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Original Research

Delivery Mode Shapes Distinct Early Gut Microbiota Dynamics in Antibiotic-Exposed Preterm Infants

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Abstract

Background: Delivery mode is a key factor that modulates gut microbial colonization in full-term infants during early life. However, in preterm infants who receive routine postnatal antibiotic exposure, it remains unclear whether and how delivery mode influences initial gut colonization. This study aimed to characterize early gut microbiota succession within the first 72 hours of life in antibiotic-exposed preterm infants and to define the impact of delivery mode on this process. **Methods:** A total of 76 preterm infants (52 delivered by cesarean section [CS], 24 by vaginal delivery [VD]) were recruited from a neonatal intensive care unit (NICU) between February 2020 and April 2022. All infants received empirical β-lactam antibiotics within 24 hours of birth for a minimum of 3 days. Fecal samples were collected longitudinally on postnatal day 1 (D1) and 3 (D3), for a total of 140 samples. 16S rRNA gene sequencing was used to analyze microbiota diversity, composition, differential taxa, and network characteristics. **Results:** Alpha diversity showed no significant intergroup differences, although temporal trends differed. Infants delivered by CS exhibited an increase in the Chao1 index, whereas those delivered by VD showed a modest decrease. Beta diversity analysis revealed no significant intergroup differences in overall microbiota structure at D1 or D3 (p = 0.114 and 0.686, respectively). However, the CS group underwent significant temporal changes in community structure between D1 and D3 (p = 0.004), whereas the VD group did not (p = 0.536). By D3, compared with the VD group, the CS group exhibited higher relative abundances of Firmicutes_D and enrichment of opportunistic pathogens, while beneficial genera remained consistently low. In contrast, at D1, the VD group had higher relative abundances of Fusobacteriota and Bacteroidota than the CS group, with *Fusobacterium_C* and *Prevotella* following the same trend at the genus level. In the VD group, the relative abundances of *Bifidobacterium* and *Bacteroides_H* increased by D3, whereas those of *Lactobacillus* and *Ligilactobacillus* decreased modestly. Microbial networks were more complex in the VD group at D1 but shifted toward greater complexity in the CS group by D3. **Conclusions:** Delivery mode shapes distinct early gut microbiota dynamics in antibiotic-exposed preterm infants. CS led to a succession pattern characterized by high-level colonization by opportunistic pathogens, whereas VD initially conferred a greater abundance of maternally derived beneficial taxa; however, this advantage diminished over time, likely due to limited maternal microbial transmission and antibiotic exposure.

Keywords: preterm infants; gut microbiota; delivery mode; antibiotic exposure

1. Introduction

As a "functional microbial organ", the gut microbiota plays a pivotal role in the growth and development of preterm infants [1]. An early, healthy microbiota establishes a critical defensive barrier, maintains intestinal mucosal integrity, prevents pathogenic colonization, and reduces the short-term risks of necrotizing enterocolitis (NEC) and late-onset sepsis (LOS) [2,3]. In the long-term, it may also decrease susceptibility to allergic diseases and metabolic syndrome [4,5]. Conversely, microbial dysbiosis can induce the secretion of proinflammatory factors (e.g., interleukin-6, interleukin-8) [6], as well as increase the risks of feeding intolerance and NEC [6,7,8]. Notably, preterm infants frequently receive antibiotics (e.g., penicillins, cephalosporins) for infection prophylaxis or treatment [9]. Such interventions not only directly eradicate susceptible beneficial bacteria (e.g., *Lactobacillus*) but also favor the enrichment of drug-resistant pathogens (e.g., methicillin-resistant *Staphylococcus* [MRS]) [10], thereby significantly disrupting natural microbial colonization and succession. A recent systematic review confirmed that antibiotic exposure in the neonatal intensive care unit (NICU) setting can markedly alter the intestinal microbiota colonization patterns in preterm infants [11]. Therefore, clarification of microbial colonization patterns in preterm infants under antibiotic exposure holds important scientific and clinical value for the optimization of microecological management strategies.

