hardly available to measure the illness severity in the current research. Therefore, as a future direction, inves-

tingating potential connection between inflammation and severity may be achieved, such as vomiting or diarrhea frequency increased in RD infants. This study was the first testing of increasing *Klebsiella*, which belonged to *Proteobacteria* in RD infants. More recent studies suggested *Klebsiella* may contribute to inflammation (33, 34), such as bronchitis, pneumonia, and meningitis (35). Therefore, it could be supposed that RD infection, as the major cause of the viral gastroenteritis might disrupt the gut microbiota and introduce more effects from *Klebsiella*.

The health can be long lasting effects due to microbiota disruption; unfortunately, the impact of disruption on health is a known limited. Bacterial gastroenteritis had previously been associated with post-infectious IBS (PI-IBS), a subset of IBS, following pathogenic *E. coli*, *Salmonella*, and *Campylobacter* infections (36). Less attention has been paid for the relationship between viral gastroenteritis and PI-IBS. Microbiota disruption existing in RD infants intestine if microbiota disruption increases the potential risk of long-term illness, following episodes of Rotaviruses needed to be researched (36). The samples examined in this study had limited the availability of the medical data of RD infant, therefore, did not measure the duration of illness. It was unavailable to measure the baseline microbiota before illness or after the path of recovery, due to lacking the information on the samples from various times following the illness onset. Based on these considerations, future study to treat these limitations may enroll human volunteers, to supervise the gut microbiota before and after RV infection, as this treatment could determine specific responses to infection.

Marchesi et al. had observed that IBD patients were correlated with lower levels of SCFAs in feces, compared with healthy controls (37). The reduction of SCFAs was due to the decrease of *Clostridium* groups in fecal samples of IBD patients (37). However, no correlation between total SCFAs of feces and RV infection had been observed in this study. The possible reason of this fact may be that no changes were found in *Clostridium* groups in RD infants feces compared with H infants feces. This study showed that the metabolism production in large intestine of RD infants had lower level of lactic acid. The metabolite of *Bifidobacterium* and *Lactobacillus* were lactic acid and acetic. Future research may focus on the relationship between the lactate and the abundance of *Bifidobacterium* and *Lactobacillus* genus because viral gastroenteritis might correlate with the change of the acetic acid.

### 5.1. Conclusions

In conclusion, because the host immunity interacts with the gut microbiota at different and complex levels, the relationship between pathologies and dysbiosis may

be built. Butto and Haller (38) had mentioned that it was worthy to debate whether the dysbiotic state is consequence of the pathology. Variation of the composition of the gut intestine microflora may happen upon exposure to infections (39). It has been proved the directly effects of the interactions between the gut microbiota and host immunity in asthma and inflammatory bowel diseases (IBD) (40, 41). Therefore, the intestinal microbiota composition of infants with RV infection and its correlation with SCFAs and lactic acid were evaluated. Rotaviral infection in infants lead to the alteration of fecal microbiota and lactic acid concentration compared with healthy infants. Fecal microbiota and metabolite may advance our understanding and treatment of RD.

### Acknowledgments

The authors would like to thank Wenkui Guo for their technical assistance and Xin-huai Zhao for writing revision. The authors also thank all the subjects, who participated in the study, and the support of Children's Hospital of Harbin city. The authors thank the anonymous referees and editors for their valuable advice.

### Footnotes

**Authors' Contribution:** Ling Guo designed experiments and wrote the paper. Dongxu Huang and Lin Li carried out experiments and analyzed sequencing data and developed analysis tools. Austin Nevin reviewed and edited the manuscript. All authors read and approved the manuscript.

**Conflict of Interests:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Ethical Approval:** This project was approved by Northeast Agricultural University and Harbin Children's Hospital in Heilongjiang Province, China. All experiments were performed with the ethical standards of Declaration of Helsinki. Additionally, the parents of infants supplied with all the written informed consent.

**Financial Disclosure:** It is not declared by the authors.

**Funding/Support:** This research was supported by the open fund of the Key Laboratory of the Dairy Sciences of the Ministry of Education at Northeast Agricultural University (2017KLDS06).

