

Original Article

Decrease in intestinal microbiome diversity at birth is related to moderate and severe bronchopulmonary dysplasia in premature infants

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ABSTRACT

Background: Bronchopulmonary dysplasia (BPD) is a prevalent respiratory disorder in premature infants. Recent studies have revealed an association between the intestinal microbiome and respiratory diseases. This study aims to explore the characteristics of the gut microbiome of premature infants with moderate and severe BPD.

Methods: The study population consisted of preterm infants with a gestational age of ≤ 34 weeks. Infants with moderate and severe BPD were selected as the case group. Stool samples were collected at birth and 28 days after delivery. The obtained samples were processed using 16sRNA technology for diversity analysis, taxonomic composition, and LEfSe analysis.

Results: In this study, 15 infants were enrolled in the BPD group, while 15 infants were included as controls. At birth, the alpha diversity of the BPD group was significantly lower compared to the control group. Infants with severe BPD had even lower diversity. The relative abundance of *Staphylococcus* in the BPD group was higher, whereas *Sphingomonas* and *Veillonella* were the predominant species in the control group. Additionally, the severe BPD group exhibited a higher abundance of *Bacillales* and *Oscillospiraceae* compared to the moderate BPD group. At 28 days after birth, there was an increase in the abundance of *Escherichia*, *Klebsiella*, and *Bifidobacterium* compared with previous levels, and a decrease in the abundance of *Herbaspirillum*.

Conclusions: The diversity of intestinal microbiota in infants with moderate and severe BPD appears to be relatively lower, and specific microbial species such as *Staphylococcus* and *Bacillales* may contribute to the pathogenesis and progression of BPD. The intestinal microbiota in infants with BPD interacts with the oxygen environment in the gut, and its early formation is influenced by maternal factors. These findings regarding the diversity and specific flora of gut microbiota could potentially offer insights into the pathogenesis of BPD.

1. Introduction

Bronchopulmonary dysplasia (BPD) is a clinical syndrome that disrupts alveolar formation and microvascular development, which has a serious impact on lung development and long-term prognosis, especially in those with a small gestational age [1]. The etiology of BPD is related to heredity, lung immaturity, mechanical ventilation, infection, inflammatory response, immunity, and other factors [2–6]. With the continuous development of research on the gut-lung axis, it has also been proposed that there may be a correlation between gut microbiota and BPD [7].

The development of the respiratory and gut microbiota occurs concurrently and postnatally due to the mucosal nature of both organs

[8]. The microbiota exhibits a consistent interaction effect across both channels. Imbalance in gut microbial homeostasis results in the disruption of the gut barrier; and various inflammatory mediators, such as interferon- γ (IFN- γ), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α), can disrupt tight junction proteins and increase epithelial permeability. This allows harmful factors to enter adjacent tissues and certain bacteria to translocate to the lung [9]. Simultaneously, mesenteric lymphatic vessels can also serve as conduits for carrying harmful factors that contribute to lung injury [10]. Furthermore, metabolites produced by intestinal flora, including short-chain fatty acids, also influence the occurrence and progression of BPD [11]. The dysbiosis of the intestinal microbiota in BPD model mice has been further validated via animal experiments, providing additional evidence for the potential

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correlation between BPD and gut microbiota [12]. However, research in this field remains limited, and there is still a lack of in-depth exploration regarding the impact of meconium on BPD. Extensive experiments are required to elucidate the precise mechanisms and causality underlying the bidirectional interaction between gut microbiota and BPD.

In this study, meconium was used as the main research sample, and 16S rRNA sequencing technique was employed to explore the characteristics of intestinal flora in infants with BPD. Additionally, the characteristics of gut microbiota in infants with different severities of BPD were also investigated.

2. Methods

Participants: This prospective cohort study was approved by the Medical Ethics Committee of the First Hospital of Jilin University (ethics No. 2018-422), and informed consent was obtained from all patients. The study encompassed premature infants (gestational age ≤ 34 weeks) who were born or admitted within 48 h after birth from January 2018 to April 2019 in the Neonatal Intensive Care Unit of Children's Medical Center, The First Hospital of Jilin University. Upon admission, comprehensive clinical data for both the mother and the neonate were collected from the electronic medical records, such as maternal medical history and antibiotic usage. Subsequently, the diagnosis, breastfeeding patterns, oral medication, and antibiotic treatments after birth were recorded. All clinical diagnoses were conducted in strict adherence to the clinical guidelines recognized by either the Chinese Medical Association or those comparable international organizations. At 28 days after birth, subjects were divided into the BPD group and the control group based on ventilator usage, and disease severity was then assessed. Meconium samples were collected from all infants within 48 h after birth to reflect the initial characteristics of intestinal flora. Because early postnatal feeding was limited and stool samples could not fully show microbiota at the onset of BPD, second stool samples were collected on the 28th day after birth. The diagnostic criteria were proposed by a consensus meeting of the National Institute of Child Health and Human Development (NICHD) in 2018 [4,13]. The assessment time for severity indicated that infants with a gestational age of less than 32 weeks are assessed at 36 weeks of postmenstrual age (PMA) or at discharge, whichever comes first. Infants with a gestational age greater than 32 weeks are assessed at 28 days after birth or at discharge.