Delivery mode is regarded as one of the most influential factors shaping the neonatal gut microbiota during early life [12,13,14]. In full-term infants, delivery by cesarean section (CS) disrupts maternal transmission of beneficial *Bacteroides* and facilitates high-level colonization by healthcare-associated opportunistic pathogens (e.g., *Enterococcus*, *Enterobacter*, and *Klebsiella*), taxa that typically harbor higher abundances of antibiotic resistance genes (ARGs) [12]. In contrast, vaginal delivery (VD) enables full-term infants to acquire microbiota (e.g., *Lactobacillus*, *Prevotella*) through exposure to the maternal birth canal [15]. The lactic acid produced by these taxa maintains a low intestinal pH, inhibiting the colonization by opportunistic pathogens [16]. Some evidence suggests that the maternal rectal microbiota is also a key source of neonatal gut microbiota colonization [17,18]. Notably, delivery mode significantly impacts the stability of subsequent microbial colonization. In fact, Bacteroides can be detected in CS full-term infants during the first week of life but are largely lost by the second week due to failure to establish sustained colonization, whereas VD infants maintain a 58% rate of sustained Bacteroides colonization [17].

However, findings from full-term infants cannot be readily generalized to preterm infants. The composition and succession trajectory of the preterm gut microbiota are markedly distinct from those of full-term infants [19]. Further complicating this issue, preterm infants are not only commonly exposed to postnatal antibiotics, but their mothers often exhibit qualitative alterations in rectal and vaginal microbiota. These factors add uncertainty and heterogeneity to early microbial colonization and its regulatory mechanisms. The vaginal microecology of mothers of preterm infants is significantly altered: core beneficial taxa (e.g., *Lactobacillus crispatus*) are reduced, disease-associated microbiota (e.g., *Gardnerella*, *Corynebacterium*) are enriched, and microbial stability is impaired [20,21,22]. Maternal rectal microbiota also shows qualitative impairments: *Bacteroides* abundance is 30%–40% lower than in mothers of full-term infants (12%–15% vs. 7%–9%), and *Bifidobacterium* abundance is similarly reduced by 25%–30% [18]. These challenges may compromise the efficiency and stability of delivery mode-driven vertical microbial transmission in preterm infants. Moreover, the first hours to days after birth represent a critical window for gut microbiota establishment and succession in early life, processes that exert profound effects on host immune system development and long-term health [23,24,25]. Unfortunately, few systematic studies have examined gut microbial characteristics in preterm infants within the first 72 hours after birth [26,27].

Based on this background, this study utilized 16S rRNA gene sequencing to longitudinally monitor preterm infants who received routine postnatal antibiotic treatment, with a focus on gut microbiota succession within the first 72 hours of life. The aim was to elucidate the potential effects of delivery mode (CS, VD) on the diversity, composition, and dominant taxa of early gut microbiota in this population. By defining associations between delivery mode and microbial colonization characteristics under antibiotic exposure, this study provides a preliminary theoretical foundation for the development of individualized microecological intervention strategies for preterm infants.

2. Materials and Methods

2.1 Recruitment of Subjects

Preterm infants (gestational age <36+0 weeks) admitted to the NICU at the Women and Children's Hospital of Ningbo University in Ningbo, China, between February 2020 and April 2022 were enrolled in this study. All infants received empirical intravenous penicillin sodium within 24 hours of birth for at least 3 consecutive days, and no other antimicrobial agents were administered. Mothers received either no antibiotics or ampicillin-sulbactam sodium within 1 week prior to delivery. All study infants were exclusively fed standard preterm formula without supplementation with commercial human milk oligosaccharides (HMOs), including 2′-fucosyllactose (2′-FL). Infants were excluded if they met any of the following criteria: (i) gestational age ≥36+0 weeks; (ii) severe gastrointestinal malformations or other major congenital anomalies; (iii) grade III intraventricular hemorrhage (IVH) or severe birth asphyxia; or (iv) early-onset sepsis (EOS).

All infants received standardized management, including nutritional and respiratory support (invasive ventilation or nasal continuous positive airway pressure [nCPAP]) tailored to clinical severity. Concurrent conditions were managed according to the Guidelines for the Management of Preterm Infants [28]. Infants were assigned to two groups based on delivery mode: the CS group and the VD group. Written informed consent was obtained from the parents or guardians of all infants prior to enrollment. The study protocol was approved by the Medical Ethics Committee of the Women and Children's Hospital of Ningbo University (registration number: EC2019-024). Due to recruitment challenges specific to preterm infants, the sample size was determined based on clinical feasibility during the study period.

