



ORIGINAL ARTICLE

Analysis of intestinal microbiota diversity in children with non-organic anorexia



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Abstract The pathogenesis of non-organic anorexia in children is not clear. This study intends to analyze intestinal bacteria to provide a relevant theoretical basis for the clinical rational selection of microecological agents. In the present study, children with non-organic anorexia were included in the anorexia group and normal healthy children in the control group. Stool samples were collected for the bioinformatics analysis after PCR and high-throughput sequencing. The results showed that the Ace, Chao, and Shannon indexes in the anorexia group were higher than those in the control group, while the Simpson index in the control group was lower than in the anorexia group. There were 14 taxa in the anorexia group and 11 taxa in the healthy control group at the phylum level, and 193 taxa in the anorexia group and 180 in the control group at the genus level. The dominant bacteria at the phylum level of the two groups were the same, while there were 16 dominant bacteria taxa in the anorexia group and 17 in the control group at the genus level. The ratio of percentage abundance of *Bacteroidetes* to that of *Firmicutes* (the B/F index) in the anorexia group was higher than in the control group. The abundance of *Bacteroidetes* in the anorexia group was higher than that in the control group, and the abundance of *Actinomycetes* in the control group was higher than that in the anorexia group. There were significant differences in 14 dominant genera between the two groups at the genus classification level. The LEfSe multilevel species difference analysis showed that at the phylum level, the significant influential bacterial taxa in the anorexia group were *Bacteroidetes* and *Actinobacteria* in the control group. At the genus level, the significant influential bacterial taxa in the anorexia group were *Bacteroides*, *Faecalibacterium*, and *Subdoligranulum*, and *Bifidobacterium*, *Blautia*, *Streptococcus*, *Lachnoclostridium*, and *Erysipelatoclostridium* in the control group. We conclude that the increase in *Bacteroides* abundance or in the B/F index and the reduction in *Bifidobacterium* abundance were related to the pathogenesis of anorexia.

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PALABRAS CLAVE

Anorexia;
Diversidad
microbiana intestinal;
Secuenciación de alto
rendimiento;
Bacterias

Diversidad microbiana intestinal en los niños con anorexia no orgánica

Resumen La patogénesis de la anorexia no orgánica en los niños no está clara. El propósito de esta investigación fue estudiar las bacterias intestinales, y proporcionar una base teórica para la selección racional de probióticos en la clínica. Se analizaron muestras fecales de un grupo de niños con anorexia no orgánica (grupo de anorexia), y de un grupo de niños sanos normales (grupo de control). Se realizó PCR y secuenciación de alto rendimiento, seguido del análisis bioinformático. En el grupo de anorexia, los índices Ace, Chao y Shannon fueron más altos que en el grupo control, mientras que el índice Simpson fue menor. Se detectaron 14 taxones en el grupo de anorexia y 11 taxones en el grupo control a nivel de *phylum*. A nivel de género, hubo 193 taxones en el grupo de anorexia y 180 taxones en el grupo control. A nivel de *phylum*, las bacterias dominantes fueron las mismas en ambos grupos, mientras que, a nivel de género, hubo 16 taxones bacterianos dominantes en el grupo de anorexia y 17 taxones bacterianos dominantes en el grupo control. La relación entre el porcentaje de abundancia de *Bacteroidetes* y el porcentaje de abundancia de *Firmicutes* en el grupo de anorexia (índice B/F) fue mayor que en el grupo control. El número de *Bacteroidetes* en el grupo de anorexia fue mayor que en el grupo control, mientras que el número de actinomicetos fue mayor en el grupo control. Hubo diferencias significativas entre los dos grupos en 14 géneros dominantes. El análisis de la diferencia de especies multinivel (LEfSe) mostró que a nivel de *phylum*, *Bacteroidetes* fue el taxón bacteriano significativamente afectado en el grupo de anorexia, en tanto que en el grupo control lo fue *Actinobacteria*. A nivel de género, los taxones bacterianos que tuvieron un efecto significativo en el grupo de anorexia fueron *Bacteroides*, *Faecalibacterium* y *Subdoligranulum*, mientras que en el grupo control fueron *Bifidobacterium*, *Blautia*, *Streptococcus*, *Lachnoclostridium* y *Erysipelatoclostridium*. Nuestra conclusión es que el aumento de la abundancia de *Bacteroides* o del índice B/F y la reducción de la abundancia de *Bifidobacterium* se asocian con la patogénesis de la anorexia.

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Introduction

Non-organic anorexia (referred to as anorexia), mostly studied in China but not in many other countries, is a nutritional disorder that differs from anorexia nervosa, the former being more common in children younger than 10 years old, and the latter often occurring in children older than 10 years old with neurological and/or psychological abnormalities^{5,6}. Anorexia can cause conditions and diseases such as malnutrition, anemia, rickets, hypoimmunity, and recurrent respiratory tract infections. It not only causes delays in growth and development, including mental development, and affects the nutritional status of children but also profoundly and adversely influences the physical health of adults¹⁵, thereby causing a heavy psychological and financial burden on the family, nation, and society. Therefore, it is particularly important to understand the pathogenesis of anorexia to be able to apply practical and effective treatments.