Inclusion Criteria: 1. Gestational age ≤ 34 weeks; 2. No evidence of digestive tract infection; 3. No severe infectious diseases, such as sepsis, suppurative meningitis, or pneumonia; 4. Stool samples were collected. Cases meeting the abovementioned criteria were included in the study.

Exclusion Criteria: 1. Gestational age > 34 weeks; 2. Congenital digestive tract malformation; 3. Death within 28 days after birth; 4. Parents did not agree to participate in the study.

Sample Collection: The samples were stored at -20°C within 24 h after collection and then transferred to -80°C for low-temperature storage until they were sent to Shanghai Parsons Biotechnology Co., Ltd., for 16S rRNA analysis.

Main Outcome Measures: The characteristics of the intestinal microbiota in the meconium of infants diagnosed with bronchopulmonary dysplasia at birth and 28 days postpartum were investigated, encompassing analysis of alpha diversity, fecal microbial community composition, and differential flora analysis.

PCR Amplification and Sequence Data Analysis: The total genomic DNA of bacteria was extracted from fecal samples, followed by PCR amplification targeting the V3–V4 region of the bacterial 16S rRNA gene. The forward primer was 338F (5'-ACTCCTACGGGAGGCAGCA-3'), and the reverse primer was 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The Illumina NovaSeq PE250 DNA library was selected as the sequencing platform to perform paired-end sequencing of community DNA fragments, and the original sequencing data were saved in FASTQ format.

Analysis of Fecal Bacteria: Sequence data analysis was primarily conducted using QIIME2 and R software (version 3.2.0). The DADA2

algorithm was employed for depriming, quality filtering, denoising, splicing, and dechimerism. After obtaining the amplicon sequence variant (ASV) abundance matrix based on the Greengenes database, the bacterial flora between groups were analyzed. Alpha diversity analysis was performed to assess the richness and diversity of the community, and a rarefaction curve was used to determine whether the current sequencing depth of each sample was sufficient to reflect the microbial diversity and community abundance within the samples. Beta diversity analysis was mainly performed using a two-dimensional sequence diagram of the non-metric multidimensional scaling (NMDS) analysis results to illustrate the differences in microbial community composition among the samples. This was supplemented by ANOSIM to assess community differences both between and within groups. Differential flora between groups were identified using LEfSe analysis, an integrated taxonomic composition analysis to identify differentially abundant taxa.

Statistical analysis: SPSS 22.0 was used to analyze the data. The measurement data in accordance with normal distribution were expressed as the mean $\pm$ standard deviation ($\bar{x} \pm s$). The two-sample t-test was used for comparisons between the two groups. The measurement data of non-conforming normal distribution were represented by the median (interquartile range) [M (P25, P75)]. The Mann-Whitney U test was used for comparisons between the two groups. $P < 0.05$ was considered statistically significant.

3. Results

According to the inclusion and exclusion criteria, a total of 15 infants with BPD were enrolled, with 6 cases classified as moderate and 9 cases as severe. To eliminate confounding factors, infants without BPD, matched with BPD infants in terms of gestational age, delivery mode, feeding mode, and duration of antibiotic use, were selected as controls (flow chart). The clinical data of the 30 preterm infants in this study are presented in Table 1. There were no statistically significant differences in maternal factors, including maternal prenatal infection, antibiotic use, or prenatal steroid administration. In terms of postnatal factors, all infants received breastfeeding as a priority, while some received formula due to insufficient breast milk. Feeding intolerance is common in premature infants, so some infants were administered probiotics. *Clostridium butyricum* and *Bifidobacterium* are commonly used in this center, and the use of probiotics was comparable across the groups. There were significant differences in invasive mechanical ventilation time between the BPD group and the control group ($p = 0.044$). However, no significant differences were found in other clinical data. Furthermore, no statistically significant difference was observed at baseline between the moderate and severe BPD groups.

Overall Characteristics of the Fecal Microbiota: Among the 60 fecal samples, a rarefaction curve was obtained (Fig. 1). The flattening observed at the ends of the curves indicates that the sample size was adequate and that the sequencing results sufficiently captured the diversity and richness present in the current sample.