2.2 Clinical Data Collection

Collected data included gestational age, birth weight, sex, perinatal history (including delivery mode, prenatal antibiotic use, asphyxia, preterm premature rupture of membranes [PPROM], and other perinatal events), and respiratory support modality within 24 hours of birth. In this study, prenatal antibiotic use was defined as prophylactic intravenous administration of ampicillin-sulbactam sodium to mothers within 1 week pre-delivery. Asphyxia was defined as follows: (i) mild: Apgar score ≤7 at 1 minute or ≤7 at 5 minutes, with umbilical arterial blood pH <7.2; (ii) severe: Apgar score ≤3 at 1 minute or ≤5 at 5 minutes, with

umbilical arterial blood pH <7.0. Clinical data were extracted from electronic medical records.

2.3 Fecal Sample Collection

Fecal samples were collected from diapers with sterile collection devices on postnatal days 1 (D1) and 3 (D3) for all infants. Samples were stored at –80 °C until processing. As samples were collected from diapers, some had insufficient volume for downstream analysis. To maximize the number of qualified samples, all enrolled infants were required to have at least one time point with a fecal sample of sufficient volume for analysis.

2.4 High-Throughput Sequencing of the 16S rRNA Gene

Genomic DNA was extracted from fecal samples using the Tiangen Fecal DNA Extraction Kit (Tiangen Biotech, Beijing, China; batch No. DP328), in accordance with the manufacturer's protocol. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Polymerase chain reaction (PCR) amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, Jiangsu, China; batch No. N411) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA; batch No. P7589). After quality control, sequencing was performed on a NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Each sample was sequenced to a depth of ≥50,000 valid reads.

2.5 Sequencing Data Analysis

Raw sequencing data were demultiplexed to assign reads to each sample. Reads were merged with FLASH (Johns Hopkins University, Baltimore, MD, USA; version 1.2.11), filtered using Fastp (Chinese Academy of Agricultural Sciences, Shenzhen, Guangdong, China; version 0.23.4), and screened for chimeric sequences with USEARCH (Drive5, Santa Barbara, CA, USA; version 11.0.667); identified chimeras were removed to obtain high-quality data. The DADA2 module in QIIME2 (https://docs.qiime2.org) was used for noise reduction, and sequences with an abundance <5 were filtered out to generate a table of amplicon sequence variants (ASVs) and their corresponding feature table. The resulting ASVs were aligned against a reference database for taxonomic annotation.

Chao1, Simpson, and Shannon indices were calculated to compare alpha diversity (α-diversity) between groups. Non-metric multidimensional scaling (NMDS) was employed to assess beta diversity (β-diversity). Linear discriminant analysis effect size (LEfSe) software was used to identify taxa, not limited to the species level, with significant intergroup differences. To investigate interspecies microbial interactions across groups, molecular ecological networks were constructed using ASV abundance data derived from 16S rRNA gene sequencing. Species associations were defined by calculating Pearson correlations between ASVs, with thresholds determined by random matrix theory (RMT). Key topological properties of the networks, including nodes, edges, and average degree, were analyzed to infer network complexity and microbial interaction patterns.

2.6 Statistical Analysis

Continuous data were reported as median (M; interquartile range [IQR]) and analyzed with Wilcoxon rank-sum tests. Categorical variables were compared using chi-square tests, and Fisher's exact test was applied when expected cell counts were <5. All statistical analyses were conducted using SPSS v25.0 (IBM, Armonk, NY, USA) and R software (R Core Team, Vienna, Austria), with $p < 0.05$ considered statistically significant. Basic statistical descriptions were performed using SPSS v25.0, whereas R was used for visualization of microbial community structures. Specifically, the R packages applied were ggplot2 (version 3.3.5; Posit, PBC, Boston, MA, USA) and vegan (version 2.6.4; Jari Oksanen & vegan development team, Oulu, Finland) for α-diversity calculations and NMDS analyses; and ggplot2 (version 3.3.5) for LEfSe visualizations.

3. Results

3.1 Characteristics of the Study Participants

A total of 76 preterm infants (47 males and 29 females) were enrolled, with a median gestational age of 30+5 weeks (IQR: 28+5 to 32+2; range: 27+2 to 35+4) and a median birth weight of 1400 g (IQR: 1232.50 to 1682.50). Based on delivery mode, 52 infants were assigned to the CS group and 24 to the VD group. A total of 140 stool samples were collected: 96 from the CS group (50 at D1 and 46 at D3) and 44 from the VD group (24 at D1 and 20 at D3).