Previous studies have shown a dysregulation of the gut microbiota in children with anorexia, and the effective rate of treatment with microecological agents can reach 87.5%. However, most studies used culture methods to identify bacteria, while 99% of the bacteria present in nature cannot be cultured. A few studies used the specific PCR method. Nevertheless, these results could not comprehensively reflect the composition of the gut microbiota in children with anorexia.

At the same time, previous studies have shown that probiotics alone or in combination with conventional therapies can be ineffective in the treatment of anorexia in children in 5.0–22.5% of cases, indicating that changes in the gut microbiota in children with anorexia may not be the only contributing factor, and precise treatment should be applied after understanding specific changes^{11,19,30}.

Therefore, this study used a molecular biology method to select gene fragments in the conserved V3 region of bacterial 16S rDNA genes to design primers and investigate the gut microbiota in children with anorexia using polymerase chain reaction (PCR) and high-throughput sequencing to test and conduct a comprehensive analysis of the intestinal microbiota of anorexic children, to then provide a theoretical basis for the selection of clinically feasible probiotics and perform subsequent drug intervention experiments²⁸.

Materials and methods**Study subjects**

Children with non-organic anorexia, who were treated in our Pediatric Health Care Center from May to October 2016, were included in the anorexia group (A), while healthy children were included in the control group (C). Observation charts were designed and used to record clinical data,

including the name, sex, age, height, weight, dietary habits, and medical history of the subjects.

The diagnostic criteria of anorexia were as follows: (1) a long-term loss of appetite, indifference toward food, inappropriate feeding, an improper diet, or a history of disorders after an illness, such as an eating time ≥ 39 min, food intake reduced by more than 1/3–1/2 compared to that before the disease onset, and a disease course over 1 month; (2) slow or no weight gain; and (3) non-organic diseases, genetic metabolic diseases, anorexia nervosa, and infection²⁰.

The inclusion criteria for the anorexia group required: (1) meeting the diagnostic criteria for anorexia. (2) No history of infection within the past week. (3) No antibiotics or gastric motility drugs used within one week before enrollment. (4) No functional or organic diseases. (5) Diseases not involving any mental or drug-related effects. (6) Age ranges from 1 to 6 years old. The inclusion criteria for the control group required meeting the conditions mentioned in sections 2–6 above.

Sample collection

The external genital area of each examined child was cleaned using an aseptic method before collecting a stool sample (5–10 g). Each sample was placed into a presterilized collection tube. Each tube was labeled with the name of the child and the sample number before being stored at -20°C for later use²².

Extraction of bacterial DNA from stool samples

Bacterial DNA was extracted from the stool samples (500 mg each) using the QIAamp stool mini kit (Qiagen, Germany), in accordance with the instruction manual²².

Amplification of the V3–V4 regions of the bacterial 16S rRNA gene

The sequence of the forward primer 338F was 5'-ACTCCTRCGGGAGGCAGCAG-3', and that of the reverse primer 806R was 5'-GGACTACCVGGGTATCTAAT-3'. Sequencing adapters A (5'-GCCTCCCTcGCGCCATCAG-3') and B (5'-GCCTTGCCAGCCCGCT CAG-3') and barcodes were added to both ends of the primers. The amplification procedure was as follows: predenaturation at 94°C for 3 min; 25 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, followed by incubation at 16°C for 10 min. Each sample was amplified in triplicate. The length of each amplification product was approximately 400 bp. After the amplification products were confirmed by 1% agarose gel electrophoresis, the triplicate samples of the amplification products were mixed and subjected to 2% agarose gel electrophoresis. The PCR products were recovered from the gel as per the instruction manual for the agarose gel DNA purification kit Ver. 2.0 (TaKaRa, China), then quantified using a NanoDrop 2000 microvolume UV spectrophotometer, and thoroughly mixed for later use^{17,22}.

Emulsion PCR (emPCR) amplification and high-throughput sequencing (454 pyrosequencing)

After the well-mixed PCR fragments were denatured and purified, each single-stranded DNA fragment with a unique-sequence was ligated to magnetic beads. The DNA fragment was then emulsified and amplified using the GS FLX titanium emPCR kit (Roche, USA). As a result, each magnetic bead contained millions of unique-sequence DNA clusters. After the emulsion was broken, the double-stranded DNA was denatured, and each magnetic bead contained unique-sequence, single-stranded DNA clusters. Magnetic beads and sequencing reagents were placed on a Pico titer plate containing 3.5 million wells made of optical fibers for detection. The sequence information was read based on the optical signals released upon oxidation of luciferin by pyrophosphate during base pairing^{17,22}.

Bioinformatics analysis^{17,22}

High-quality optimized sequences were filtered from the original sequences, obtained by sequencing, using barcodes and primers and were truncated to an even length (150 bp). Then, the SILVA tool was used for comparison, and sequences were clustered to generate operational taxonomic units (OTUs) with similarities greater than 97%.

The bacterial sequences were compared to the 16S rRNA sequence data available in GenBank and the Ribosomal Database Project (RDP) to obtain the taxonomic information. Afterwards, all the sequences in each OTU were compared to find the closest taxa for a particular OTU.

Based on the data table in the tax.summary.a folder, the R language tools were used to plot the taxa composition and community composition. Hierarchical clustering was performed based on the distance matrix between samples.

