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The Effects of Feeding Type on the Gut Microbiota of Neonates and Early Infants

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ABSTRACT

Aim: To explore the effects of feeding type on the intestinal microbiota of early infants.

Methods: We enrolled 100 newborns and performed stool sample sequencing analysis of the 16S rRNA gene at 3 days and 30–42 days after birth. The composition of the intestinal microbiota was analyzed. One hundred newborns were divided into three groups according to feeding type: breastfed, partially breastfed, and formula feeding.

Results: In all 3 day old samples, *Escherichia–Shigella*, *Streptococcus*, *Staphylococcus*, and *Bifidobacterium* were the prominent genera and there were no statistical differences among the three groups. In the 30–42 day old samples, the prominent genera were *Bifidobacterium*, *Lactobacillus*, *Escherichia–Shigella* and *Bacteroides* in the breastfed group; *Bifidobacterium*, *Escherichia–Shigella*, *Lactobacillus* and *Bacteroides* in the partially breastfed group; *Escherichia–Shigella*, *Bacteroides*, *Bifidobacterium*, and *Clostridium* in the formula feeding group. All genera showed statistically significant differences among the three groups ($P < 0.05$). By 30–42 days of age, however, microbiota from the infant stool clustered and showed significant reorganization. Microbiota were detected in all first-pass meconium samples, but their alpha diversity was low relative to that of the 30–42 day old group in all three feeding type groups.

Conclusions: Intestinal microbiota in early infants differed according to feeding type. 16S sequencing technology is effective and reliable for the detection of intestinal microbiota in early infants.

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Introduction

The intestinal microbial system is large and complex, including the majority of bacteria and previously neglected species such as viruses, archaea, and eukaryotes [1]. Microbiota, including intestinal colonizing bacteria, play an important regulatory role in human metabolism, cell differentiation, and immune function [2]. The composition of the intestinal microbiota is influenced by a variety of factors, both genetic and environmental, such as infections, the application of antibiotics, and changes in dietary structure [3,4]. In the maternal uterus, the fetus has a sterile gut and acquires bacterial colonization at birth through exposure to microorganisms in the mother's vagina, feces, and skin. From colonization to maturation, intestinal microorganisms are vulnerable to various factors, such as feeding type, gestational age, antibiotics, and delivery methods, especially during infancy and childhood [5]. Under the influence of these factors, there are more changes in the human body, such as obesity [6].

It is well known that the early infant gut microbiota plays an important role in normal development and is important for future health [7,8]. However, it is not clear when and how the gut microbial community develops and colonizes during early development and what unique species and functional markers are required. Although there have been some studies on the effects of different feeding types on infant gut microbiota, few studies on this factor have been conducted in China, and the early infant gut microbiota library is under construction.

In this study, to clarify the possible effects of different feeding types on the gut microbiota of infants in early life, we recruited 100 newborns and continued follow-up until 6 weeks after birth. Stool samples were taken within 3 days and between 30 and 42 days after birth for 16S sequencing analysis to detect the gut microbiota.

Subjects and methods

Study subjects we recruited 100 newborns born in the obstetrics department of Xiaoshan Affiliated Hospital of Wenzhou Medical University from April 2019 to August 2021, of which 41 were breastfed, 32 were partially breastfed, and 27 were formula feeding (48 boys and 52 girls). All newborns were 37–42 weeks of gestational age, had a birth weight of 2.5–4 kg, had premature rupture of membranes less than 18 hours before birth, and were not on perinatal antibiotics. There were no statistical differences between the three groups with respect to gestational age, birth weight, and premature rupture of membranes. The detailed information is shown in Table 1.

Stool samples were collected within 3 days of birth and at 30-42 days after birth. The gut microbiota was analyzed using 16S sequencing technology to compare the effects of different feeding types on the gut microbiota of infants in neonates and early life.

The study was approved by the Ethics and Safety Committee of the Xiaoshan Affiliated Hospital of Wenzhou Medical University (Protocol Number: 2019-XS-06). Informed consent was obtained from the subjects' parents. The study protocol followed the ethical standards set by the Medical Ethics Committee.