## References

- Salonen A, Lahti L, Salojarvi J, Holtrop G, Korpela K, Duncan SH, et al. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J*. 2014;**8**(11):2218–30. doi: [10.1038/ismej.2014.63](https://doi.org/10.1038/ismej.2014.63). [PubMed: [24763370](https://pubmed.ncbi.nlm.nih.gov/24763370/)]. [PubMed Central: [PMC4992075](https://pubmed.ncbi.nlm.nih.gov/PMC4992075/)].
- Matamoros S, Gras-Leguen C, Le Vacon F, Potel G, de La Cochetiere MF. Development of intestinal microbiota in infants and its impact on health. *Trends Microbiol*. 2013;**21**(4):167–73. doi: [10.1016/j.tim.2012.12.001](https://doi.org/10.1016/j.tim.2012.12.001). [PubMed: [23332725](https://pubmed.ncbi.nlm.nih.gov/23332725/)].
- Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol*. 2010;**10**(3):159–69. doi: [10.1038/nri2710](https://doi.org/10.1038/nri2710). [PubMed: [20182457](https://pubmed.ncbi.nlm.nih.gov/20182457/)].
- Arboleya S, Binetti A, Salazar N, Fernandez N, Solis G, Hernandez-Barranco A, et al. Establishment and development of intestinal microbiota in preterm neonates. *FEMS Microbiol Ecol*. 2012;**79**(3):763–72. doi: [10.1111/j.1574-6941.2011.01261.x](https://doi.org/10.1111/j.1574-6941.2011.01261.x). [PubMed: [22126419](https://pubmed.ncbi.nlm.nih.gov/22126419/)].
- Ang L, Arboleya S, Lihua G, Chuihui Y, Nan Q, Suarez M, et al. The establishment of the infant intestinal microbiome is not affected by rotavirus vaccination. *Sci Rep*. 2014;**4**:7417. doi: [10.1038/srep07417](https://doi.org/10.1038/srep07417). [PubMed: [25491920](https://pubmed.ncbi.nlm.nih.gov/25491920/)]. [PubMed Central: [PMC4261165](https://pubmed.ncbi.nlm.nih.gov/PMC4261165/)].
- Li JT, Wei J, Guo HX, Han JB, Ye N, He HY, et al. Development of a human rotavirus induced diarrhoea model in Chinese mini-pigs. *World J Gastroenterol*. 2016;**22**(31):7135–45. doi: [10.3748/wjg.v22.i31.7135](https://doi.org/10.3748/wjg.v22.i31.7135). [PubMed: [27610023](https://pubmed.ncbi.nlm.nih.gov/27610023/)]. [PubMed Central: [PMC4988310](https://pubmed.ncbi.nlm.nih.gov/PMC4988310/)].
- Chenoll E, Casinos B, Bataller E, Buesa J, Ramon D, Genoves S, et al. Identification of a peptide produced by bifidobacterium longum CECT 7210 with antirotaviral activity. *Front Microbiol*. 2016;**7**:655. doi: [10.3389/fmicb.2016.00655](https://doi.org/10.3389/fmicb.2016.00655). [PubMed: [27199974](https://pubmed.ncbi.nlm.nih.gov/27199974/)]. [PubMed Central: [PMC4855034](https://pubmed.ncbi.nlm.nih.gov/PMC4855034/)].
- Istrate C, Hagbom M, Vikstrom E, Magnusson KE, Svensson L. Rotavirus infection increases intestinal motility but not permeability at the onset of diarrhea. *J Virol*. 2014;**88**(6):3161–9. doi: [10.1128/JVI.02927-13](https://doi.org/10.1128/JVI.02927-13). [PubMed: [24371070](https://pubmed.ncbi.nlm.nih.gov/24371070/)]. [PubMed Central: [PMC3957942](https://pubmed.ncbi.nlm.nih.gov/PMC3957942/)].
- Hagbom M, Sharma S, Lundgren O, Svensson L. Towards a human rotavirus disease model. *Curr Opin Virol*. 2012;**2**(4):408–18. doi: [10.1016/j.coviro.2012.05.006](https://doi.org/10.1016/j.coviro.2012.05.006). [PubMed: [22722079](https://pubmed.ncbi.nlm.nih.gov/22722079/)].
- Nelson AM, Walk ST, Taube S, Taniuchi M, Houpt ER, Wobus CE, et al. Disruption of the human gut microbiota following Norovirus infection. *PLoS One*. 2012;**7**(10):e48224. doi: [10.1371/journal.pone.0048224](https://doi.org/10.1371/journal.pone.0048224). [PubMed: [23118957](https://pubmed.ncbi.nlm.nih.gov/23118957/)]. [PubMed Central: [PMC3484122](https://pubmed.ncbi.nlm.nih.gov/PMC3484122/)].