Statistical analysis

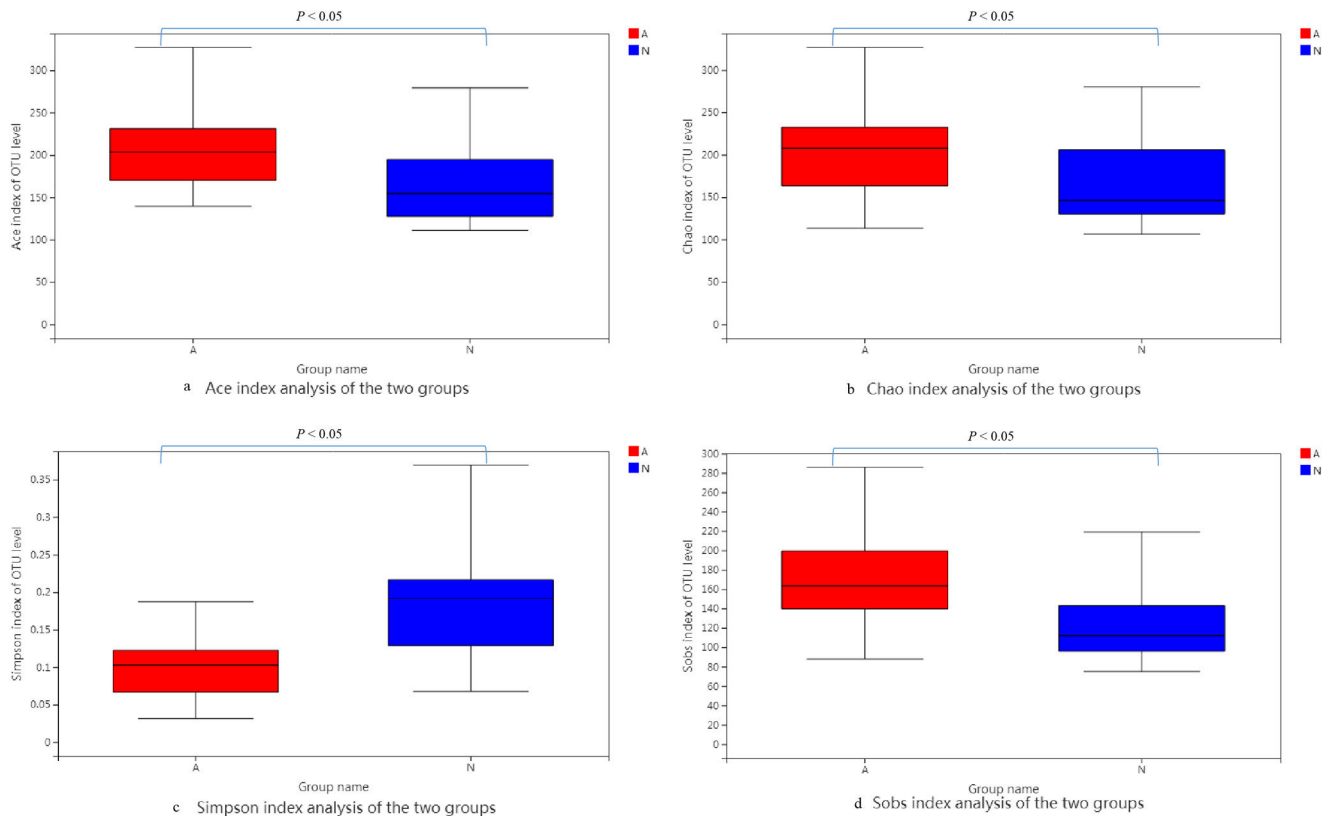
Weight/height index was used for the evaluation of the children's physical growth and the fifth division method was adopted³⁵. When weight/height is below P3, it is considered low-grade malnutrition; when the value is between P3 and P25, it is considered middle and lower class. When the value is between P25 and P75, it is considered middle class; when the value is between P75 and P97, it is considered middle and higher class, and when the value is higher than P97, it indicates high-grade obesity.

Data with a normal distribution were expressed as $\bar{x} \pm s$. Comparisons between the two groups were performed using the unpaired samples *t*-test. The rank-sum test was used when the variance was heterogeneous after calibration. Data without a normal distribution were expressed as P50 (P25 – P75) and analyzed using the rank-sum test, a non-parametric test method.

$\alpha = 0.05$, $p < 0.05$ indicates a statistically significant difference.

Table 1 Clinical characteristics of group A and C.

	A (n=24)	C (n=25)	Statistical analysis	p
Gender (male)	13	14	$\chi^2 = 2.0$	0.368
Age ($\bar{x} \pm s$, year)	4.07 ± 1.21	4.24 ± 1.30	$t = -0.506$	0.615
Weight/height level ($\bar{x} \pm s$, P)	13.08 ± 8.09	38.67 ± 19.02	$t = -6.064$	0

**Figure 1** α-Diversity analysis of the two groups.

Results

Clinical data

A total of 24 pediatric patients and 25 healthy children were included in the study. The clinical data are shown in Table 1. There were no differences in age and gender between the anorexia group and the control group. The children were not obese. The weight/height level of the anorexia group was medium to low, and that of the control group was medium. After the statistical analysis, it was found that the weight/height level of the anorexia group was lower than that of the normal group.

