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Effects of Antibiotic Treatment and Probiotics on the Gut Microbiome of 40 Infants Delivered Before Term by Cesarean Section Analysed by Using 16S rRNA Quantitative Polymerase Chain Reaction Sequencing

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

DEF 1 **Chen Gong**
G 1 **Liqi Yang**
C 1 **Kangkang Liu**
F 2 **Shichun Shen**
B 1 **Qixing Zhang**
B 1 **Han Li**
A 1 **Yan Cheng**

1 Department of Pediatrics, The Second Affiliated Hospital of Anhui Medical University, Hefei, Anhui, P.R. China

2 Department of Cardiology, The Second Affiliated Hospital of Anhui Medical University, Hefei, Anhui, P.R. China

Corresponding Author: Yan Cheng, e-mail: chengyan1971@163.com

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Background: This study aimed to investigate the effects on the gut microbiome of 40 infants delivered before term by cesarean section between antibiotic treatment and probiotics as assessed by 16S rRNA quantitative polymerase chain reaction (qPCR) sequencing.


Material/Methods: We divided 40 premature infants delivered by cesarean section into 4 groups according to exposure to antibiotics or probiotics: N group (No-probiotics and No-antibiotics), A group (antibiotics), P group (probiotics), and the AP group (antibiotics+probiotics). Fecal samples were collected on days 1, 3, and 10, and the microflora data were generated using 16S rRNA qPCR sequencing technology. The BugBase tool was used for phenotype prediction, the Tax4Fun tool was used for function prediction, and iPath software was used to predict the metabolic pathways of intestinal bacteria.

Results: Antibiotics increased the abundance of pathogenic bacteria and reduced the replication and repair function ($P=0.049$), nucleotide metabolism function ($P=0.047$), and the purine metabolism pathways ($P<0.05$) of the gut microbiota. Probiotics increased the abundance of beneficial bacteria and the cellular community prokaryote function ($P=0.042$) and contributed to the *Bifidobacteria* biofilm formation. Probiotics alleviated the damage of antibiotics to the composition and function of the gut microbiota.

Conclusions: The findings from this study showed that antibiotic treatment of preterm infants born by cesarean section changed the gut microbiome, but that the use of probiotics could restore the normal microbiome, which supports that restoration of the normal gut microbiota may be achieved with probiotics.

Keywords: **Microbiota • Cesarean Section • Infant, Premature • Antibiotic Prophylaxis • RNA, Ribosomal, 16S • Probiotics**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/928467>

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Background

The human gut microbiota, which is composed of complex microbial communities, is a complex ecosystem shaped by evolution, where the combination of host and bacteria promote a delicate balance evolved to modulate immune responses and promote health. Understanding the process of colonization by gastrointestinal microorganisms involves defining host physiology and immunity, and the neonatal period is the essential and susceptible stage in this process [1].

Premature infants are born between 28 and 37 weeks of gestational age. Compared with full-term infants, the intestinal microflora of premature infants is established significantly later, the abundance and diversity of microflora are lower, and there are more types and contents of pathogenic bacteria [2,3]. However, the gut microbiota of premature infants is characterized by low microbial diversity and high inter-individual variation [4]. Many studies have indicated that this discrepancy might be caused by frequent exposure of preterm infants to various medical treatments [5,6]; delivery mode and use of antibiotics are the most common of these [2,7]. Cesarean section decreases the abundance of *Bifidobacteria* and increases the abundance of pathogenic bacteria in the early gut microbiota of infants, and taxa strongly associated with respiratory infections are more abundant in infants delivered by cesarean section [8]. Moreover, a more substantial proportion of antibiotic resistance genes were found in intestinal microbial tests in cesarean-section infants in the first year of life [9]. Use of antibiotics not only reduces the levels of *Bifidobacteria* in the gut microbiota of premature infants, but also increases the levels of enterobacteria and the risk of sepsis and necrotizing enterocolitis (NEC) in premature infants [10,11]. Through high-throughput sequencing, research has shown that the combined use of ampicillin and gentamicin has significant effects on the evolution of infant gut flora in early life [12]. Premature infants are often exposed to the medically-induced factors of antibiotics application and cesarean section. Therefore, the gut flora of premature infants is more prone to various disorders, which affects the development of substance metabolites and the immune system, causing further diseases [13].

Many studies have demonstrated that sepsis, NEC, and feeding intolerance are closely related to gut microbiota imbalance in premature infants [14-16]. Furthermore, brain injury in premature infants and nervous system development are related to gut microbiota disorders [17]. Hence, it is important to intervene in the gut microbiota of premature infants as early as possible. Use of probiotics is currently the most commonly used intervention method. Probiotics increase beneficial bacteria and decrease pathogenic bacteria in preterm infants [18], and can reduce the risk of NEC and feeding intolerance in preterm infants, as well as reducing the expression level of inflammatory

factors to minimize brain damage [19,20]. Research has shown that long-term probiotic supplementation can help restore normal microbiota in 3-month-old antibiotic-treated infants [21].

However, previous studies did not deeply explore the effects of probiotics supplementation in early life on the gut flora of infants delivered before term by cesarean section and treated with antibiotics. The 16S rRNA quantitative polymerase chain reaction (qPCR) sequencing method is mainly used to identify strains through sequencing and analysis of the specific 16S rRNA high-variation region. It is an important technology for the study of the microbial community and identifies broad levels of community composition. Therefore, the present prospective study aimed to investigate the effects on the microbiome of 40 infants delivered before term by cesarean section between antibiotic treatment and probiotics by use of 16S rRNA quantitative PCR sequencing.

Material and Methods

Participant selection

In this study, 40 premature infants born by cesarean section at 28 to 37 weeks of gestational age were recruited with parental informed consent. Among the 40 premature infants, 20 premature infants had high-risk factors for infection (eg, premature rupture of membranes over 18 h, inhaled contaminated amniotic fluid or meconium during labor, and clinical manifestations suspected of early infection) and needed antibiotic treatment based on the clinical guidelines for the use of antibiotics. There were 20 premature infants without infectious factors and without antibiotic treatment. This study was conducted in strict accordance with the Helsinki Declaration and was approved by the Ethics Committee of the Second Affiliated Hospital of Anhui Medical University (approval no. Pj2016-05-01).

Exclusion criteria were: severe asphyxia at birth, neonatal respiratory distress syndrome (NRDS), genetic metabolic diseases, congenital pyloric obstruction, congenital megacolon, and other digestive tract malformations.