NMDS analysis provided a visual representation of the similarities and disparities within the overall microbial community among different samples. At birth, the control group exhibited a low degree of similarity among its samples, characterized by a dispersed distribution. In contrast, the community similarity in the BPD group was higher. At 28 days after birth, the microbial communities in the control group showed increased similarity, suggesting a trend toward a unified structure. Meanwhile, the BPD group's samples remained scattered, diverging from the control group's pattern (Fig. 2a and b). The community structure of infants born via cesarean section was more similar than that of infants born vaginally, and maternal antibiotic use did not significantly affect the community structure (Fig. 2c and d). The stress values of the NMDS results were all less than 0.2, indicating that the analysis result is reliable. Additionally, ANOSIM analysis confirmed significant between-group dissimilarity ($p = 0.041, 0.007$). The alpha diversity analysis revealed that the Chao1, observed species, and Shannon indices of the

Table 1
Clinical data of all groups.

group	Control	BPD	P value	Moderate BPD	Severe BPD	P value
n	15	15		6	9	
gestational age(day)	213 ± 9.79	209.4 ± 13.84	0.406	203.83 ± 14.25	213.11 ± 13.02	0.215
Gender(male)	10	9	0.500	4	5	0.545
birth weight(kg)	1.60 ± 0.27	1.37 ± 0.38	0.081	1.38 ± 0.41	1.36 ± 0.38	0.947
Apgar 1min	7.13 ± 0.91	6.47 ± 1.5	0.167	6.83 ± 1.17	6.22 ± 1.72	0.462
delivery mode(vaginal)	9	11	0.350	6	5	0.092
antenatal corticosteroid	10	9	0.500	2	7	0.206
PROM	5	7	0.355	3	4	0.622
Maternal antibiotic use	8	10	0.355	3	7	0.406
Maternal antibiotic use time	1.93 ± 2.78	2.27 ± 2.54	0.735	1.5 ± 2.07	2.78 ± 2.81	0.331
feed mode(breast milk)	13	12	0.763	4	8	0.698
Antibiotic application time	7.87 ± 6.09	8.67 ± 5.35	0.705	7.5 ± 7.25	9.44 ± 3.94	0.511
Classes of Antibiotics			0.249			0.363
Probiotic (Bifidobacterium)	8	10	0.305	4	4	0.315
Mechanical ventilation time	1.47 ± 2.20	6.40 ± 8.77	0.044*	7.17 ± 9.6	5.89 ± 8.73	0.794
NRDS	8	13	0.109	5	8	0.657
cytomegalovirus infection	4	6	0.350	2	4	0.545
neonatal asphyxia	4	6	0.350	1	5	0.168
anemia	8	11	0.225	4	5	0.545

x ± s: mean ± standard deviation; M (P25, p75): median (interquartile spacing).

* = P < 0.05, P < 0.05 was considered statistically significant.

BPD: bronchopulmonary dysplasia; PROM: premature rupture of fetal membranes.

NRDS: neonatal respiratory distress syndrome.

Classes of Antibiotics: Antibiotic-free; cephalosporin; semi-synthetic penicillins; or both; The time metric is quantified in days.

Probiotic use: Bifidobacterium, clostridium butyricum, or both.

Mechanical ventilation: invasive.

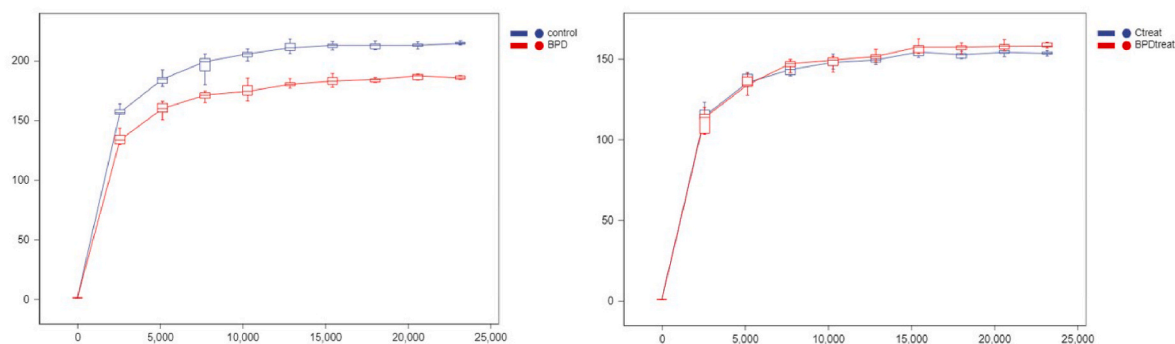


Fig. 1. The horizontal axis of the rarefaction curve represents the total number of randomly selected sequences in each sample, the vertical axis represents the number of ASVs observed at the corresponding depth, and the length of this curve reflects the amount of sample sequencing. The left figure presents the rarefaction curve at birth, and the right one at 28 days postnatal. A flatter curve indicates that the sequencing result is sufficient to reflect the diversity contained in the current sample.

BPD group were significantly lower than those of the control group at birth. Furthermore, severe BPD showed lower microbial diversity than moderate BPD (Table 2.1). However, at 28 days after birth, no significant difference in α -diversity was observed, either between the BPD group and the control group or among infants with moderate and severe BPD (Table 2.2).