α-Diversity analysis

Figure 1 shows that the Ace, Chao, and Shannon indexes in the anorexia group were higher than those in the control group, while the Simpson index was lower in the control group than in the anorexia group.

β-Diversity analysis

Taxa composition analysis

The bacterial composition number of the anorexia group at the phylum, class, order, family, and genus levels was 14, 24, 36, 66, 193, and in the control group was 11, 21, 30, 59, 180. The number of the same bacterial compositions in the two groups was 11, 20, 28, 55, 169.

Analysis of community composition

The bacterial taxa (i.e., community composition) and their abundance in each sample at the species level are shown in Figure 2.

Differential analysis of dominant bacteria

Differences in the bacteria with abundance greater than 1% at different levels were analyzed between the two groups.

Figure 3a shows that the abundance of *Bacteroidetes* in the anorexia group was higher than that in the control group, and the abundance of *Actinobacteria* in the control group was higher than that in the anorexia group at phylum



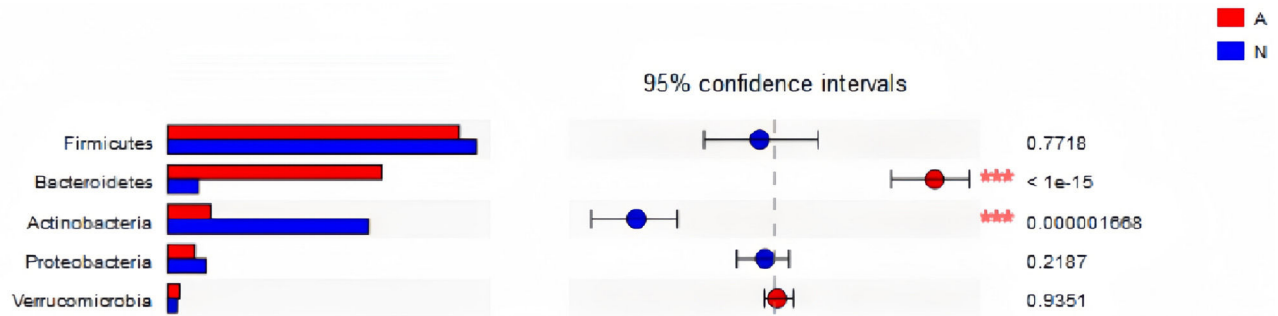


Fig.3a Significance analysis of preponderance bacteria between groups at Phylum level

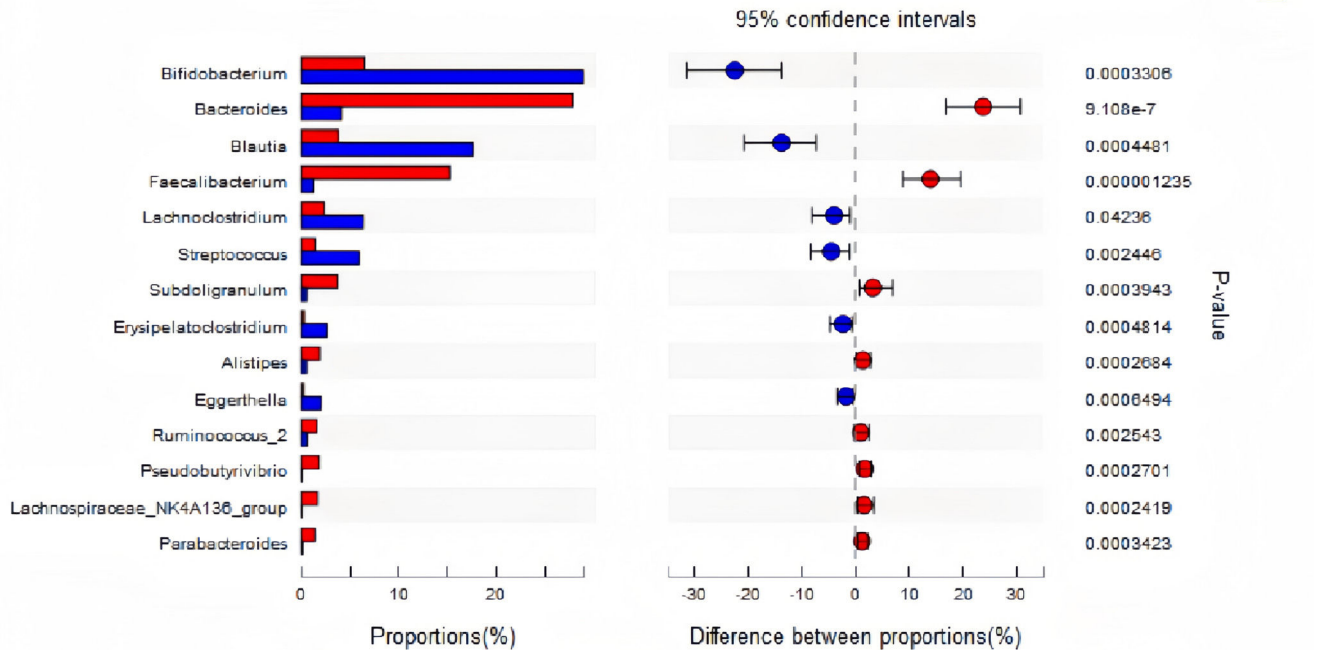


Fig.3b Significance analysis of preponderance bacteria between groups at Genus level

Fig.3 Significance analysis of preponderance bacteria between groups at different levels

Figure 3 Significance analysis of predominant bacteria between groups at different levels.