Experimental Design

Twenty premature infants treated with antibiotics were randomized to 2 groups, one group with probiotics and the other group without probiotics (10 cases in each group): the AP group (antibiotics + probiotics) and the A group (antibiotics). Another 20 preterm infants without antibiotic treatment were also randomized to 2 groups, one with probiotics and the other without probiotics (10 cases in each group): the P group (probiotics) and the N group (No-probiotics and No-antibiotics).

Antibiotic usage method: In this study, Cefotaxime (North China Pharmaceutical Hebei Huamin Pharmaceutical Company limited, H10960279) was selected. Premature infants of the AP group and A group were given antibiotic treatment within 24 h of birth. The antibiotic dosage was 50 milligrams per kilogram of body weight, twice daily, and the duration of therapy was 7 days.

Probiotics usage method: The probiotic was Bifid triple viable powder (Shanghai Shangyao Xinyi Pharmaceutical Factory Company limited, S10970104). One gram of the probiotic powder contains a mixture of cells (quantified as colony-forming units, CFU) of *Bifidobacterium longum* (1×10^7 CFU), *Lactobacillus acidophilus* (1×10^7 CFU), and *Enterococcus faecalis* (1×10^7 CFU). Premature infants of the AP group and P group were given oral probiotics on day 3 after birth. The probiotic dosage was 1 gram per time, twice daily, and the duration of therapy was 7 days.

To exclude the influence of feeding mode, the premature infants in this study were all fed with formula milk.

Stool Specimen Collection

Fecal samples from the 4 groups of premature infants were collected on days 1, 3, and 10. Day 10 fecal specimens were samples after 7 days of exposure to probiotics or antibiotics. The fecal samples were immediately stored in a sterilized frozen test tube, which was transferred to the refrigerator at -80°C in liquid nitrogen for preservation.

Detection of the Gut Microbiota

Genome DNA Extraction and PCR Amplification

The genomic DNA of stool samples was extracted by the cetyl trimethyl ammonium bromide (CTAB) method as described by Tang et al [22]. We used 1% agarose gel to detect the concentration and purity of DNA. The genomic DNA diluted to 1 ng/ μL with sterile water was then used as a template. The corresponding primer (16S V3-V4: 341F-806R, 5'-CCTAYGGGRBGCASCAG and 5'-GGACTACNNGGGTATCTAAT) with the barcode were selected to amplify the 16S rRNA genes of regions (16S V3-V4). All PCR reactions were carried out in 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s, followed by a final cycle of 72°C for 5 min. We mixed the same volume of 1 \times loading buffer (contained SYB green) with PCR products and performed electrophoresis on 2% agarose gel for detection. PCR products were mixed in equidensity ratios. Then, the mixture of PCR products

was purified using a GeneJET™ Gel Extraction Kit (Thermo Scientific) [23]. The target bands were cut and recovered after PCR products were mixed and purified. Then, the library was built and sequenced on the IonS5™XL sequencing platform (ThermoFisher Scientific, Waltham, MA, USA).

Operational Taxonomic Units (OTUs) Cluster and Species Annotation

Based on the effective data obtained by quality control, filtering, and chimera removal of the original sequencing results, Uparse software (Uparse v7.0.1001) was used to screen representative sequences for each OTU. Then, the Silva Database was used based on the Mothur algorithm to annotate taxonomic information for the representative sequences. Finally, we standardized the abundance information of the OTU and performed data analysis.

Experimental Data Analysis

QIIME (version 1.7.0) was used to calculate alpha diversity index, including the abundance-based coverage estimator (ACE) and Chao1, and the results were displayed by R software (Version 2.15.3). Spearman correlation analysis was performed using *vegdist* function and *mantel* function in the *Vegan* package in R. In addition, the BugBase tool was used for phenotype prediction of intestinal flora, the Tax4Fun tool was used to predict the function of gut microbiota, and *iPath* software was used to predict the metabolic pathways of intestinal bacteria.

SPSS 19.0 (Version SPSS Inc., Chicago, IL, USA) was used to analyze the basic information of the samples. The statistical significance of differences was evaluated by one-way ANOVA (SNK method) and chi-square test. The results were expressed as the mean \pm SD. $P < 0.05$ was considered statistically significant.

Results

Participant Characteristics

A total of 40 premature infants were enrolled in this study, all of whom were delivered by cesarean section. A total of 120 stool samples were collected. SPSS 19.0 software was used for statistical analysis of the basic information of the premature infants in the 4 groups, and the results indicated that there were no significant differences among groups in birth weight, gestational age, and sex ($P > 0.05$) (Table 1).

Table 1. Baseline characteristics of each group.

	N (n=10)	A (n=10)	P (n=10)	AP (n=10)
Birth weight* (kg)	2.23±0.27	2.02±0.40	2.10±0.35	2.19±0.51
Gestational age* (week)	35.08±0.45	33.94±1.56	34.32±1.50	33.86±2.21
Sex* n (%)				
Male	6 (60%)	5 (50%)	6 (60%)	7 (70%)
Female	4 (40%)	5 (50%)	4 (40%)	3 (30%)

Data are presented as means±standard deviation or percentages as appropriate. n – number; N – the N group (No-probiotics and No-antibiotics); A – the A group (antibiotics); P – the P group (probiotics); AP – the AP group (antibiotics+probiotics). * P>0.05.