Table1: Description of information on the 100 study subjects

Group	breastfed	partially breastfed	formula feeding	P-value
n	41	32	27	
Sex (%)				0.987
boys	20(48.8%)	15(46.9%)	13(48.1%)	
girls	21(51.2%)	17(53.1%)	14(51.9%)	
Gestational age (w)	39.2 ± 0.8	39.2 ± 0.9	38.9 ± 0.8	0.378
premature rupture of membranes (h)	3.2 ± 2.3	3.3 ± 2.4	3.1 ± 1.9	0.939
Birth weight (g)	3109.5± 317.5	3073.1 ± 258.1	3128.9 ± 254.0	0.740

Methods

Sequencing procedure: After the fresh infant stool sample was collected by the parents, about 200 mg was transferred into a 2 ml centrifuge tube with1 ml RNAlater, mixed thoroughly, stored at 4 °C for 8–12 hours, then stored at −80 °C and transported on dry ice to Hangzhou Bio-science Biotechnology Company for 16S sequencing detection. Genomic DNA was extracted and detected using 1 % agarose gel electrophoresis. PCR amplification, fluorescence quantification, MiSeq library construction, and sequencing were performed.

Bioinformatic analysis process

From the extracted DNA, the hypervariable regions of the 16S ribosomal gene were sequenced and an interactive cloud analysis of the microbiota diversity was conducted. α-diversity (diversity within samples) and β-diversity (between samples) were evaluated using the operational taxonomic unit (OTU) table. The Sobs index was determined as α-diversity, and its significance was calculated using the Student's t-test. Qiime 1.9.1 was used to generate cumulative distribution plots of β-diversity distances.

Statistical analysis

SPSS software (version 24.0) was used for statistical analysis. Student's t-test was used for normally distributed data and the Wilcoxon signed-rank test was used for non-normally distributed data in the two-way comparison between groups. Differences were considered statistically significant at P values < 0.05.

Results

The results of microbiota testing of samples collected from the three groups (breastfed, partially breastfed, and formula feeding groups) at birth are shown in Figure 1. For samples collected within 3 days after birth, the most abundant genera in all three groups were *Escherichia–Shigella*, *Streptococcus*, *Staphylococcus*, and *Bifidobacterium*, with no statistical difference between the OTU groups (all P > 0.05).

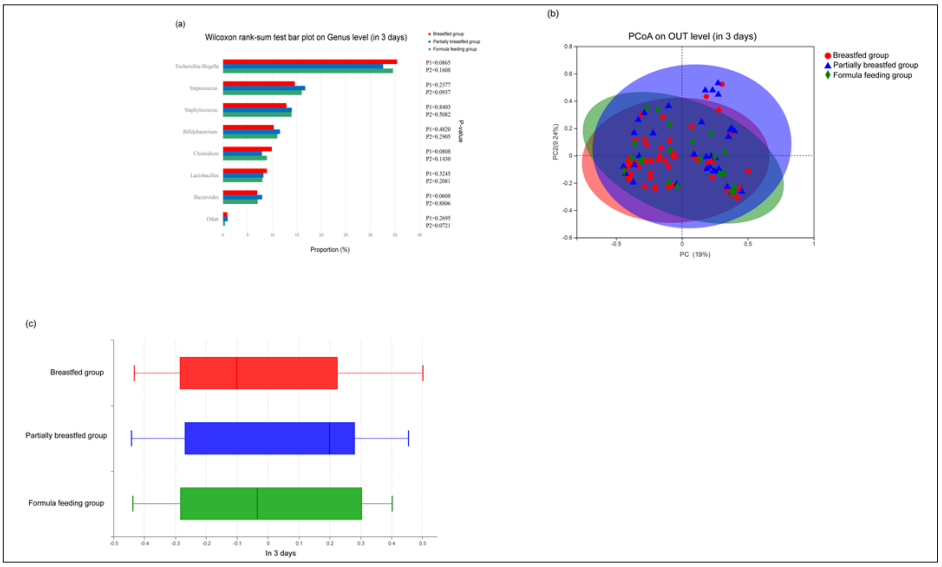

Figure 1: Microbiota test results for samples collected from three groups (breastfed group, partially breastfed group, and formula feeding group) within 3 days after birth.