Table 2 Comparison of B/F index between group A and C.

B/F index	A (n=24)	C (n=25)	Statistical analysis	p
$\bar{x} \pm s$	0.73 ± 0.328	0.10 ± 0.056	$t = 9.97$	0

in the control group *Bifidobacterium*, *Blautia*, *Streptococcus*, *Lachnospiraceae*, and *Erysipelatoclostridium* (Fig. 4a) were the significant influential taxa, and *Bifidobacterium* had the most influential effect (Fig. 4b).

Discussion

The results of the α -diversity analysis indicate that the abundance and diversity of intestinal bacteria in the anorexia group were greater than those in the control group, that is, the α -diversity of the control group was lower than that in

the anorexia group. Most studies have shown that increased diversity of the intestinal microbiota is beneficial to human health, whereas decreased diversity of the intestinal microbiota is mostly related to disease states, such as obesity, diabetes, allergic diseases, inflammatory bowel disease, and autism, as well as the use of antibiotics^{2,10,13,34}. In general, greater richness of the microbiota corresponds to a better ecotype of the colony and a stronger ability to resist the invasion of foreign bacteria or other microorganisms. Therefore, the results of this study contradict traditional research conclusions. While Duan⁸ and Zhou⁴⁰ found no difference in the α -diversity of the intestinal microbiota between