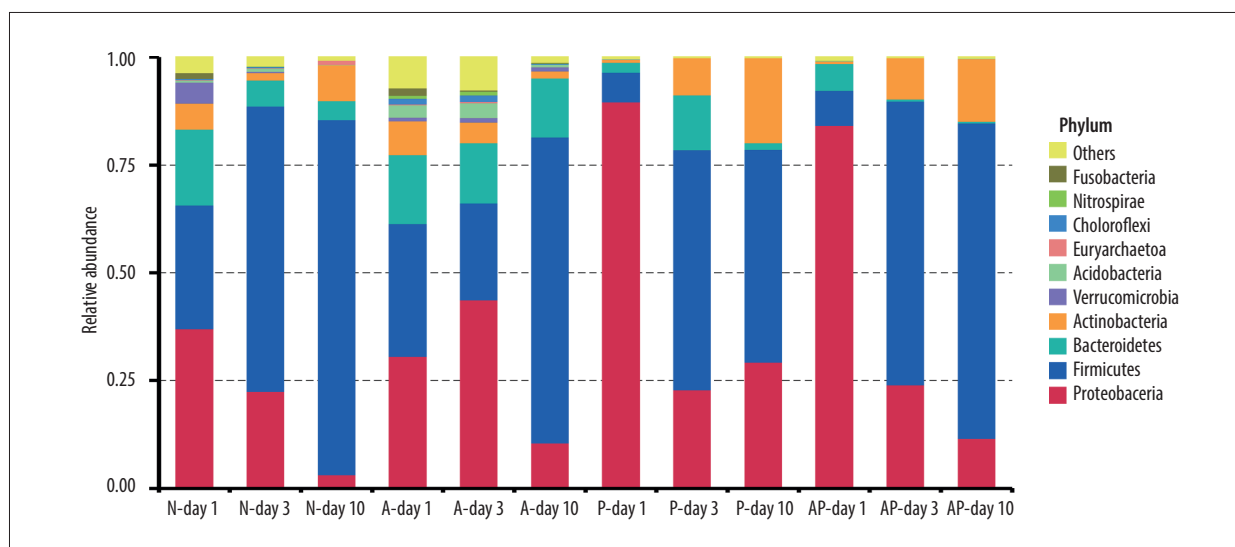


Figure 1. The composition of gut microbiome of preterm infants delivered by cesarean section in the 4 groups at 1, 3, and 10 days: Ten most abundant bacteria at the phylum level. N – the N group (No-probiotics and No-antibiotics); A – the A group (antibiotics); P – the P group (probiotics); AP – the AP group (antibiotics+probiotics). Day1 – One day after birth; day3 – Three days after birth; day10 – Ten days after birth.

Characteristic of Gut Microbiota in Premature Infants Delivered by Cesarean Section

After 16S rRNA sequencing, a total of 10 878 species of OTU were found in all the fecal specimens of the premature infants, and 57 phyla and 1058 genera were identified. At the phylum level, the gut microbiota of all of the premature infants delivered by cesarean section was mainly composed of Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, accounting for more than 90% of the microorganisms in the fecal samples. At the phylum level, the gut microbiota of the 4 groups of premature infants showed a trend of decreasing Proteobacteria and increasing Firmicutes over time (days 1, 3, and 10), and Firmicutes were dominant at day 10 (Figure 1).

Differences in Composition of the Gut Microbiota on Day10

Since the premature infants had been exposed to probiotics or antibiotics for 7 days at day10, we compared the effects of antibiotics or probiotics at day 10 on the composition of the gut microbiota. At the genus level, in the A-day10 group, the abundance of unidentified Enterobacteriaceae and Parabacteroides was higher, whereas the abundance of Bifidobacterium was lower than in the N-day10 group. Compared with the N-day10 group, the abundance of Bifidobacterium, Lactobacillus, and Enterococcus were higher in the P-day10 group (Figure 2A).

To discuss the effect of probiotics on the composition in the gut microbiota of premature infants delivered by cesarean section treated with antibiotics, LfSe (linear discriminant analysis) was used to analyze the biomarkers (the major bacteria causing the difference between the 2 groups) of groups AP-day10 and A-day10. The results indicated that 24 biomarkers (order:

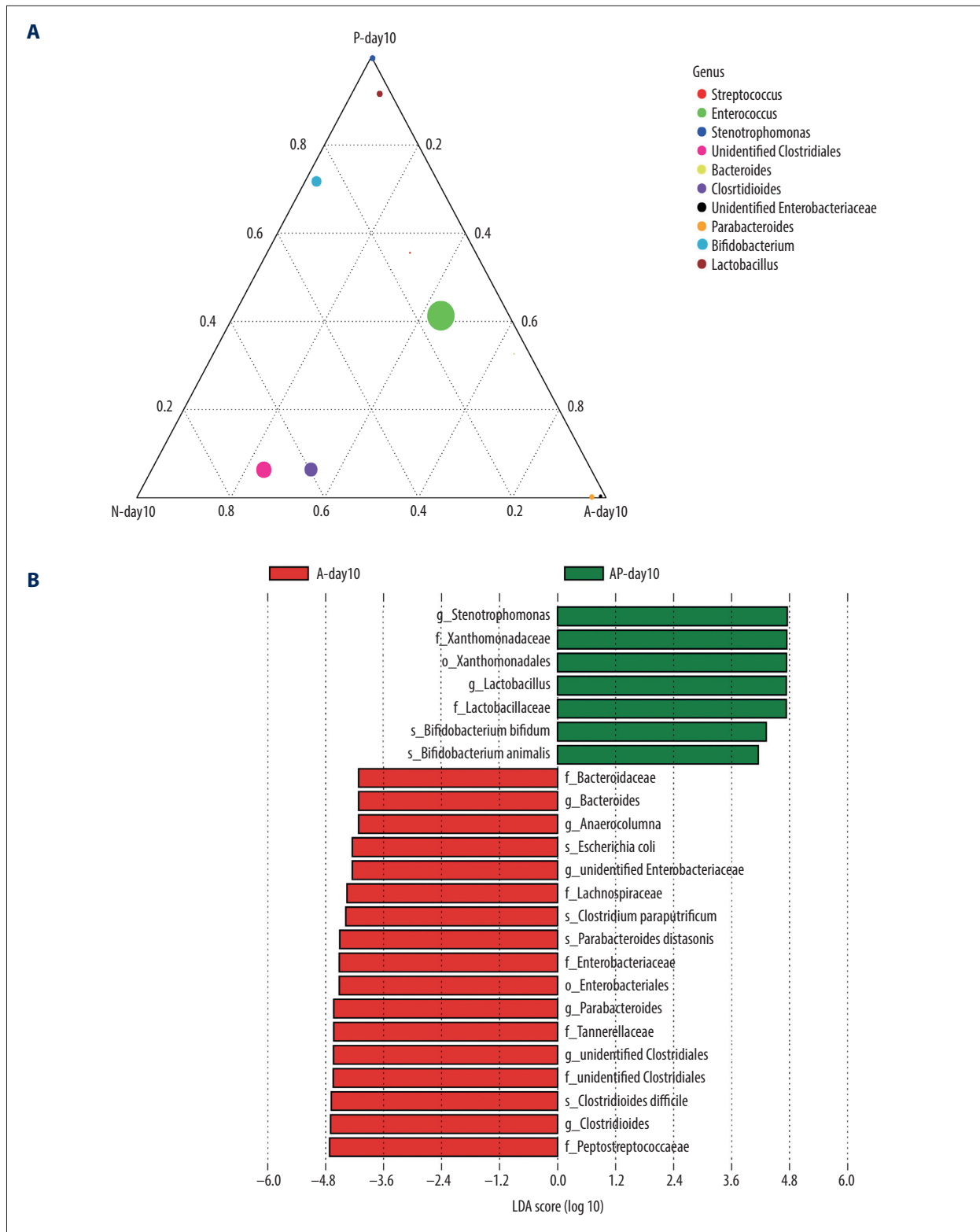


Figure 2. Differences of gut microbiome composition of preterm infants delivered by cesarean section in the 4 groups at 10 days. **(A)** Differences in N-day10, A-day10 and P-day10 group at the genus level. **(B)** Significance difference Biomarker between the A-day10 and AP-day10 groups. N – the N group (No-probiotics and No-antibiotics); A – the A group (antibiotics); P – the P group (probiotics); AP – the AP group (antibiotics+probiotics). Day10 – Ten days after birth.