(a) Wilcoxon rank-sum test bar plot between the three groups at genus level. (b) Principal coordinate analysis (PCoA) on unweighted UniFrac distances between the neonatal microbiota is shown along the first two principal coordinate (PC) axes. Each point represents a sample, and the closer the two sample points are, the more similar the species composition of the two samples is. (c) PCoA box diagram representing the discrete distribution of different groups of samples on the PC1 axis and colored by feeding method: red, breastfed group; blue, partially breastfed group; and green, formula feeding group.

The results of microbiota testing of samples from the three groups (breastfed, partially breastfed, and formula feeding groups) at 30–42 days after birth are shown in Figure 2. The predominant genera in the stools of the breastfed group were *Bifidobacterium*, *Lactobacillus*, *Escherichia–Shigella*, and *Bacteroides*. *Bifidobacterium*, *Escherichia–Shigella*, *Lactobacillus*, and *Bacillus mimicus* in the partially breastfed group. The predominant genera in the formula feeding group were *Escherichia–Shigella*, *Bacillus mimicus*, *Bifidobacterium*, and *Clostridium*. Statistical differences were found between the OTU groups compared with each bacterial group (all $P < 0.05$).

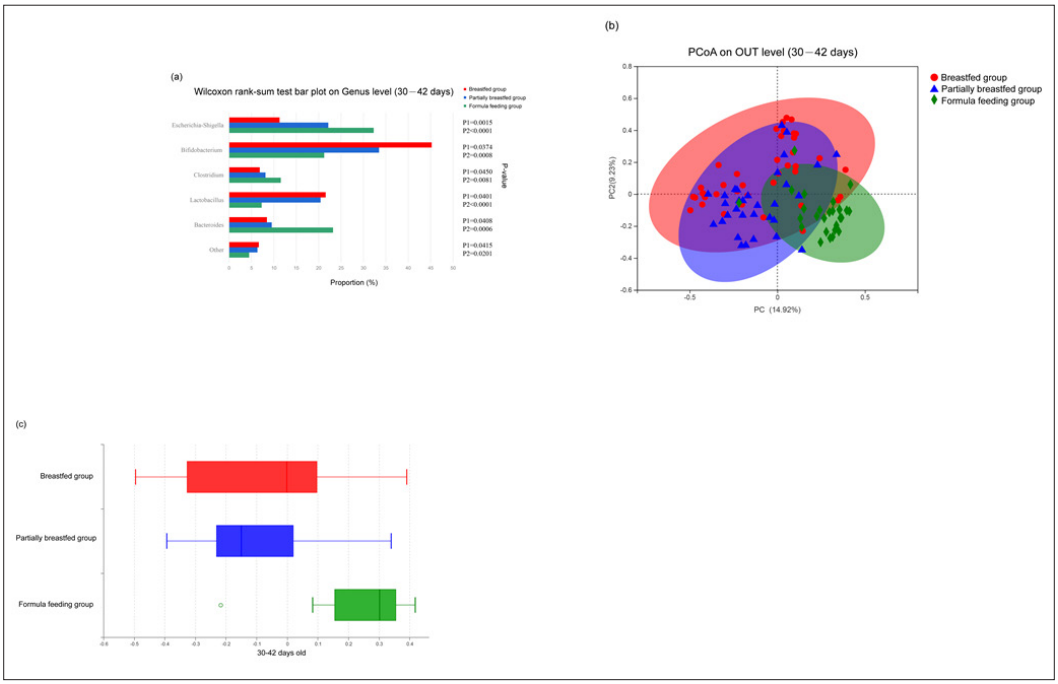

Figure 2: Microbiota test results of samples collected from three groups (breastfed group, partially breastfed group, and formula feeding group) at 30–42 days after birth