Different Flora: Based on the composition and sequence distribution of each sample at various taxonomic levels, the distribution of higher relative abundance intestinal flora among the control, BPD, moderate, and severe groups was determined (Fig. 3e and f). In both the BPD and control groups, Proteobacteria was the dominant phylum at birth, while the abundance of Firmicutes gradually increased over time. At 28 days after birth, the abundances of *Escherichia*, *Klebsiella* and *Bifidobacterium* were higher compared to their levels at birth, while the abundances of *Herbaspirillum*, *Serratia* and *Bacteroides* were lower. LEfSe analysis demonstrated that *Staphylococcus* had a higher relative abundance in the BPD group compared to the control group at birth, while *Sphingomonas* and *Veillonella* had lower relative abundance (Fig. 3a). On day 28 after birth, *Burkholderiaceae*, *Herbaspirillum* and *Pelomonas* showed higher relative abundance in the BPD group than in the control

group, while *Peptostreptococcaceae* and *Lactobacillaceae* exhibited lower relative abundance in the BPD group (Fig. 3b). Additionally, *Bacillales* and *Oscillospiraceae* exhibited higher abundance in severe BPD cases compared to moderate cases at birth (Fig. 3c). According to the delivery mode at birth, *Enterococcaceae* and *Enterobacteriaceae* were dominant in naturally delivered infants (Fig. 3d).

4. Discussion

In this study, fecal samples were prospectively collected from pre-term infants with a gestational age of ≤ 34 weeks. The characteristics of fecal microbiota and differential flora in meconium and at 28 days of life in preterm infants with moderate and severe BPD were described. Meconium reflects the characteristics of the intestinal microbiota in infants with BPD prior to the influence of various therapeutic factors. At birth, the diversity index of meconium flora in the BPD group was found to be lower compared to the control group, and this decrease in diversity correlated with the severity of BPD. The presence of a diverse gut microbial community enhances immune self-tolerance and promotes healing, establishing an equilibrium that facilitates an efficient response

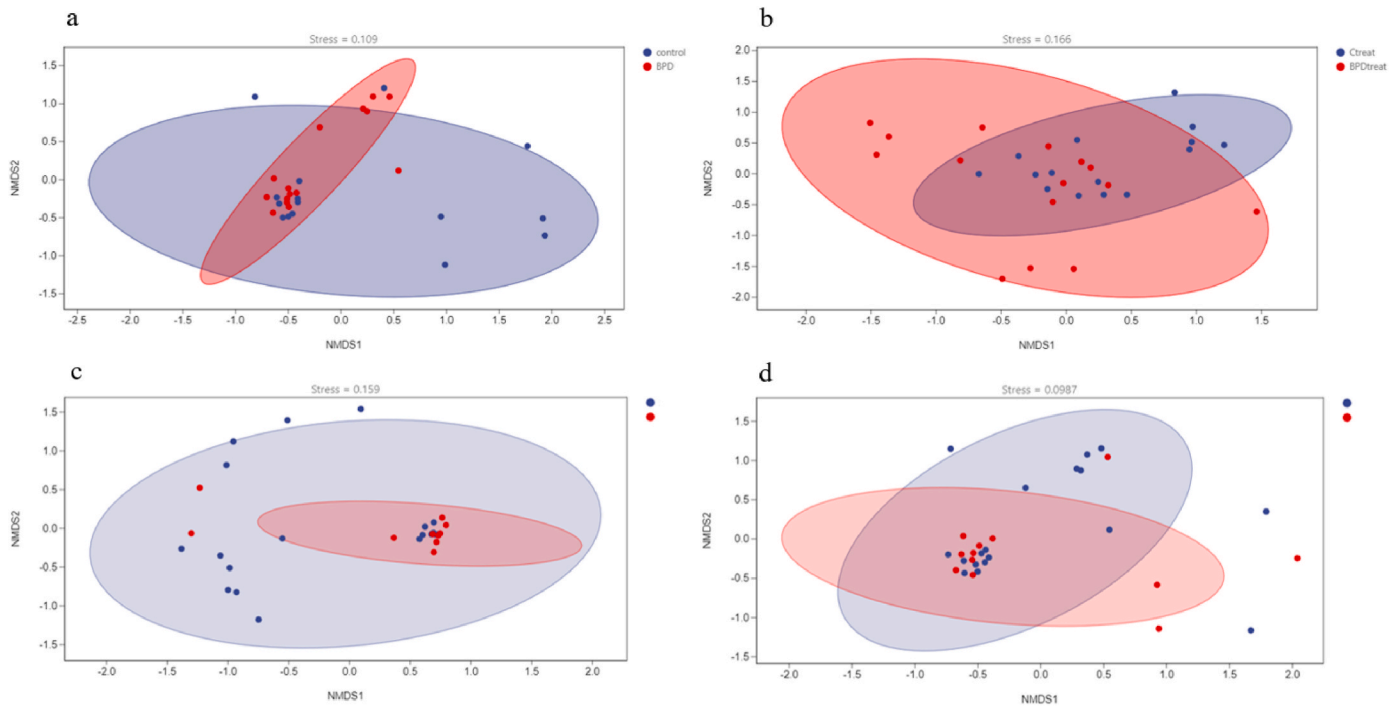


Fig. 2. NMDS Analysis was employed to assess the community similarity. a: control group and BPD group at birth; b: control group and BPD group at 28 days after birth; c: red represents cesarean delivery and blue represents vaginal delivery; d: red indicates no antibiotics and blue indicates antibiotics applied; proximity of samples belonging to the same group indicates a higher level of community resemblance.