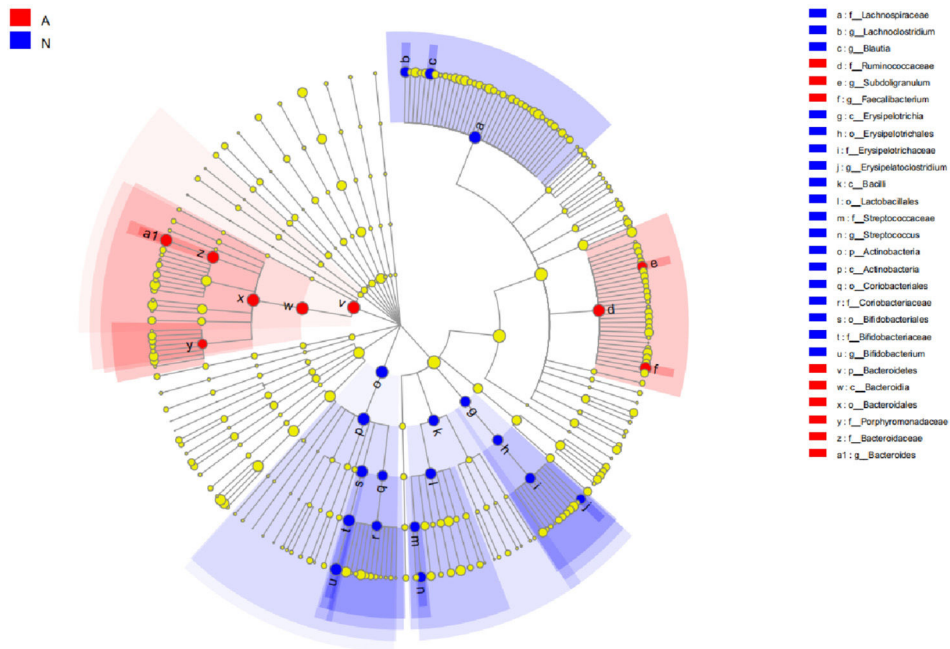


Fig.4a The tree map of Lefse multilevel species hierarchy

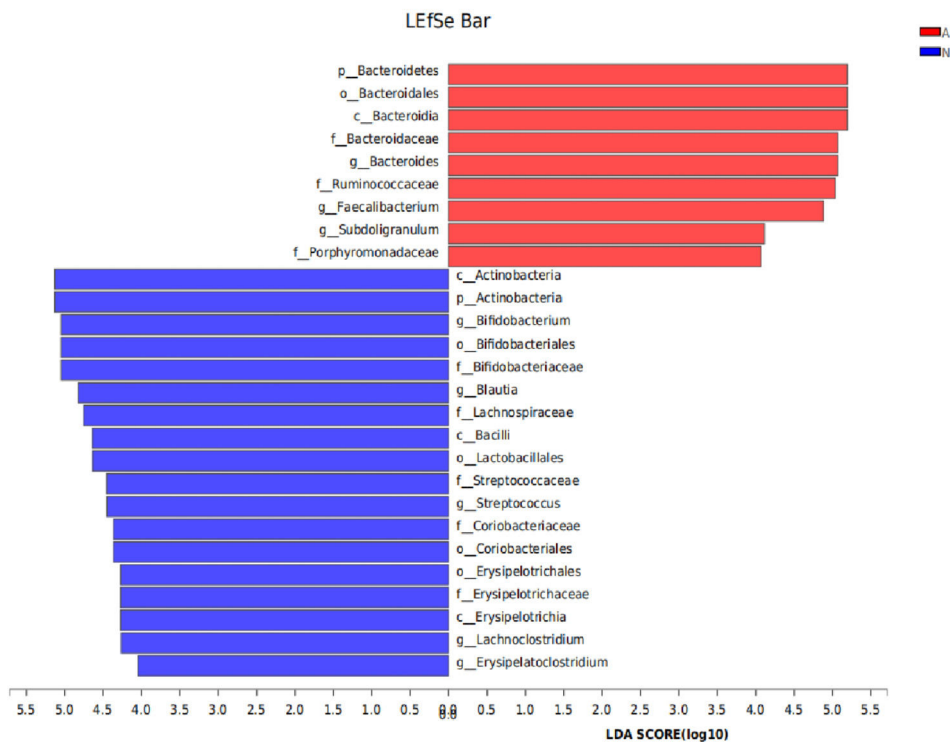


Fig.4b LDA data sheet

Figure 4 LefSe multilevel species difference analysis.

infants with breast milk jaundice and infants without jaundice, Normann²⁵ found that the α -diversity indexes of the intestinal microbiota did not differ between the necrotizing enterocolitis group and the control group. Furthermore, a 10-year follow-up study²⁴ showed that in a high-risk female population, higher vaginal bacterial diversity was associated with an increased risk of HIV infection. These findings indicate that higher bacterial diversity is not necessarily better

and that a change in intestinal bacterial α -diversity may not have significant positive correlations with the occurrence, development, and severity of disease. Furthermore, in this article, there were five significant bacterial species in the control group, while three significant bacterial species in the anorexia group at the genus level by the LefSe multilevel species difference analysis in Figure 4. That is the control group has more dominant microbial communities than

the anorexia group, which may suggest that the β -diversity analysis is more meaningful for bacterial communities with significant effects than for all the bacteria present in the gut which showed by α -diversity analysis.

The results of the analysis of taxa composition and the analysis of community composition indicate that the gut microbiota in the two groups included a variety of bacteria and were characterized by community diversity. High-throughput sequencing can be used to understand the gut microbiota composition comprehensively³³.

The dominant bacterial phyla in the two groups were identical and included *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, which is consistent with data in previous reports showing that 98% of the gut microbiota belong to four phyla, namely, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*^{7,21,39}.

Turnbaugh and Ley^{16,32} confirmed that a decrease in *Bacteroides* or a decrease in the B/F index may promote obesity, and Lim¹⁸ found that the B/F index in the intestines of overweight patients was decreased. By contrast, Turnbaugh³¹ found that the genus *Bacteroides*, which is rich in genes encoding carbohydrate-metabolizing enzymes, was the main constituent of the intestinal microbiota in emaciated subjects. Research has shown^{3,23,29} that *Firmicutes* can help the human body absorb heat from food more effectively, then gradually convert food into fat and can regulate the expression of energy storage-related genes, while *Bacteroidetes* can effectively break down carbohydrates. Therefore, individuals with a high abundance of *Bacteroides* in their gut microbiota are less prone to gaining weight. The children included in the study were not obese and the abundance of *Bacteroidetes* in the anorexia group was higher than that in the control group, and the B/F index in the anorexia group was higher than that in the control group. These results may indicate that an increase in *Bacteroides* or in the B/F index can promote decreases in body weight.

Studies have shown that an early depletion of intestinal *Bifidobacteria* is associated with severe acute malnutrition. Kalliomaki's study showed that the proportion of intestinal *Bifidobacteria* in overweight children were lower than in normal-weight children of the same age. *Bifidobacterium*, a typical probiotic, can promote colon health by producing organic acids such as acetate and lactate and can inhibit pathogens. Anaerobes, especially *Bifidobacteria*, are the major protective organisms in the maturation of a healthy gut microbiota. Thus, an increase in *Bacteroides* abundance and a reduction in *Bifidobacterium* abundance may be related to the occurrence of anorexia. The effective rate of *Bifidobacteria* in the treatment of infantile anorexia is reported to be 77.5–93.2%. Therefore, for pediatric patients with anorexia, *Bifidobacteria* may be selected for treatment prior to the analysis of the intestinal microbiota^{9,12,14,36}.

Furthermore, as the LEfSe multilevel species difference analysis showed in this study, the significant influential bacterial taxa in the anorexia group was *Bacteroidetes* and *Actinobacteria* in the control group at the phylum level. Moreover, at the genus level, *Bacteroides* had the most influential effect in the anorexia group and *Bifidobacteria* in the control group. These findings support the fact that *Bacteroidetes* are involved in the onset of anorexia, and *Bifidobacterium* plays a major role in maintaining a normal intestinal microbiota^{17,22}.

However, each individual has a unique gut microbiota^{1,38}. In the control group, although the overall percentage of *Bifidobacterium* abundance (29.75%) was the highest, *Bifidobacterium* spp. were not necessarily the dominant bacteria that ranked first in every sample. For example, the most dominant bacterial genera were *Lachnoclostridium* in N4, *Streptococcus* in N21, and *Blautia* in N23. In addition, interactions occur among various dominant genera; in particular, when the abundance levels are similar [for example, the percentage of *Bifidobacterium* abundance (20.83%) was the same as that of *Blautia* abundance (20.83%) in N10, synergistic and restrictive interactions will be stronger, with the dominant genera jointly participating in the onset of disease or jointly maintaining the normal structure and function of the gut microbiota^{4,27}. These interactions may explain why the conditions of some children with anorexia are not improved after treatment with *Bifidobacteria* as the most dominant bacteria may not be *Bifidobacteria* (or not solely *Bifidobacteria*) in these children but rather *Bifidobacteria* and other bacteria that together constitute the normal gut microbiota^{26,31,37}. Therefore, supplementation with *Bifidobacteria* alone is not sufficient, and different probiotics must be selected based on the intestinal characteristics of each individual.