- Sassone-Corsi M, Nuccio SP, Liu H, Hernandez D, Vu CT, Takahashi AA, et al. Microcins mediate competition among Enterobacteriaceae in the inflamed gut. *Nature*. 2016;**540**(7632):280–3. doi: [10.1038/nature20557](https://doi.org/10.1038/nature20557). [PubMed: [27798599](https://pubmed.ncbi.nlm.nih.gov/27798599/)]. [PubMed Central: [PMC5145735](https://pubmed.ncbi.nlm.nih.gov/PMC5145735/)].
- Tazume S, Takeshi K, Saidi SM, Ichoroh CG, Mutua WR, Waiyaki PG, et al. Ecological studies on intestinal microbial flora of Kenyan children with diarrhoea. *J Trop Med Hyg*. 1990;**93**(3):215–21. [PubMed: [2348500](https://pubmed.ncbi.nlm.nih.gov/2348500/)].
- Fei P, Li L, Cai X, Zhang X, Bai HJ, Jiang YJ, et al. Differences in the biodiversity of the fecal microbiota of infants with rotaviral diarrhea and healthy infants. *Jundishapur J Microbiol*. 2016;**9**(4):e32356. doi: [10.5812/jjm.32356](https://doi.org/10.5812/jjm.32356). [PubMed: [27279991](https://pubmed.ncbi.nlm.nih.gov/27279991/)]. [PubMed Central: [PMC4895788](https://pubmed.ncbi.nlm.nih.gov/PMC4895788/)].
- Shu Q, Qu F, Gill HS. Probiotic treatment using Bifidobacterium lactis HN019 reduces weanling diarrhea associated with rotavirus and Escherichia coli infection in a piglet model. *J Pediatr Gastroenterol Nutr*. 2001;**33**(2):171–7. doi: [10.1097/00005176-200108000-00014](https://doi.org/10.1097/00005176-200108000-00014). [PubMed: [11568519](https://pubmed.ncbi.nlm.nih.gov/11568519/)].
- Plummer S, Weaver MA, Harris JC, Dee P, Hunter J. Clostridium difficile pilot study: effects of probiotic supplementation on the incidence of C. difficile diarrhoea. *Int Microbiol*. 2004;**7**(1):59–62. [PubMed: [15179608](https://pubmed.ncbi.nlm.nih.gov/15179608/)].
- Chen SY, Tsai CN, Lee YS, Lin CY, Huang KY, Chao HC, et al. Intestinal microbiome in children with severe and complicated acute viral gastroenteritis. *Sci Rep*. 2017;**7**:46130. doi: [10.1038/srep46130](https://doi.org/10.1038/srep46130). [PubMed: [28397879](https://pubmed.ncbi.nlm.nih.gov/28397879/)]. [PubMed Central: [PMC5387401](https://pubmed.ncbi.nlm.nih.gov/PMC5387401/)].
- Arboleya S, Stanton C, Ryan CA, Dempsey E, Ross PR. Bosom buddies: The symbiotic relationship between infants and bifidobacterium longum ssp. Longum and ssp. Infantis. Genetic and probiotic features. *Annu Rev Food Sci Technol*. 2016;**7**:1–21. doi: [10.1146/annurev-food-041715-033151](https://doi.org/10.1146/annurev-food-041715-033151). [PubMed: [26934170](https://pubmed.ncbi.nlm.nih.gov/26934170/)].
- Blottiere HM, Buecher B, Galmiche JP, Cherbut C. Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. *Proc Nutr Soc*. 2003;**62**(1):101–6. doi: [10.1079/PNS2002215](https://doi.org/10.1079/PNS2002215). [PubMed: [12740064](https://pubmed.ncbi.nlm.nih.gov/12740064/)].
- Sanderson IR. Short chain fatty acid regulation of signaling genes expressed by the intestinal epithelium. *J Nutr*. 2004;**134**(9):2450S–4S. doi: [10.1093/jn/134.9.2450S](https://doi.org/10.1093/jn/134.9.2450S). [PubMed: [15333741](https://pubmed.ncbi.nlm.nih.gov/15333741/)].
- Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: Fermentation and short chain fatty acids. *J Clin Gastroenterol*. 2006;**40**(3):235–43. doi: [10.1097/00004836-200603000-00015](https://doi.org/10.1097/00004836-200603000-00015). [PubMed: [16633129](https://pubmed.ncbi.nlm.nih.gov/16633129/)].