Table 2
alpha diversity index.

2.1 At birth					
Group	n	Chao1	Observed_species	Shannon	Simpson
control	15	241.97 ± 51.68	218.38 ± 52.02	3.51 ± 0.65	0.78 ± 0.11
BPD	15	185.96 ± 54.93	171.91 ± 49.06	2.92 ± 0.75	0.70 ± 0.12
P value		0.009*	0.018*	0.031*	0.064
moderate BPD	6	197.31 ± 44.21	186.21 ± 39.49	3.55 ± 0.71	0.79 ± 0.13
severe BPD	9	178.39 ± 62.43	162.37 ± 54.60	2.50 ± 0.41	0.63 ± 0.07
P value		0.534	0.376	0.014*	0.04*
2.2 28 days after birth					
group	n	Chao1	Observed_species	Shannon	Simpson
control	15	193.21 ± 51.84	183.78 ± 61.00	3.36 ± 1.28	0.77 ± 0.16
BPD	15	173.21 ± 49.75	145.94 ± 54.06	3.18 ± 1.08	0.71 ± 0.21
P value		0.290	0.083	0.688	0.410
moderate BPD	6	166.43 ± 37.47	152.85 ± 34.19	3.39 ± 1.02	0.75 ± 0.20
severe BPD	9	152.70 ± 69.83	141.33 ± 65.75	3.05 ± 1.16	0.68 ± 0.23
P value		0.669	0.702	0.570	0.521

Chao1 and Observed_species indices that focus on the richness of the community, as well as both the Shannon and Simpson indices, which consider community abundance and evenness (the difference of abundance among members). * = P < 0.05.

to pathogens [14]. The establishment of stable communities and prevention of excessive proliferation of single species are facilitated by a high diversity of gut microbiota [15]. Preterm infants, particularly those with BPD, exhibit reduced diversity in their gut microbial composition and they are unable to generate appropriate microbial signals necessary for immune homeostasis stimulation [16]. Decreased diversity increases

the risk of bacterial dysbiosis, leading to excessive colonization by opportunistic pathogens and triggering an inflammatory response that negatively impacts alveolar and microvascular development, ultimately contributing to the progression of BPD. Research has also found reduced diversity of gut microbiota in BPD infants [17]. However, the timing of sample collection varies across studies, leading to differing time points at which decreased diversity is observed. This may be attributed to the dynamic nature of gut microbiota, differing characteristics of NICU populations, inherent environmental microbes, and variations in post-natal treatment protocols. In this study, although meconium could minimize the impact of postnatal treatment factors on gut microbiota, maternal factors also significantly influenced it. In a cohort of 126 preterm infants born via cesarean section at <32 weeks of gestation, there were no significant differences in microbial diversity in the amniotic fluid among infants with varying severity of BPD. However, moderate and severe BPD showed a predominance of 16S rRNA genes belonging to the *Escherichia-Shigella* cluster [18]. In the placental microbiome analysis, a decline in species diversity was observed in placentas with severe chorioamnionitis [19], further evidencing the impact of maternal factors on gut microbiota. Additionally, based on the mode of delivery, cesarean-born infants exhibited more similar gut microbiota characteristics at birth, while the meconium microbiota of vaginally delivered infants showed a more dispersed distribution, predominantly consisting of *Enterococcus* and *Enterobacter*. This might be associated with the relatively sterile environment during cesarean delivery.

As demonstrated by differential microbiota analysis, the abundance of *Staphylococcus* in the BPD group at birth was significantly higher than in the control group, while abundances of *Sphingomonas* and *Veillonella* were lower. *Staphylococcus* is a genus of gram-positive, facultatively anaerobic coccoid bacteria, which is widely distributed in nature and found on the skin of humans and animals, as well as in body cavities connected to the external environment. Common species include *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Staphylococcus capitis*, most of which are non-pathogenic or opportunistic pathogens. They are significant sources of nosocomial infections,