(a) Wilcoxon rank-sum test bar plot between the three groups at genus level. (b) Principal coordinate analysis (PCoA) on unweighted UniFrac distances between the early infants' microbiota is shown along the first two principal coordinate (PC) axes. Each point represents a sample, and the closer the two sample points are, the more similar the species composition of the two samples is. (c) PCoA box diagram. Represents the discrete distribution of different groups of samples on the PC1 axis and colored by feeding method: red, breastfed group; blue, partially breastfed group; and green, formula feeding group.

Comparison of the Sobs indices of microbiota from the three groups within 3 days after birth and at 30–42 days after birth is shown in Figure 3. The sample alpha diversity was significantly higher in all three groups at 30–42 days than in the respective groups within 3 days after birth, and the test comparing the Sobs index between the two groups showed statistically significant differences ($P < 0.001$).

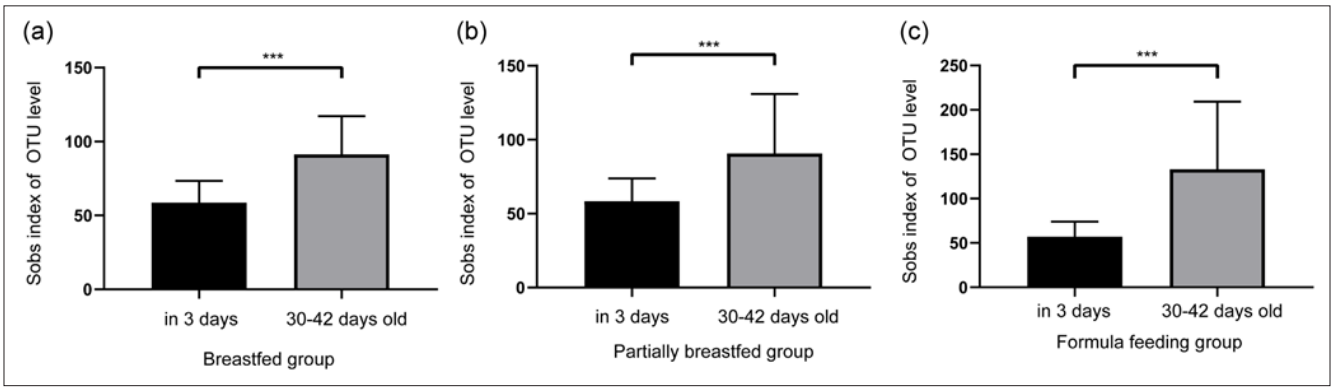

Figure 3: Comparison of Sobs index of microbiota in three groups of samples within 3 days of birth and at 30–42 days after birth

(a) Comparison of Sobs index between the breastfed group samples collected within 3 days of birth and at 30–42 days after birth. (b) Comparison of Sobs index between the partially breastfed group samples collected within 3 days of birth and at 30–42 days after birth. (c) Comparison of Sobs index between the formula feeding group samples collected within 3 days of birth and at 30–42 days after birth.

Discussion

In this study, we chose the study endpoint of 30–42 days after birth because infants at that age have limited human contact and have not been exposed to a variety of environmental microorganisms[9]. Feeding status refers to the status at the time of stool collection. Breastfed in this article refers to giving breast milk to the infant, whether it is direct feeding or bottle feeding after expression.