- Huda-Faujan N, Abdulmir AS, Fatimah AB, Anas OM, Shuhaimi M, Yazid AM, et al. The impact of the level of the intestinal short chain Fatty acids in inflammatory bowel disease patients versus healthy subjects. *Open Biochem J*. 2010;**4**:53–8. doi: [10.2174/1874091X01004010053](https://doi.org/10.2174/1874091X01004010053). [PubMed: [20563285](https://pubmed.ncbi.nlm.nih.gov/20563285/)]. [PubMed Central: [PMC2887640](https://pubmed.ncbi.nlm.nih.gov/PMC2887640/)].
- Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*. 2011;**469**(7331):543–7. doi: [10.1038/nature09646](https://doi.org/10.1038/nature09646). [PubMed: [21270894](https://pubmed.ncbi.nlm.nih.gov/21270894/)].
- Takaishi H, Matsuki T, Nakazawa A, Takada T, Kado S, Asahara T, et al. Imbalance in intestinal microflora constitution could be involved in the pathogenesis of inflammatory bowel disease. *Int J Med Microbiol*. 2008;**298**(5-6):463–72. doi: [10.1016/j.ijmm.2007.07.016](https://doi.org/10.1016/j.ijmm.2007.07.016). [PubMed: [17897884](https://pubmed.ncbi.nlm.nih.gov/17897884/)].
- Sha S, Xu B, Wang X, Zhang Y, Wang H, Kong X, et al. The biodiversity and composition of the dominant fecal microbiota in patients with inflammatory bowel disease. *Diagn Microbiol Infect Dis*. 2013;**75**(3):245–51. doi: [10.1016/j.diagmicrobio.2012.11.022](https://doi.org/10.1016/j.diagmicrobio.2012.11.022). [PubMed: [23276768](https://pubmed.ncbi.nlm.nih.gov/23276768/)].
- Hao YT, Wu SG, Xiong F, Tran NT, Jakovic I, Zou H, et al. Succession and fermentation products of grass carp (Ctenopharyngodon idellus) hindgut microbiota in response to an extreme dietary shift. *Front Microbiol*. 2017;**8**:1585. doi: [10.3389/fmicb.2017.01585](https://doi.org/10.3389/fmicb.2017.01585). [PubMed: [28871246](https://pubmed.ncbi.nlm.nih.gov/28871246/)]. [PubMed Central: [PMC5566599](https://pubmed.ncbi.nlm.nih.gov/PMC5566599/)].
- Costa MC, Stampfli HR, Allen-Vercoe E, Weese JS. Development of the faecal microbiota in foals. *Equine Vet J*. 2016;**48**(6):681–8. doi: [10.1111/evj.12532](https://doi.org/10.1111/evj.12532). [PubMed: [26518456](https://pubmed.ncbi.nlm.nih.gov/26518456/)].
- Li AJ, Hou BL, Li MX. Cell adhesion, ammonia removal and granulation of autotrophic nitrifying sludge facilitated by N-acyl-homoserine lactones. *Bioresour Technol*. 2015;**196**:550–8. doi: [10.1016/j.biortech.2015.08.022](https://doi.org/10.1016/j.biortech.2015.08.022). [PubMed: [26295441](https://pubmed.ncbi.nlm.nih.gov/26295441/)].
- Jia HR, Geng LL, Li YH, Wang Q, Diao QY, Zhou T, et al. The effects of Bt CryIIe toxin on bacterial diversity in the midgut of Apis mellifera ligustica (Hymenoptera: Apidae). *Sci Rep*. 2016;**6**:24664. doi: [10.1038/srep24664](https://doi.org/10.1038/srep24664). [PubMed: [27090812](https://pubmed.ncbi.nlm.nih.gov/27090812/)]. [PubMed Central: [PMC4835784](https://pubmed.ncbi.nlm.nih.gov/PMC4835784/)].
- Dostal A, Baumgartner J, Riesen N, Chassard C, Smuts CM, Zimmermann MB, et al. Effects of iron supplementation on dominant bacterial groups in the gut, faecal SCFA and gut inflammation: A randomised, placebo-controlled intervention trial in South African children. *Br J Nutr*. 2014;**112**(4):547–56. doi: [10.1017/S0007114514001160](https://doi.org/10.1017/S0007114514001160). [PubMed: [24916165](https://pubmed.ncbi.nlm.nih.gov/24916165/)].
- Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, et al. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes*. 2011;**60**(11):2775–86. doi: [10.2337/db11-0227](https://doi.org/10.2337/db11-0227). [PubMed: [21933985](https://pubmed.ncbi.nlm.nih.gov/21933985/)]. [PubMed Central: [PMC3198091](https://pubmed.ncbi.nlm.nih.gov/PMC3198091/)].