Conclusion

The increase in *Bacteroides* abundance or in the B/F index and the reduction in *Bifidobacterium* abundance were related to the pathogenesis of anorexia.

CRediT authorship contribution statement

All authors have read and approved the final version of the manuscript, and they all declared that they have no competing interests.

Ethical considerations

The study was approved by the relevant ethics committee of the Affiliated Hospital of Zunyi Medical College (2015 No. 01). Furthermore, prior to the study, a signed informed consent was obtained from the children's guardians.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

References

- Adak A, Khan MR. An insight into gut microbiota and its functionalities. *Cell Mol Life Sci*. 2019;76:473–93.
- Andoh A, Nishida A. Alteration of the gut microbiome in inflammatory bowel disease. *Digestion*. 2023;104:16–23.
- Beam A, Clinger E, Hao L. Effect of diet and dietary components on the composition of the gut microbiota. *Nutrients*. 2021;13:2795.
- Blanton LV, Charbonneau MR, Salih T, Barratt MJ, Venkatesh S, Ilkaveya O, Subramanian S, Manary MJ, Trehan I, Jorgensen JM, Fan YM, Henrissat B, Leyn SA, Rodionov DA, Osterman AL, Maleta KM, Newgard CB, Ashorn P, Dewey KG, Gordon JI. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science*. 2016;351:1–18.
- Caso JR, Graell M, Navalón A, MacDowell KS, Gutiérrez S, Soto M, Leza JC, Carrasco JL, Marsá MD. Dysfunction of inflammatory pathways in adolescent female patients with anorexia nervosa. *Prog Neuropsychopharmacol Biol Psychiatry*. 2020;96:109727.
- Datta N, Matheson BE, Citron K, Van Wye EM, Lock JD. Evidence based update on psychosocial treatments for eating disorders in children and adolescents. *J Clin Child Adolesc Psychol*. 2023;52:159–70.
- de Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. *Gut*. 2022;71:1020–32.
- Duan M, Yu J, Feng J, He Y, Xiao S, Zhu D, Zou Z. 16S ribosomal RNA-based gut microbiome composition analysis in infants with breast milk jaundice. *Open Life Sci*. 2018;13:208–16.
- Duan W-L, Fei J-Y. Clinical study on the treatment of infantile anorexia with live combined *Bifidobacterium* and *Lactobacillus* tablets. *Chin J Microecol*. 2012;24:529–30.
- Gomaa EZ. Human gut microbiota/microbiome in health and diseases: a review. *Antonie Van Leeuwenhoek*. 2020;113:2019–40.
- Huang Y-Q, Zhao F-F. *Bifidobacterium* triple viable powder combined with zinc gluconate for the treatment of anorexia in children and its effect on intestinal flora. *Chin J Microecol*. 2019;31:435–8.
- Iddrisu I, Monteagudo-Mera A, Poveda C, Pyle S, Shahzad M, Andrews S, Walton GE. Malnutrition and gut microbiota in children. *Nutrients*. 2021;13:2727.
- Igudesman D, Crandell JL, Corbin KD, Hooper J, Thomas JM, Bulik CM, Pence BW, Pratley RE, Kosorok MR, Maahs DM, Carroll IM, Mayer-Davis EJ. Associations of dietary intake with the intestinal microbiota and short-chain fatty acids among young adults with type 1 diabetes and overweight or obesity. *J Nutr*. 2023;153:1178–88.
- Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict over-weight. *Am J Clin Nutr*. 2008;87:534–8.
- Khademian M, Farhangpajouh N, Shahsanaee A, Bahreynian M, Mirshamsi M, Kelishadi R. Effects of zinc supplementation on subscales of anorexia in children: a randomized controlled trial. *Pak J Med Sci*. 2014;30:1213–7.
- Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. 2006;444:1022–3.
- Li D, Feng Y, Tian M, Ji J, Hu X, Chen F. Gut microbiota-derived inosine from dietary barley leaf supplementation attenuates colitis through PPAR γ signaling activation. *Microbiome*. 2021;9:1–22.
- Li M, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, Zhang Y, Shen J, Pang X, Zhang M, Wei H, Chen Y, Lu H, Zuo J, Su M, Qiu Y, Jia W, Xiao C, Smith LM, Yang S, Holmes E, Tang H, Zhao G, Nicholson JK, Li L, Zhao L. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci U S A*. 2008;105:2117–22.
- Li Y-X, Shi Z-G, Wu L-P, Qi H. Effect of spleen depletion on intestinal microecology in young rats with anorexia based on the theory of “spleen main mobility”. *Pharmacol Clin Med Chin*. 2019;35:106–11.
- Liu W-X, Lang J-F, Zhang Q-F. Risk factors of anorexia in children. *Chin J Contemp Pediatr*. 2016;18:1119–21.
- Liu X, He G, Lan Y, Guo W, Liu X, Li J, Liu A, He M, Liu X, Fan Z, Zhang Y. Virome and metagenomic analysis reveal the distinct distribution of microbiota in human fetal gut during gestation. *Front Immunol*. 2023;13:1079294.
- Luo H, Li M, Wang F, Yang Y, Wang Q, Zhao Y, Du F, Chen Y, Shen J, Zhao Q, Zeng J, Wang S, Chen M, Li X, Li W, Sun Y, Gu L, Wen Q, Xiao Z, Wu X. The role of intestinal stem cell within gut homeostasis: focusing on its interplay with gut microbiota and the regulating pathways. *Int J Biol Sci*. 2022;18:5185–206.
- Malesza IJ, Malesza M, Walkowiak J, Mussin N, Walkowiak D, Aringazina R, Bartkowiak-Wieczorek J, Mądry E. High-fat, western-style diet, systemic inflammation, and gut microbiota: a narrative review. *Cells*. 2021;10:3164.
- McClelland RS, Lingappa JR, Srinivasan S, Kinuthia J, John-Stewart GC, Jaoko W, Richardson BA, Yuhas K, Fiedler TL, Mandaliya KN, Munch MM, Mugo NR, Cohen CR, Baeten JM, Celum C, Overbaugh J, Fredricks DN. Evaluation of the association between concentrations of key vaginal bacteria and the increased risk of HIV acquisition in African women from five cohorts: a nested case-control study. *Lancet Infect Dis*. 2018;18:30056–60.
- Normann E, Fahlén A, Engstrand L, Lilja HE. Intestinal microbial profiles in extremely preterm infants with and without necrotizing enterocolitis. *Acta Paediatr*. 2013;102:129–36.
- Peng Q, Huo D, Xu Z, Hu Q, Zhang J. Structural characteristics of intestinal microbial community and its association with diet of Li people. *Microbiol Bull*. 2017;44:2624–33.
- Ponde NO, Lortal L, Ramage G, Naglik JR, Richardson JP. *Candida albicans* biofilms and polymicrobial interactions. *Crit Rev Microbiol*. 2021;47:91–111.
- Sah S, Bordoloi P, Vijaya D, Amarnath SK, Sheela Devi C, Indumathi VA, Prashanth KJ. Simple and economical method for identification and speciation of *Staphylococcus epidermidis* and other coagulase negative *Staphylococci* and its validation by molecular methods. *Microbiol methods*. 2018;149:106–19.
- Stojanov S, Berlec A, Štrukelj B. The influence of probiotics on the *Firmicutes/Bacteroidetes* ratio in the treatment of obesity and inflammatory bowel disease. *Microorganisms*. 2020;8:1715.
- Tidjani Alou M, Naud S, Khelaifia S, Bonnet M, Lagier JC, Raoult D. State of the art in the culture of the human microbiota: new interests and strategies. *Clin Microbiol Rev*. 2020;34, e00129–19.
- Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature*. 2009;457:480–4.
- Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;444:1027–31.
- Varesi A, Campagnoli LIM, Fahmideh F, Pierella E, Romeo M, Ricevuti G, Nicoletta M, Chirumbolo S, Pascale A. The interplay between gut microbiota and Parkinson’s disease: implications on diagnosis and treatment. *Int J Mol Sci*. 2022;23:12289.
- Wiertsema SP, van Bergenhenegouwen J, Garssen J, Knipfels LMJ. The interplay between the gut microbiome and the immune system in the context of infectious diseases throughout life and the role of nutrition in optimizing treatment strategies. *Nutrients*. 2021;13:886.
- World health organization. WHO Anthro (version 3.2.2, January 2011) and macros [EB/OL] (2011-01). Available from: <http://www.who.int/childgrowth/software/en/> [12.05.16].