Comparisons of baseline clinical characteristics between the two groups, including sex, birth weight, gestational age, prenatal antibiotic use, PPROM, abnormal amniotic fluid, placental abnormalities, asphyxia, Apgar score, and postnatal invasive respiratory support, revealed no statistically significant differences (all $p > 0.05$, Table 1). The study flowchart is shown in Fig. 1.

3.2 α-Diversity and β-Diversity of Gut Microbiota

A comparative analysis of gut microbial α-diversity in preterm infants by delivery mode at D1 and D3 is presented in Fig. 2. In the CS group, species richness (Chao1 index) showed a trend to increase slightly from D1 to D3, whereas the VD group exhibited a slight decrease over the same period. For species evenness (Simpson index) and overall diversity (Shannon index), both groups showed an increasing trend from D1 to D3, with a greater increase in the CS group than in the VD group. However, no significant intergroup difference was observed.

Fig. 3 shows the β-diversity of the gut microbial community in the two groups. At both D1 and D3, sample distri-

Table 1. Characteristics of infants based on delivery mode (CS vs. VD).

Characteristic	CS group (n = 52)	VD group, (n = 24)	*p*-value
Sex (Male/Female)	35/17	12/12	0.149
Birth weight, g (M, P25~P75)	1810 (1520~2150)	1450 (1250~1900)	0.862
Gestational age, weeks (M, P25~P75)	33+1 (32+2~34+0)	30+5 (28+5~31+5)	0.075
Prenatal antibiotic use	16 (30.77)	12 (50.00)	0.106
PPROM	19 (36.54)	8 (33.33)	0.786
Abnormal amniotic fluid	9 (17.31)	7 (29.17)	0.238
Placental abnormality	15 (28.85)	9 (37.50)	0.451
Mild asphyxia	4 (7.69)	4 (16.67)	0.253
1-minute Apgar score (M, P25~P75)	9.00 (8.00~9.00)	8.00 (8.00~9.00)	0.504
5-minute Apgar score (M, P25~P75)	10.00 (9.00~10.00)	9.00 (9.00~10.00)	0.292
Invasive respiratory support, D1	31 (59.62)	13 (54.17)	0.655

Data are presented as M (interquartile range [IQR]) or number (percentage). CS, cesarean section; VD, vaginal delivery; M, median; PPROM, preterm premature rupture of membranes; D1, postnatal day 1.