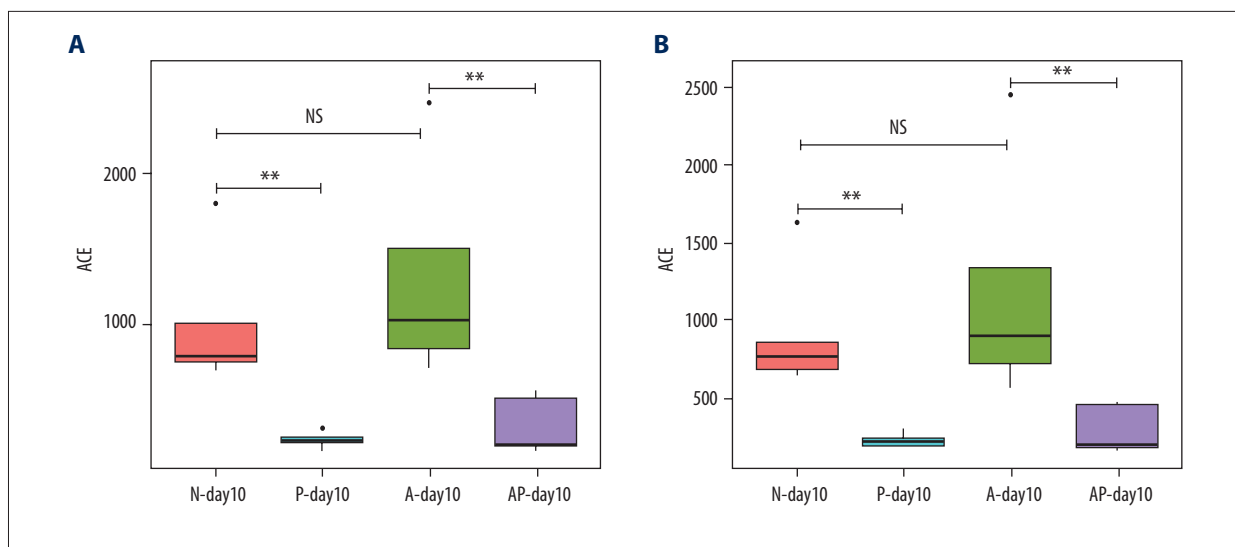


Figure 3. Alpha diversity of gut microflora in the 4 groups at day10. (A) Abundance-based coverage estimator (ACE) index. (B) Chao1 index. N - the N group (No-probiotics and No-antibiotics); A - the A group (antibiotics); P - the P group (probiotics); AP - the AP group (antibiotics+probiotics). Day10 - Ten days after birth. NS - means no statistical differences. ** $P<0.01$.

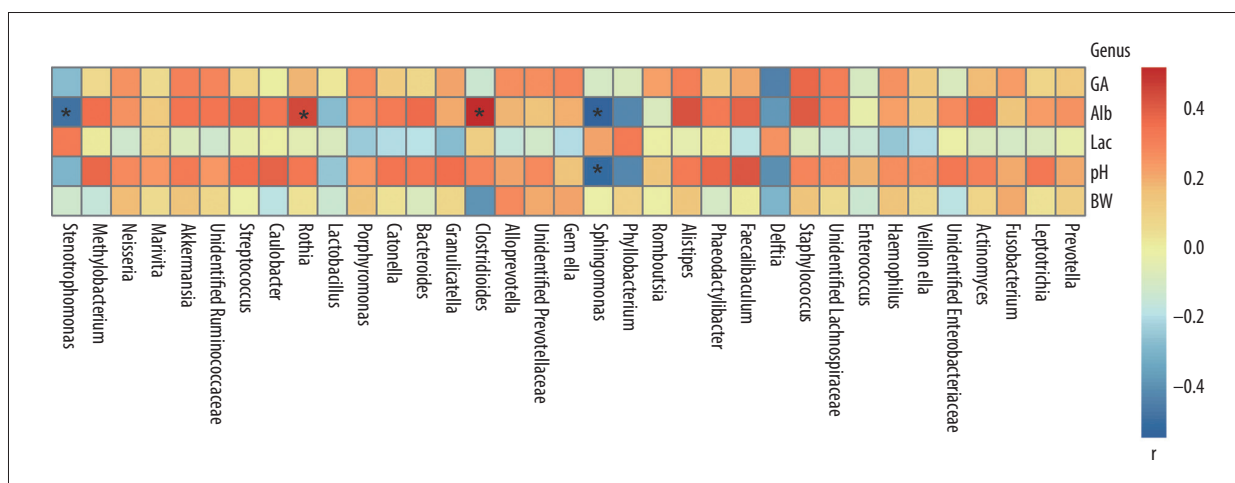


Figure 4. Correlation between gut microbiota and environmental factors of all preterm infants delivered by cesarean section in this study at the genus level. BW - birth weight; GA - gestational age; Alb - blood albumin; Lac - blood lactate; pH - blood pH at birth. r - correlation. * $P<0.05$.

2, family: 8, genus: 8, species: 6) were found, and the biomarkers of the AP-day10 group primarily contained *Lactobacillus* (genus level), *Bifidobacterium bifidum*, and *Bifidobacterium* animals (species level), among others. The A-day10 group mainly included unidentified *Enterobacteriaceae*, unidentified *Clostridiales* (genus level), *Escherichia coli*, *Clostridium parvutrophicum*, and *Clostridium difficile* (species level) (Figure 2B).

Analysis of the Diversity of the Gut Microbiota

The ACE index and Chao1 index were used to evaluate the alpha diversity of the gut microbiota on day 10 in the 4 groups of preterm infants delivered by cesarean section. The results

showed that there were no statistically significant differences in the ACE index and Chao1 index values between the N-day10 group and the A-day10 group ($P>0.05$). Compared with the N-day10 group, the ACE index and Chao1 index of the P-day10 group were significantly lower ($P<0.01$). Similarly, the ACE and Chao1 indexes in the AP-day10 group were lower than in the A-day10 group ($P<0.01$) (Figure 3A, 3B).