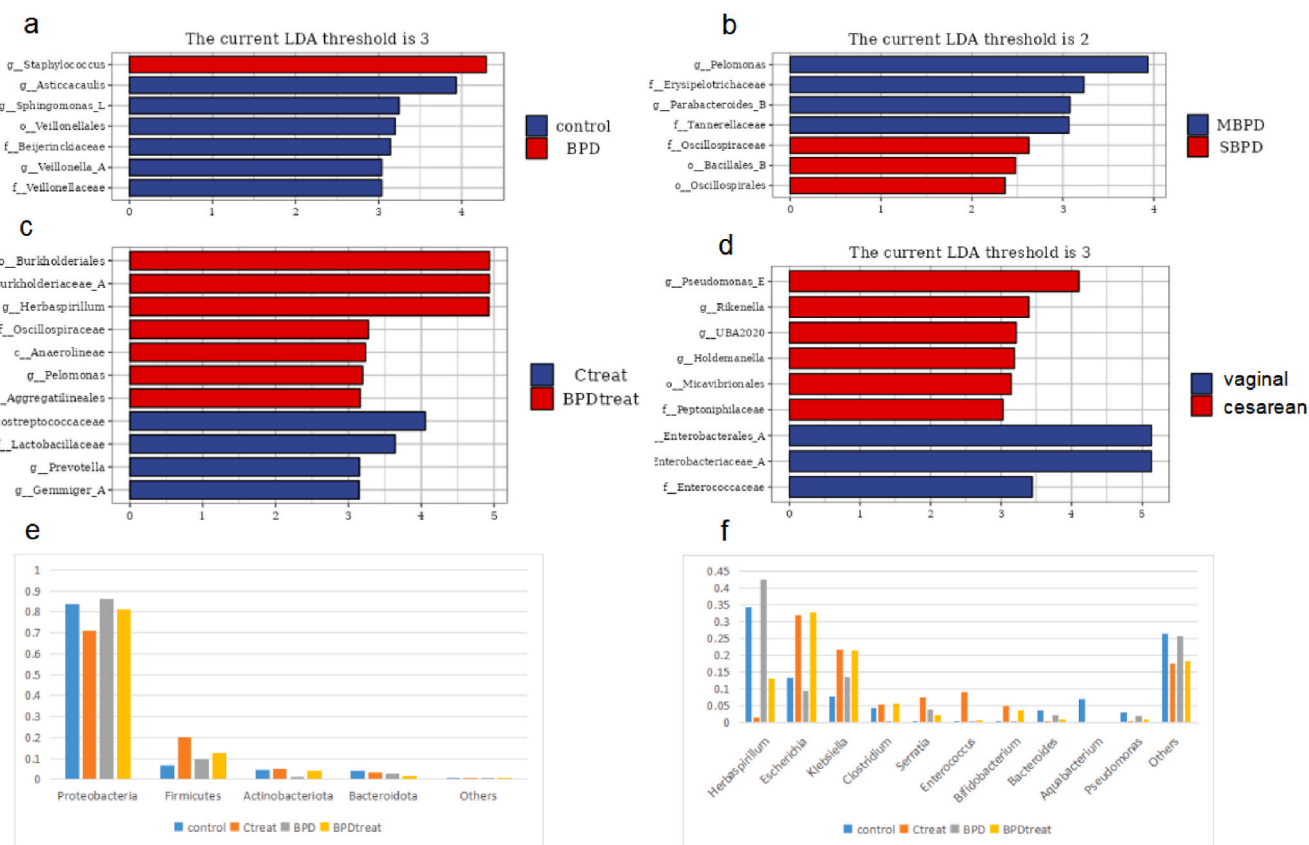


Fig. 3. a-d are lefse analysis: a is the difference between the BPD group and the control group at birth; b is the difference between the moderate BPD group and the severe BPD group at birth; c is 28 days after birth, red is the BPD group and blue is the control group; and d is the differences in mode of delivery; e and f are taxonomic composition analysis, where e is phylum level, f is genus level.

contributing to superficial skin infections and infections associated with indwelling medical devices and these could lead to ventilator-associated pneumonia [20].

Current in vitro studies have indicated that *Staphylococcus epidermidis* may promote inflammation in human alveolar epithelial cells by stimulating the expression of pro-inflammatory mediators [21]. Unbalanced inflammatory responses may disrupt the pulmonary capillary-alveolar unit, leading to lung injury and the development of BPD. Different strains of *Staphylococcus epidermidis* have varying pro-inflammatory capacities. Clinical studies have shown that the early airway microbiota is predominantly composed of *Staphylococcus* [22]. Additionally, *Staphylococcus* 16S rRNA has been detected in the amniotic fluid of preterm infants [18], suggesting that *Staphylococcus* can quickly colonize the gut. The early colonization of preterm infants' gut microbiota is related to the maternal amniotic fluid and airway microbiota. Research on BPD-associated microbiota should integrate a maternal-airway-gut perspective. The mechanisms by which gut *Staphylococcus* contributes to BPD still require further investigation.

The higher levels of *Sphingomonas* in the control group represent a strictly aerobic bacterium, while *Veillonella* is an anaerobic bacterium. *Sphingomonas* can survive for long periods in disinfected environments, whereas *Veillonella* typically resides in the oral cavity, intestines, and respiratory tracts of humans and animals. Although some studies indicated that *Sphingomonas* could contaminate sterile fluids like intravenous medications and dialysis solutions, potentially leading to nosocomial infections, their relatively low abundance in this study did not necessarily suggest a pathogenic or protective role.