36. Wu Q-B. Application of probiotics in children's intestines. *Chin J Child Health Care*. 2018;26:813–5.
37. Zang K, Jiangyan, Sun Y, Yan Y, Chen Q, Zhao L, Zhao P, Cui W, Ma X. Study on the adjustment of microbial abundance level of intestinal disease population by probiotics. *Food Sci*. 2018;39:133–43.
38. Zeb F, Osaili T, Obaid RS, Naja F, Radwan H, Cheikh Ismail L, Hasan H, Hashim M, Alam I, Sehar B, Faris ME. Gut microbiota and time-restricted feeding/eating: a targeted biomarker and approach in precision nutrition. *Nutrients*. 2023;15:259.
39. Zhang J, Guo Z, Xue Z, Sun Z, Zhang M, Wang L, Wang G, Wang F, Xu J, Cao H, Xu H, Lv Q, Zhong Z, Chen Y, Qimuge S, Menghe B, Zheng Y, Zhao L, Chen W, Zhang H. A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *ISEM J*. 2015;9:1979–90.
40. Zhou J-L, Zhou S-M, Wang M-B, Cheng Y-W, Dai D-L, Cai H-B, Zhu Z-S, Zou Y, Ding J. Study on metagenomics of intestinal flora in children with breast milk jaundice. *Chin J Microecol*. 2016;28:893–8.