Correlation Analysis of Environmental Factors

To study the influence of environmental factors on the gut flora of premature infants delivered by cesarean section, the Spearman method was used to analyze the relationship

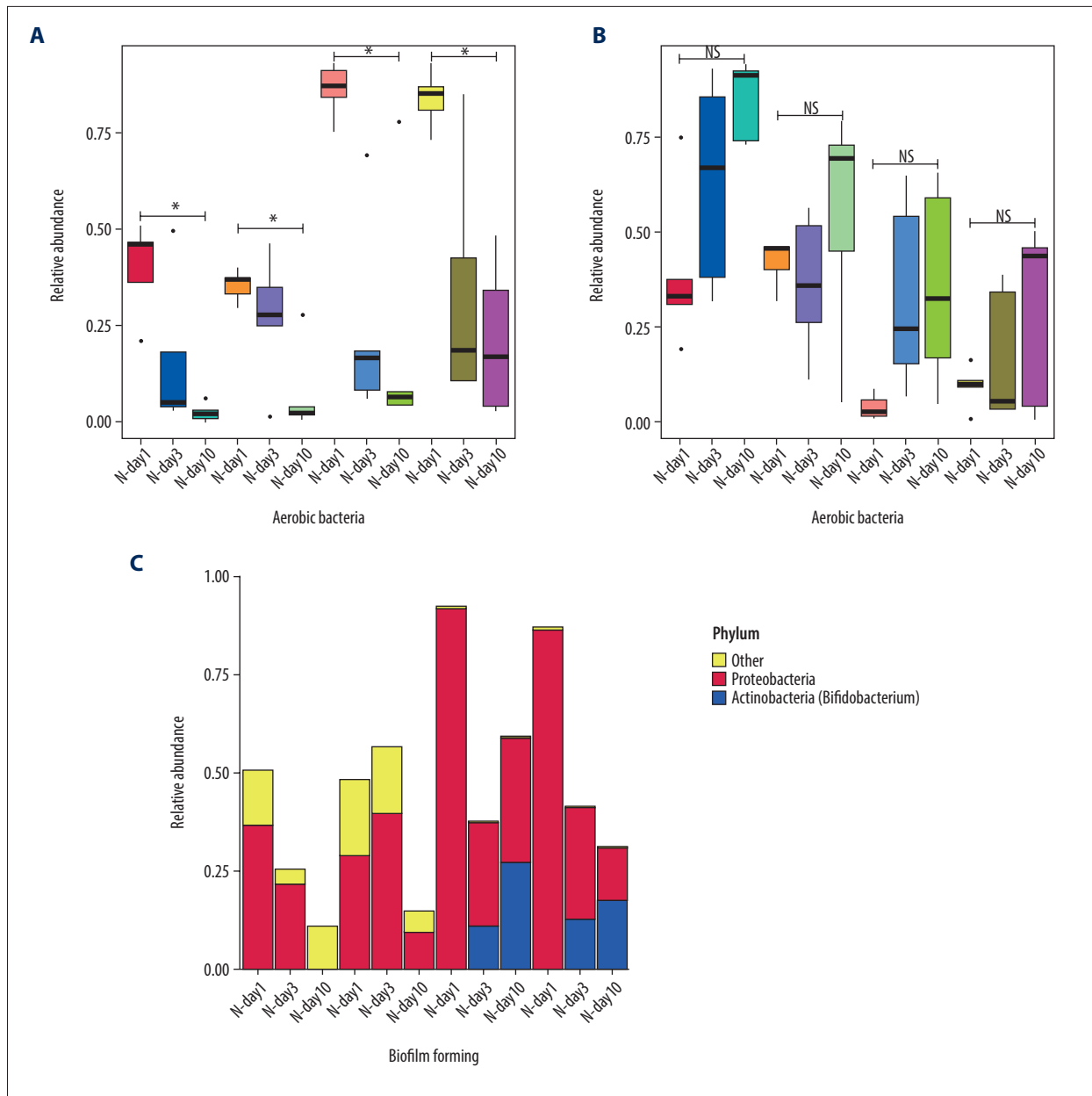


Figure 5. Predicted phenotype of gut microbiota of preterm infants delivered by cesarean section in the 4 groups at 1, 3, and 10 days. **(A)** Aerobic bacteria relative abundance. **(B)** Anaerobic bacteria relative abundance. **(C)** biofilm-forming bacteria relative abundance. N - the N group (No-probiotics and No-antibiotics); A - the A group (antibiotics); P - the P group (probiotics); AP - the AP group (antibiotics+probiotics). Day1 - One day after birth; day3 - Three days after birth; day10 - Ten days after birth. NS - means no significant differences. * $P < 0.05$.

between the gut microbiota and 5 environmental factors: birth weight, gestational age, blood albumin (Alb), blood lactate, and blood pH at birth.

Experimental results show that, at the genus level, the gut microbiota of premature infants was mainly significantly correlated with Alb. *Stenotrophomonas* ($P=0.03$, $r=-0.47$) and *Shingomonas* ($P=0.01$, $r=-0.53$) were negatively correlated

with *Alb. Rothia* ($P=0.04$, $r=0.45$) and *Clostridioides* ($P=0.01$, $r=0.52$) were positively correlated with Alb (**Figure 4**).

Phenotypic Prediction of the Gut Microbiota

BugBase was used to predict the phenotype of gut microbiota. We found that the time trend of intestinal microecology of the 4 groups of premature infants from days 1 to 3 to 10