From the analysis of disease severity, a 2017 study revealed that infants with more severe BPD exhibited higher turnover rates of airway microbial bacterial communities [23]. This suggests that even among

infants diagnosed with BPD, those with varying degrees of severity possess distinct microbial community compositions. This study demonstrates that neonates with severe BPD exhibit lower gut biodiversity than those with moderate BPD in terms of gut microbiota composition at birth. Moreover, infants with severe BPD exhibit a high abundance of Bacillales and Oscillospiraceae at birth. Bacillales is an order within the class Bacilli, which includes many Gram-positive bacteria, such as *Listeria* and *Staphylococcus*. The higher abundance of *Staphylococcus* in the BPD group at birth might be associated with the severe BPD group.

After treatment, no significant difference in microbial diversity was observed between the BPD group and the control group. Some studies have the same view [24], yet not all studies have detected these differences [25,26]. The microbial evolution of the gut exhibits substantial variability within the early life critical window, which commences with the vertical transmission of microorganisms from mother to fetus and persists until the first year of life [27]. During this period, environmental factors such as feeding method, oral probiotic supplementation and antibiotic usage can significantly impact microbial structure and community composition; however, these effects tend to decline over time. The initial similarity of the intestinal communities in the control group was relatively low at birth, but gradually increased over time. These results suggest that the intestinal microbiota of healthy preterm infants continued to progress towards a similar community structure even under the influence of various treatment factors, whereas this trend is impeded by the development of BPD and that differences widen over time.

In term infants, the initial aerobic environment primarily supports aerobes and facultative anaerobes. Subsequently, due to bacterial consumption, intestinal oxygen levels decline rapidly, facilitating the proliferation of strictly anaerobic species from the Actinobacteria,

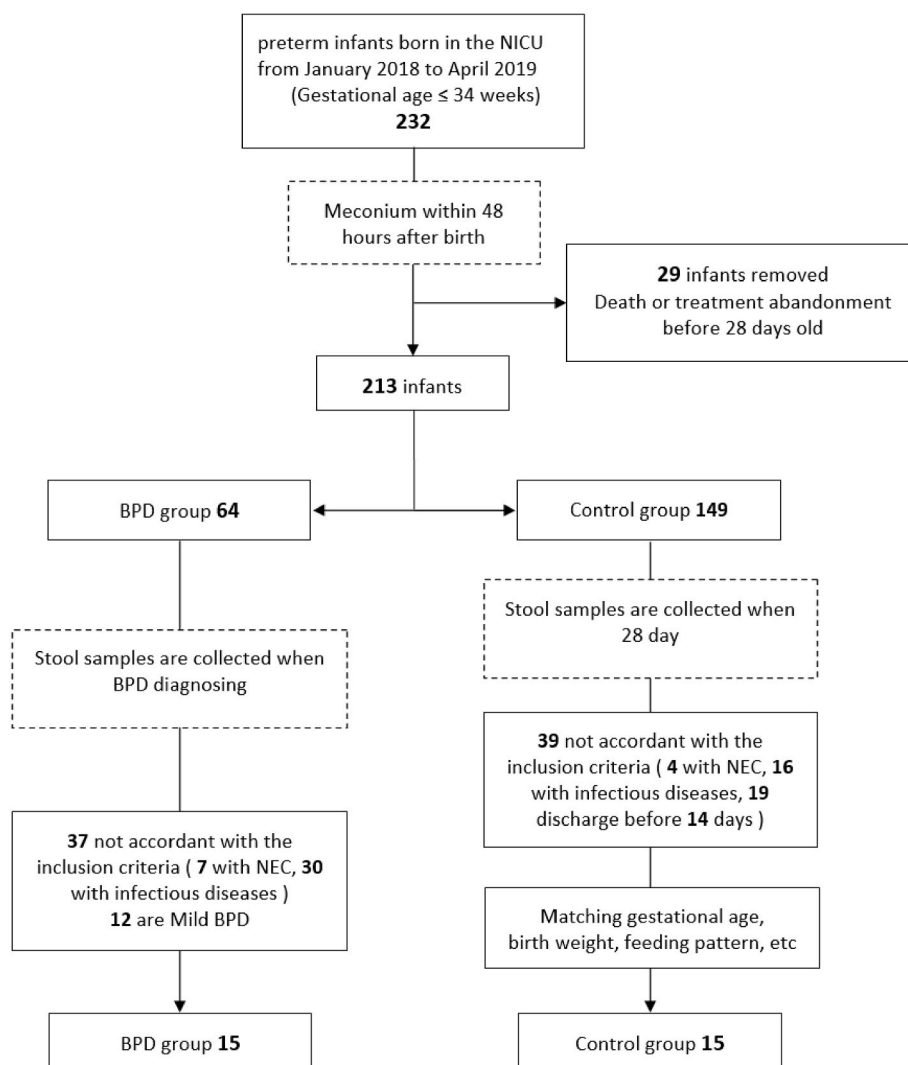


Fig. 4. Flow chart.