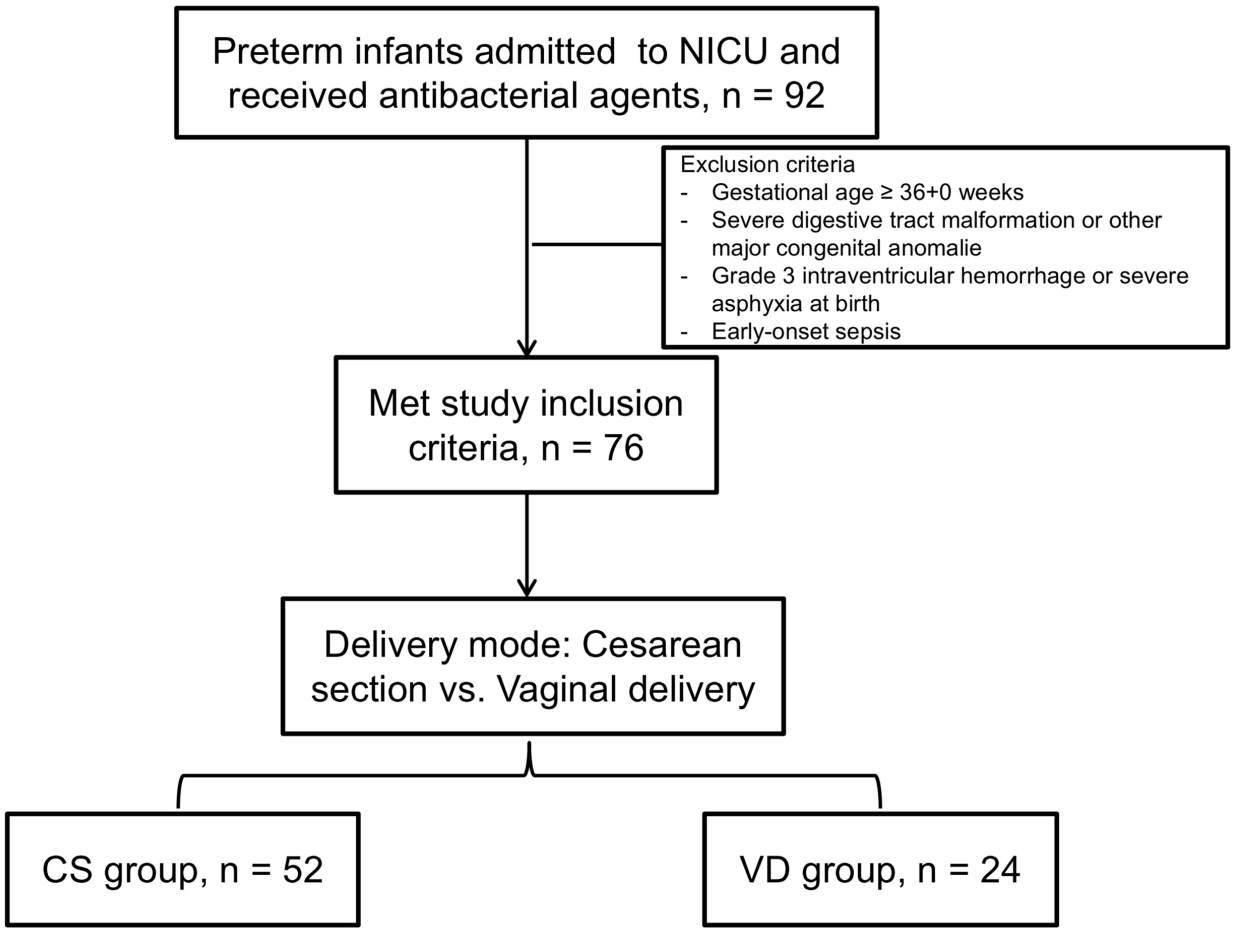

Fig. 1. Study design and participant flowchart. NICU, neonatal intensive care unit.

butions for the CS and VD groups largely overlapped, with no significant intergroup differences (p = 0.114 and 0.686, respectively; Fig. 3A,B). These findings indicate that delivery mode did not significantly alter the overall gut microbiota structure of preterm infants at these time points. However, the CS group exhibited significant temporal changes in gut microbiota structure between D1 and D3 (p = 0.004; Fig. 3C). In contrast, no significant temporal difference was detected in the VD group (p = 0.536; Fig. 3D).