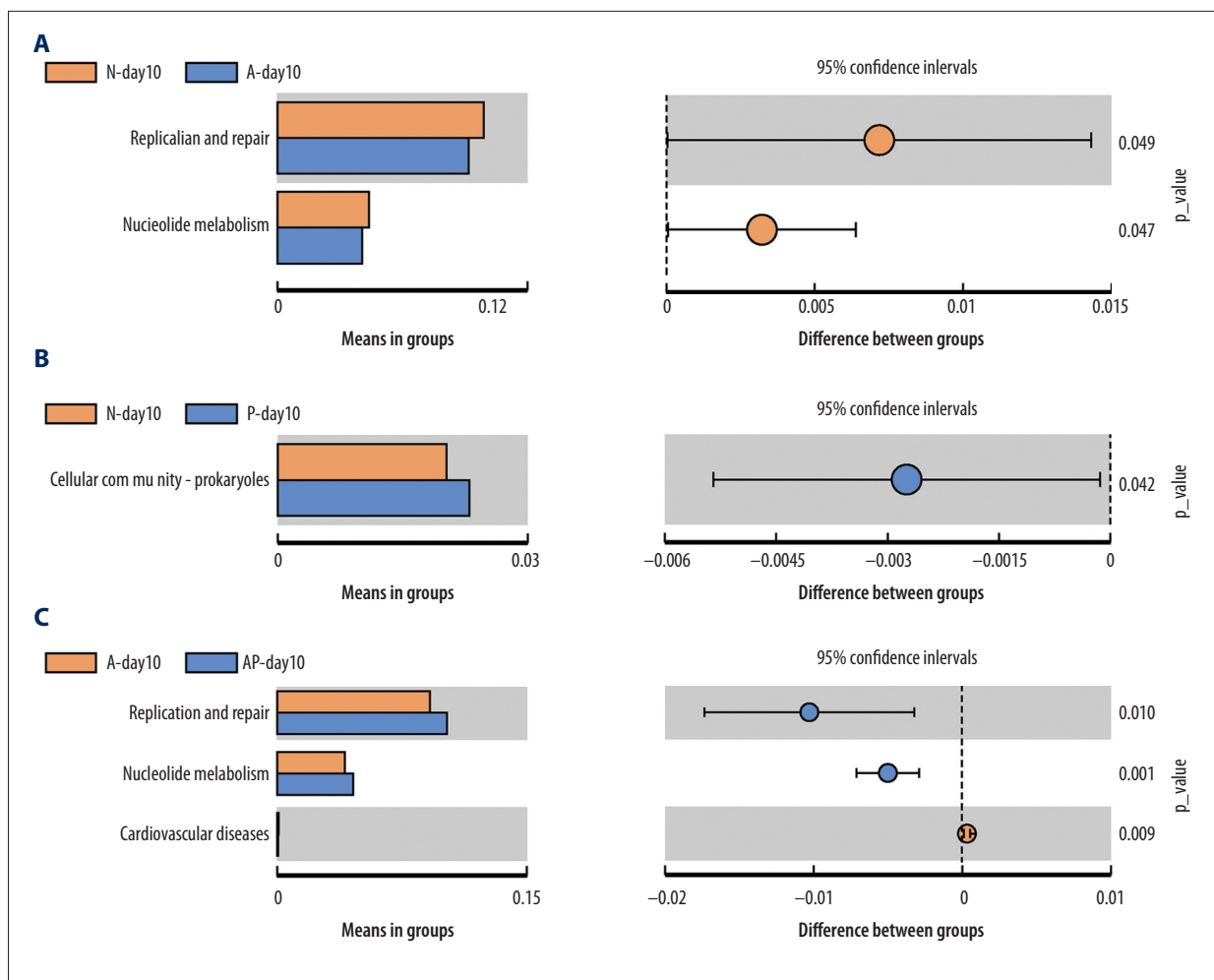


Figure 6. Differences of gut microbiome function of preterm infants delivered by cesarean section in the 4 groups at 10 days. (A) Difference between N-day10 and A-day10 groups. (B) Difference between N-day10 and P-day10 groups. (C) Difference between A-day10 and AP-day10 groups. N – the N group (No-probiotics and No-antibiotics); A – the A group (antibiotics); P – the P group (probiotics); AP – the AP group (antibiotics+probiotics). Day10 – Ten days after birth. $P < 0.05$ means significant differences.

showed a gradual decrease in the abundance of aerobic bacteria, and there were significant differences between day 1 and day 10 in the 4 groups ($P < 0.05$) (Figure 5A). There was a gradual increase in the abundance of anaerobic bacteria, but there were no significant differences between day 1 and day 10 in the 4 groups ($P > 0.05$) (Figure 5B).

Figure 5C shows the relative abundance diagram of biofilm-forming bacteria in each group. It was found that the biofilm formation in groups N-day10 and A-day10 was mainly Proteobacteria, and the biofilm formation in groups P-day10 and AP-day10 using probiotics was Proteobacteria and Actinobacteria (mainly Bifidobacteria) (Figure 5C).

Function Prediction Analysis of Intestinal Flora

Tax4Fun software was used to predict the function of the gut microbiota, and the functions with significant differences among the groups were compared ($P < 0.05$). The results of function prediction showed that the replication and repair ($P = 0.049$) and nucleotide metabolism ($P = 0.047$) of the N-day10 group were significantly higher than in the A-day10 group (Figure 6A). The cellular community *Prokaryotes* ($P = 0.042$) of the P-day10 group were significantly higher than in the N-day10 group (Figure 6B). In addition, compared with the A-day10 group, replication and repair ($P = 0.01$) and nucleotide metabolism ($P = 0.001$) of the AP-day10 group were significantly higher, whereas the cardiovascular disease ($P = 0.009$) was lower (Figure 6C).