31. Willing BP, Dicksved J, Halfvarson J, Andersson AF, Lucio M, Zheng Z, et al. A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology*. 2010;**139**(6):1844-1854. doi: [10.1053/j.gastro.2010.08.049](https://doi.org/10.1053/j.gastro.2010.08.049). [PubMed: 20816835].
32. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host Microbe*. 2007;**2**(2):19-29. doi: [10.1016/j.chom.2007.06.010](https://doi.org/10.1016/j.chom.2007.06.010). [PubMed: 18005726].
33. Gu DX, Huang YL, Ma JH, Zhou HW, Fang Y, Cai JC, et al. Detection of colistin resistance gene mcr-1 in hypervirulent klebsiella pneumoniae and escherichia coli isolates from an infant with diarrhea in China. *Antimicrob Agents Chemother*. 2016;**60**(8):5099-100. doi: [10.1128/AAC.00476-16](https://doi.org/10.1128/AAC.00476-16). [PubMed: 27270278]. [PubMed Central: PMC4958155].
34. Zhou YF, Tao MT, Huo W, Liao XP, Sun J, Liu YH. In vivo pharmacokinetic and pharmacodynamic profiles of antofloxacin against Klebsiella pneumoniae in a neutropenic murine lung infection model. *Antimicrob Agents Chemother*. 2017;**61**(5). doi: [10.1128/AAC.02691-16](https://doi.org/10.1128/AAC.02691-16). [PubMed: 28264844]. [PubMed Central: PMC5404557].
35. Montesinos Sanchis E, Aguilera Alonso D, Martinez Sebastian L. Klebsiella pneumoniae bacteremia complicating adenovirus gastroenteritis. *Pediatr Infect Dis J*. 2016;**35**(4):467. doi: [10.1097/INF.0000000000001045](https://doi.org/10.1097/INF.0000000000001045). [PubMed: 26967686].
36. Schwille-Kiuntke J, Enck P, Zendler C, Krieg M, Polster AV, Klosterhalfen S, et al. Postinfectious irritable bowel syndrome: Follow-up of a patient cohort of confirmed cases of bacterial infection with Salmonella or Campylobacter. *Neurogastroenterol Motil*. 2011;**23**(11):e479-88. doi: [10.1111/j.1365-2982.2011.01779.x](https://doi.org/10.1111/j.1365-2982.2011.01779.x). [PubMed: 21883703].
37. Marchesi JR, Holmes E, Khan F, Kochhar S, Scanlan P, Shanahan F, et al. Rapid and noninvasive metabonomic characterization of inflammatory bowel disease. *J Proteome Res*. 2007;**6**(2):546-51. doi: [10.1021/pr060470d](https://doi.org/10.1021/pr060470d). [PubMed: 17269711].
38. Butto LF, Haller D. Dysbiosis in intestinal inflammation: Cause or consequence. *Int J Med Microbiol*. 2016;**306**(5):302-9. doi: [10.1016/j.ijmm.2016.02.010](https://doi.org/10.1016/j.ijmm.2016.02.010). [PubMed: 27012594].
39. Moloney RD, Desbonnet L, Clarke G, Dinan TG, Cryan JF. The microbiome: Stress, health and disease. *Mamm Genome*. 2014;**25**(1-2):49-74. doi: [10.1007/s00335-013-9488-5](https://doi.org/10.1007/s00335-013-9488-5). [PubMed: 24281320].
40. Weber J, Illi S, Nowak D, Schierl R, Holst O, von Mutius E, et al. Asthma and the hygiene hypothesis. Does cleanliness matter? *Am J Respir Crit Care Med*. 2015;**191**(5):522-9. doi: [10.1164/rccm.201410-1899OC](https://doi.org/10.1164/rccm.201410-1899OC). [PubMed: 25584716].
41. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett*. 2014;**588**(22):4223-33. doi: [10.1016/j.febslet.2014.09.039](https://doi.org/10.1016/j.febslet.2014.09.039). [PubMed: 25307765]. [PubMed Central: PMC5050012].