Firmicutes, and Bacteroidetes phyla, which gradually establish a typical adult gut microbiome composition [28]. Although the participants in this study were preterm infants, they exhibited changes similar to those in term infants. In both groups, the abundance of *Herbaspirillum* (aerobic bacteria) decreased, while *Escherichia* (facultative anaerobic), *Klebsiella* (facultative anaerobic), and *Bifidobacterium* (strictly anaerobic) gradually increased. This indicates that the turnover of gut microbiota is closely related to oxygen levels. In the comparison of differential microbiota, the dominant taxa in the control group were mainly anaerobic bacteria, whereas the BPD group was dominated by aerobic bacteria. Considering the treatment protocols in our center, there were no significant differences between the two groups in terms of feeding methods, types of oral probiotics, or duration of antibiotic use. However, the BPD group had a longer duration of invasive ventilator use. Prolonged oxygen therapy may delay the transition of gut microbiota towards anaerobic dominance. The proliferation of gut microbiota interacts with the intestinal oxygen environment and is associated with the occurrence and progression of BPD. The current protocol for modulating the gut microbiota involves oral probiotics. Previous studies have shown that *Bifidobacterium* can reduce inflammation and oxidative stress in BPD [29]. The oral probiotics used in our center contain *Bifidobacterium*, but there was no significant difference in the abundance of *Bifidobacterium* between the two groups. In contrast, the abundance of *Lactobacillaceae* was higher in the control group than in

the BPD group. *Lactobacillaceae*, belonging to the phylum Firmicutes, has various functions, including maintaining microbial balance, lowering cholesterol levels, enhancing the immune response, and promoting nutrient absorption. An animal study demonstrated that administering *Lactobacillus plantarum* by gavage for two weeks significantly increased anti-inflammatory metabolites in BPD rats, improved intestinal permeability, reduced lung injury, and enhanced lung development [30]. Therefore, modulating gut homeostasis through probiotics may have beneficial effects in alleviating the progression of BPD.

Although this study has proposed the differential flora, research on the association between BPD and the intestinal flora remains scarce. Besides exploring postnatal factors, maternal factors are of crucial importance in shaping the microbial characteristics of infants with BPD. Maternal factors include dietary patterns, nutritional status, and maternal diseases during pregnancy, as well as the administration of antibiotics and other medications [31]. Moreover, prospective studies have revealed distinct bacterial profiles in the amniotic fluid of infants with different degrees of BPD severity [18]. Based on the maternal medical history, there were no significant differences among the groups. However, further investigation into other maternal factors is necessary. The intrauterine contact between the fetus and the mother mainly occurs through the placenta, umbilical cord, and amniotic fluid, which promotes the development of swallowing function and the gastrointestinal system. It has been found that meconium microflora is similar to

the microbial community in the maternal amniotic fluid and placenta [31,32]. Animal experiments have suggested a potential relationship between the microbes in the amniotic fluid/placenta and the maternal gut microbiota [33]. Therefore, it can be considered that maternal gut microbiota serves as a bridge influencing meconium microbiota composition. Studies have shown that mothers with inflammatory bowel disease (IBD) exhibit low diversity of gut microbiota in early pregnancy; and their offspring also show low diversity within the first week after birth [34], further confirming the impact of maternal gut microbiota on offspring health. In-depth exploration of structural characteristics of maternal gut microbiota during pregnancy, along with the monitoring of overall health status, is of great significance for the prevention of BPD.

The present study has limitations: there were restricted longitudinal controls within the same patient cohort. Assessing biodiversity scales based on relative rather than absolute value may present difficulties. The gut microbiota is affected by multiple factors, including population characteristics, environmental conditions, and treatment protocols. Consequently, further research is required to validate the reliability of these findings. For further studies, we will enlarge the sample size to perform a more comprehensive analysis of infants with mild, moderate, and severe BPD, and continue to investigate the effects of maternal factors on the intestinal microbiota of infants with BPD.

5. Conclusions

In summary, preterm infants with moderate and severe BPD tend to exhibit reduced gut microbiota diversity at birth, with specific taxa such as *Bacillus* and *Staphylococcus* potentially having a role in the onset and progression of BPD. The establishment of gut microbiota in preterm infants is likely influenced by maternal factors, including maternal gut microbiota, amniotic fluid composition, and placental microbiota. The gut microbiota in BPD infants appears to interact with the intestinal oxygen environment; and the use of probiotics may potentially improve BPD by adjusting gut microbial characteristics. These findings regarding the diversity and specific flora of gut microbiota potentially offer insights into the pathogenesis of BPD, but further research is required to confirm and clarify these associations.

Contributions

Xin Fu, Yan fang Jang and Hui Wu designed the study. Xin Fu, Yan fang Jang, Dan Dang, Xintong Hu, conducted the research. Xin Fu, Dan Dang, Xintong Hu acquired and analyzed the data. Xin Fu wrote the first draft of the manuscript. Xin Fu, Yan fang Jang and Hui Wu critically reviewed the manuscript. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare no conflict of interest.

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