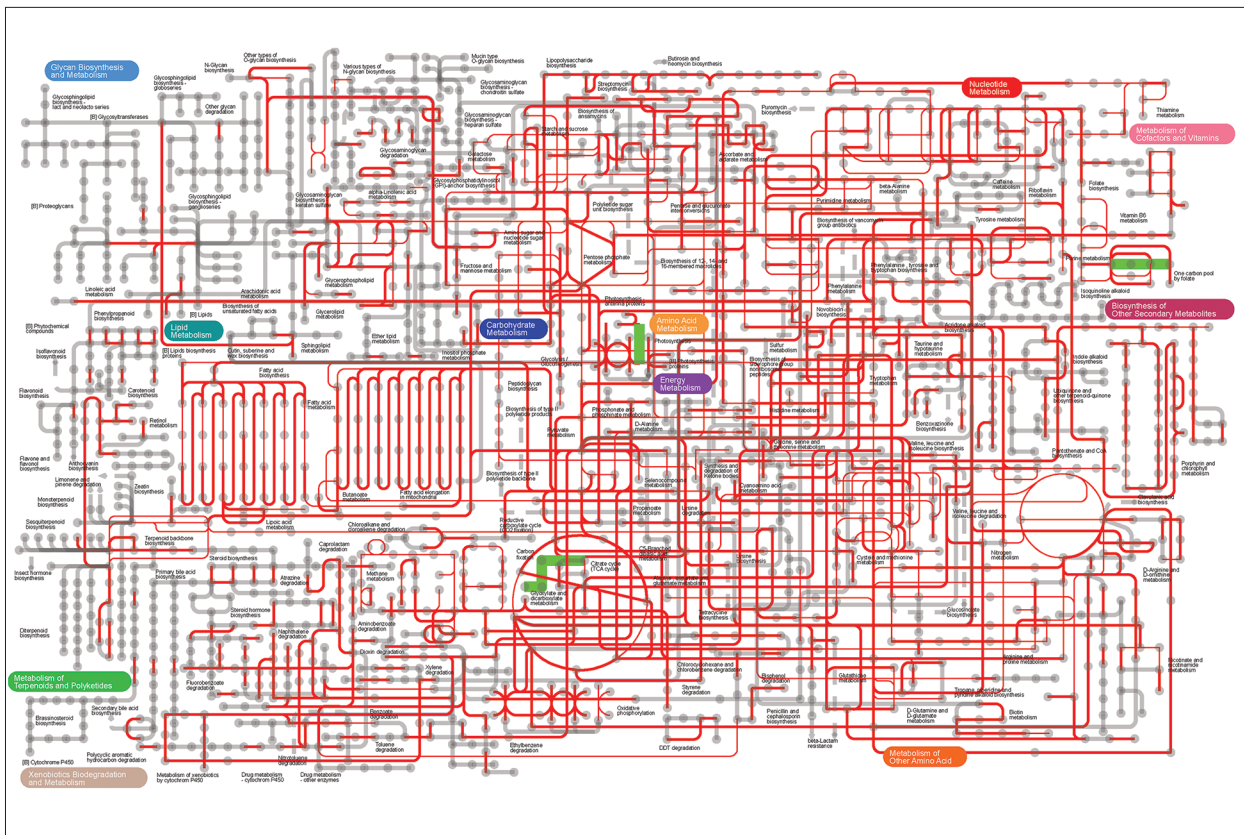


Figure 7. Metabolic pathway prediction between N-day10 and A-day10 groups based on KOs. Red lines are the same metabolic pathway of 2 groups, and Green lines are the metabolic pathway with significant differences of 2 groups ($P < 0.05$). KOs – Kyoto Encyclopedia of Genes and Genomes Orthologous Groups; N – the N group (No-probiotics and No-antibiotics); A – the A group (antibiotics). Day10 – Ten days after birth.

Metabolic Pathway Prediction Analysis

Given the differences in nucleotide metabolism function between groups N-day10 and A-day10, the metabolism function of the 2 groups was further investigated. The approach was based on Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologous groups extracted from fecal samples of groups N-day10 and A-day10 to predict and map metabolic pathways. The results showed that the purine metabolism (map00230), one-carbon pool by folate (map00670), photosynthesis (map00195), and carbon fixation pathways in *Prokaryotes* (map00720) of the A-day10 group were significantly lower than in the N-day10 group (Figure 7).

Discussion

In this study, we investigated the composition and metabonomics of gut microbiota in premature infants delivered by cesarean section using 16S rRNA Quantitative Polymerase Chain Reaction Sequencing techniques. The results showed that early application of antibiotics significantly changed the gut microbiota,

antibiotics reduced the abundance of *Bifidobacterium* and attenuated the replication and repair function, the nucleotide metabolism function, and the one-carbon pool by folate pathway of the gut microbiota. The short-term probiotics supplementation could correct the damage of antibiotics to intestinal flora composition and function of premature infants born by cesarean section in early life. Additionally, the characteristics of intestinal flora transformation were also found in premature infants delivered by cesarean section.

The gut microbiota of premature infants born by cesarean section was mainly composed of 4 significant phyla, which is consistent with the literature [24]. In addition, the variational tendency from *Proteobacteria* to *Firmicutes* was observed at 1, 3, and 10 days. Antibiotics and probiotics did not change the transformation.

On day 10, the composition of the gut microbiota showed differences in abundance at the genus level. Compared with the N-day10 group, the A-day10 group receiving antibiotics had a higher abundance of unidentified *Enterobacteriaceae*, while the abundance of *Bifidobacterium* was lower. A study of

short-term use of ampicillin and gentamicin in term infants has shown that the antibiotic-treated infants had significantly higher proportions of *Enterobacteriaceae* and significantly lower proportions of *Bifidobacteriaceae* at week 4 [12]. In our study, the same changes occurred in intestinal flora composition at 10 days of antibiotic treatment for premature infants delivered by cesarean section. This may indicate that the intestinal flora of premature infants born by cesarean section is affected by antibiotics earlier. Furthermore, the abundance of *Parabacteroides* in the A-day10 group was also higher. *Parabacteroides* is a symbiotic bacterium that can regulate the host mucosal immune system [25], and has been shown to be resistant to cephalosporins and ampicillin antibiotics [26]. Therefore, the increased abundance of *Parabacteroides* in the antibiotic group may be the result of the self-protection of the gut flora of premature infants. A study has shown that probiotics increases the abundance of *Bifidobacterium* in the gut microbiota of premature infants [27]. In the P-day10 groups receiving probiotics in this experiment, in addition to the higher numbers of *Bifidobacterium* compared to those of the N-day10, the abundance of *Lactobacillus* had also increased significantly. These results indicated that antibiotics increased the abundance of harmful intestinal bacteria and decreased the abundance of beneficial bacteria in premature infants born by cesarean section, whereas probiotics contributed to an increase in beneficial bacteria.

LEfSe was used to analyze the biomarkers of AP-day10 and A-day10 groups, so as to assess the effect of probiotics on the gut microbiota of premature infants delivered by cesarean section treated with antibiotics. The results showed that the AP-day10 group contained more beneficial bacteria (*Lactobacillus*, *Bifidobacterium bifidum*), while the A-day10 group contained more pathogenic bacteria (*Escherichia coli*, *Clostridium paraputrificum*, *Clostridium difficile*). Studies have shown that long-term probiotic supplementation can help restore the proportion of *Bifidobacteria* and *Lactobacillus* in antibiotic-treated infants at the age of 3 months [21]. However, our present study suggests that the same effect could be reached in the antibiotic-treated premature infants born by cesarean sections by short-term probiotics supplementation in early life. Late-onset sepsis (LOS) is considered to be the result of the spread of symbiotic bacteria in the skin or intestines of premature infants, the most important of which are *Staphylococcus* and *Escherichia coli* [28]. Studies have shown that preterm infants at risk of infection are often treated with empirical antibiotics until early-onset sepsis (EOS) is ruled out. Paradoxically, although antibiotics reduce the incidence of early-onset sepsis (EOS), they may increase the risk of late-onset sepsis (LOS) by altering the intestinal microbiota of preterm infants [7,29]. In the present study, there were fewer *Escherichia coli* in the AP-day10 group than in the A-day10 group, suggesting that early use of probiotics in premature infants treated with antibiotics could

reduce *Escherichia coli*, which may reduce the risk of late-onset sepsis (LOS) in premature infants. Moreover, *Clostridium difficile* is believed to be one of the main factors of antibiotic-associated diarrhea [30]. This study found that numbers of *Clostridium difficile* was significantly reduced in the antibiotic group after using probiotics, indicating that probiotics has a certain preventive effect on *Clostridium difficile*-associated diarrhea, which is consistent with previous studies [31].