The results of the study showed no significant differences in microbial genus composition among the three groups in samples collected within 3 days after birth. This is considered to be related to the short time after birth during which feeding practices have not yet had an impact, with these dominant microbiota coming from the maternal intestine and skin. *Bifidobacterium* and *Lactobacillus* were the main microbiota in the breastfed group at 30–42 days after birth, which is consistent with the results of several other studies [10-12]. Breastfeeding is an important way to transfer maternal microbiota to infants after birth. Breastfeeding allows maternal microorganisms, including bifidobacteria, *Lactobacillus*, *Staphylococcus*, and *Streptococcus*, to continuously transfer to the infant's intestine and accelerate the proliferation and maturation of the intestinal microbiota. In particular, breast milk contains oligosaccharides as a substrate for certain microorganisms to obtain energy, and bifidobacteria are the main species that can use oligosaccharides to proliferate, establish a balanced microecological environment, resist the proliferation of pathogenic microorganisms, and regulate the mucosal barrier function of the intestine and immune function [13].

Bifidobacterium is a member of the actinomycetes, and its main end products, acetate and lactate, are important sources of energy for colon cells [14]. Bifidobacteria produce essential nutrients, including riboflavin and folic acid and produce short-chain fatty acids that prevent pathogens, reduce diarrhea, increase nutrient absorption, and stimulate the immune system [15,16]. *Lactobacillus* can regulate the normal flora of the gastrointestinal tract, maintain the micro-ecological balance, improve gastrointestinal function, and lower serum cholesterol. Lactobacillus also inhibits the adhesion and growth of gastrointestinal pathogenic bacteria such as *Escherichia–Shigella* and the formation of an antibacterial intestinal barrier [17,18].

The dominant organism in the formula feeding group was *Escherichia–Shigella*, which showed significantly higher abundance than in the breastfed and partially breastfed groups. *Escherichia–Shigella* is considered to be a precursor to the development of pathology and a potential health risk [19]. Although microorganisms were detected in all samples within 3 days after birth, their alpha-diversity was significantly lower compared to samples at 30–42 days, independent of feeding type. This is consistent with the previous findings of Chu et al., and suggests that the gut microbiota is enriched with age [9]. However, the mode of delivery of the newborn and the maternal diet have been reported to influence the early infant gut microbiota. The lack of analysis of the effects of these factors is a shortcoming of this study.

In conclusion, our results show that the gut microbiota of infants in the breastfed group was dominated by probiotics, while that of the infants in the formula feeding group was dominated by pathogenic bacteria, and the partially breastfed group was intermediate. In addition to the microbiota, biologically active substances in breast milk, including immunoglobulins, cytokines, chemokines, growth factors, hormones, and lactoferrin, are dynamically present throughout lactation and provide immune protection to the infant [20]. Some studies have also found that children who are exclusively breastfed have better cognitive development [21]. Furthermore, Ding and Schloss found that feeding type in infancy determines the composition of bacterial communities in adulthood [22]. Even brief exposure to formula may disrupt the normal colonization of the infant's intestinal microbiota [23]. Therefore, we recommend that infants should be exclusively breastfed for as long as possible in the early years of life for the benefit of their health thereafter.

The 16S rRNA gene contains nine highly variable regions with genus or species specificity. Therefore, 16S rRNA can be used as a characteristic nucleic acid sequence and is considered the most suitable indicator for bacterial phylogeny and taxonomic identification [24]. 16S rRNA amplicon sequencing, analysis, and strain identification are important tools for studying the composition and structure of microorganisms in environmental samples [25]. At present, 16S sequencing technology is gradually being developed in China. For example, Nanjing Jinling Hospital used 16S sequencing technology to detect animal models of constipation in mice[26]. Shanghai Jiao Tong University uses 16S sequencing technology to detect weight loss through dietary intervention [27]. This study applied 16S sequencing technology to identify gut microbiota in early infants, to provide an accurate and scientific data resource for establishing a gut microbiota database for early infants, and to provide an objective basis for interventions to compensate for the adverse effects of different feeding types on the gut microbiota of early infants, such as routine probiotic treatment for formula-feeding infants.

Author contributions

KYP, ZXC, LFX and HYH contributed to data collection, analysis and writing of the manuscript. KYP contributed to study design and editing of the manuscript.

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethical Statement

The Institutional Review Board of The First People's Hospital of Xiaoshan District approved this study (Protocol Number: 2019-XS-06).

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