In the analysis of gut microbiota diversity, a previous study showed no significant changes in the gut microbial diversity at week 4 for term infants given early, short-term antibiotic treatment [12]. A study has also shown that antibiotic exposure in the first few days after birth did not significantly change the diversity of the gut microbiota in premature infants [32]. Therefore, there was no significant difference in the gut microbiota diversity of antibiotic-treated premature infants delivered by cesarean section, which may be due to the short duration of antibiotics use. Consistent with previous studies, the 2 groups of preterm infants treated with probiotics in this experiment had significantly lower alpha diversity of gut flora, which may account for the decrease in the number of harmful bacteria [18].

Correlation analysis of environmental factors showed that, at the genus level, Alb was the major factor affecting the composition of the gut microbiota, and the *Sphingomonas* was negatively correlated with Alb. Earlier studies have shown that the *Sphingomonas* is closely related to the occurrence of NEC [33,34]. Therefore, whether there is an association between the Alb level at birth and NEC in premature infants warrants further study. A previous study has suggested that gestational age is a factor affecting the intestinal microecology of premature infants [10]. However, the relationship between gut microbiota and gestational age in this experiment was not apparent, which might be related to the relatively centralized gestational age of premature infants collected in this study.

The phenotype prediction analysis showed that the intestinal microecology of premature infants delivered by cesarean section showed a trend of transformation from aerobic bacteria to anaerobic bacteria over time. This may be because the gut is rich in oxygen at birth, when it is suitable for aerobic bacteria to grow, and anaerobic bacteria begin to grow in large quantities after oxygen is used up. In addition, biofilm formation of *Actinobacteria* (mainly *Bifidobacteria*) was observed in groups P-day10 and AP-day10 receiving probiotics. Biofilm formation is a protective growth pattern that is highly resistant to antibiotics and harsh environments [35,36]. The stable vegetation of *Bifidobacteria* is thought to regulate the immune function of intestinal mucosa [37], and affect the biofilm formation of *Escherichia coli* [38].

The results of function prediction found that both antibiotics and probiotics had significant effects on the gut microbiota function of premature infants delivered by cesarean section. A study on the gut microbiota of mice treated with antibiotics revealed that the use of antibiotics decreases the translation, replication, recombination, and repair functions [39]. Similar to the gut microbiota experiment in mice, the replication and repair function in preterm infants delivered by cesarean section receiving antibiotics (A-day10 group) was also significantly reduced. In addition, the nucleotide metabolism function of the A-day10 group was significantly lower than that of the N-day10 group in this experiment, indicating the adverse effect of the antibiotics on the gut microbiota function of preterm infants in cesarean section. Regarding the effects of probiotics, the cellular community of *Prokaryotes* was significantly higher in the P-day10 group using probiotics than in the N-day10 group. A study has shown that intestinal health is related to the diversity of prokaryotic communities, and the reduction of prokaryotic biodiversity is associated with inflammatory bowel disease [40]. Moreover, compared with the A-day10, the AP-day10 group had higher replication and repair and nucleotide metabolism, indicating that the use of probiotics alleviates the adverse effects of antibiotics on the function of gut microbiota. Furthermore, premature infants have a high incidence of cardiovascular disease, and the gut microbiota is believed to be related to cardiovascular disease. Some studies have shown that the use of probiotics can reduce the risk of cardiovascular disease [41,42]. In this experiment, the cardiovascular diseases function in the AP-day10 group was lower than in the A-day10 group, showing that early use of probiotics may prevent cardiovascular diseases in premature infants treated with antibiotics.

The results of metabolic pathway prediction in N-day10 and A-day10 groups showed that the purine metabolic pathway and one-carbon pool by folate pathway were significantly lower in cesarean section preterm infants treated with antibiotics compared with that in the N-day10 group. A previous study showed that the gut microbiota is involved in purine metabolism, and abnormal purine metabolism may increase uric acid [43]. In this study, the purine metabolism pathway in group A-day10 was lower than that in group N-day10, which may be due to the disorder of gut microbiota caused by using antibiotics, thus affecting the purine metabolism pathway. The differences in purine metabolic pathways may be the reason that the nucleotide metabolism function of the A-day10 group was lower than in the N-day10 group. In addition, the one-carbon pool by folate pathway in group A-day10 was also significantly lower

than in the N-day10 group. Folic acid is one of the essential B vitamins involved in DNA synthesis and repair [9]. In this experiment, the replication and repair function of the A-day10 group was lower than that of the N-day10 group. We speculate that this function might be affected by the abnormal metabolic pathway. Therefore, the use of antibiotics has an adverse effect on some metabolic pathways of intestinal flora in premature infants delivered by cesarean section.

To the best of our knowledge, the present study is the first to indicate that probiotics supplementation can restore the composition and function of gut flora in antibiotic-treated premature infants delivered by cesarean section. However, there are some limitations to our research that need to be considered. For example, although the high-throughput sequencing of 16S rRNA is currently the most widely used technique in analysis of the microbial community, it is limited in resolution compared with metagenomics [44]. In addition, to ensure the accuracy of the experiment, all the 40 premature infants by cesarean section selected in this study were fed with formula milk, which may ignore the effect of breastfeeding on gut flora. Accordingly, future studies should assess the effects of probiotics or antibiotics on intestinal flora of breastfed premature infants delivered by cesarean section. Metagenomics and proteomics may also be combined to further explore this topic in future studies.

Conclusions

In conclusion, the findings from this study showed that antibiotic treatment of preterm infants born by cesarean section changed the composition and function of gut microbiome, but that the use of probiotics could restore the normal microbiome, which supports that restoration of the normal gut microbiota may be achieved with probiotics. Furthermore, the relationship between *Sphingomonas* and Alb was found for the first time in this study, providing a novel viewpoint for the study of NEC in premature infants.

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Conflict of Interest

None.

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