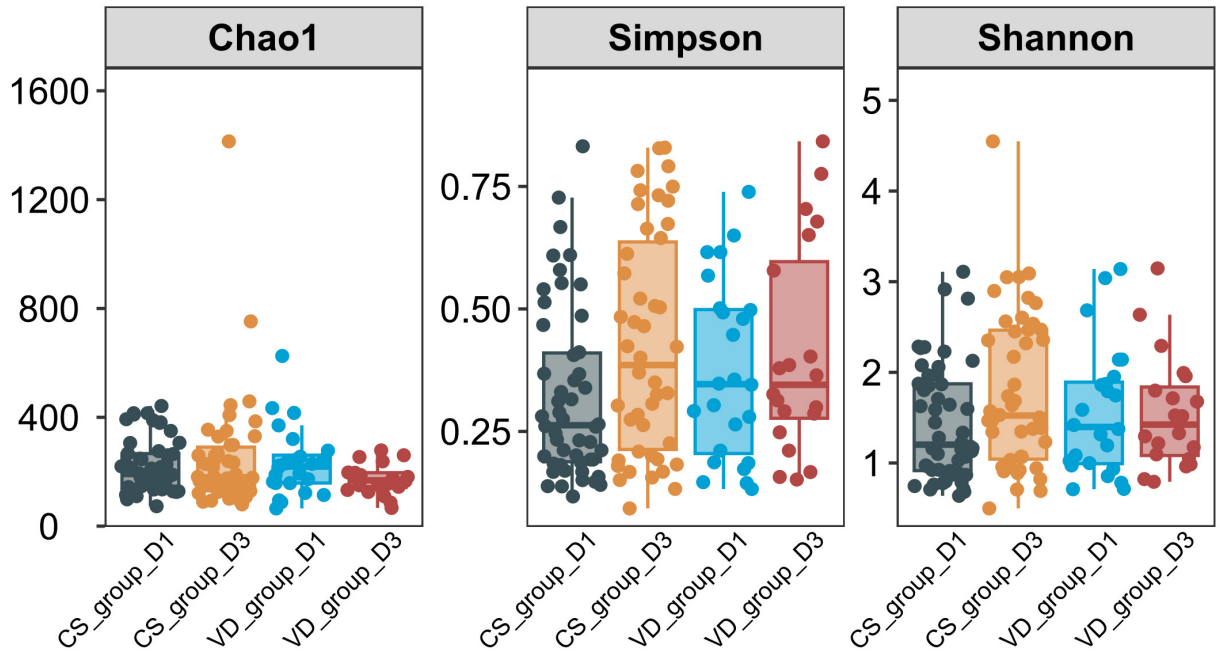

Fig. 2. Distribution of α-diversity indices of gut microbiota in preterm infants by delivery mode at D1 and D3. Species richness (Chao1), species evenness (Simpson), and species diversity (Shannon). The absence of statistical annotations in the figure indicates no significant differences between or within groups (all $p > 0.05$). α-diversity, alpha diversity; D3, postnatal day 3.

3.3 Composition Analysis of Gut Microbiota

At the phylum level, Proteobacteria was the dominant phylum in both groups at D1 and D3. In the CS group (Fig. 4A), the five most abundant phyla at D1 were Proteobacteria (91.18%), Firmicutes_D (6.32%), Firmicutes_A (0.87%), Bacteroidota (0.73%), and Actinobacteriota (0.39%). By D3, the CS group showed a reduction in Proteobacteria abundance (77.29%) and an increase in Firmicutes_D (18.84%), whereas Firmicutes_A (2.28%), Bacteroidota (0.90%), and Actinobacteriota (0.24%) remained among the next most abundant. In the VD group (Fig. 4B), the top five phyla at D1 were Proteobacteria (84.85%), Firmicutes_D (6.88%), Fusobacteriota (3.31%), Bacteroidota (3.18%), and Firmicutes_A (0.91%). At D3, Proteobacteria remained the most abundant phylum (77.97%) in the VD group, followed by Firmicutes_D (13.05%), Bacteroidota (4.68%), Actinobacteriota (1.13%), and Firmicutes_A (1.01%).

At the genus level, temporal shifts in the top five most abundant genera were observed in both groups. In the CS group (Fig. 4C), *Pseudomonas_E* was the most abundant genus at D1 (78.11%), followed by *Sphingomonas_L* (3.34%), *Klebsiella* (3.06%), *Escherichia* (3.04%), and *Ureaplasma* (2.69%). By D3, *Pseudomonas_E* abundance decreased to 60.90%, whereas *Enterococcus_B* (9.47%), *Staphylococcus* (6.75%), *Klebsiella* (5.41%), and *Escherichia* (3.56%) became the next most abundant genera. In the VD group (Fig. 4D), *Pseudomonas_E* also ranked first at D1 (69.70%), followed by *Escherichia* (8.09%), *Sphingomonas_L* (3.62%), *Fusobacterium_C* (3.31%), and *Prevotella* (2.62%). At D3, *Pseudomonas_E* remained dominant (59.73%), and the top five genera were *Escherichia* (8.93%), *Staphylococcus* (6.08%), *Klebsiella* (5.40%), and *Prevotella* (3.84%).

Notably, the relative abundance of *Bifidobacterium* in the CS group exhibited a downward trend, declining from 0.22% at D1 to 0.06% at D3 (Fig. 4C). Furthermore, taxa including *Lactobacillus* and *Bacteroides_H* were consistently detected at very low levels and did not rank among the top 20 most abundant genera in the CS group. In the VD group (Fig. 4D), the relative abundances of *Bifidobacterium* and *Bacteroides_H* increased from 0.08% and 0.05% at D1 to 0.84% and 0.49% at D3, respectively, which indicates a colonization trend. However, the relative abundances of *Lactobacillus* and *Ligilactobacillus* decreased from 0.15% to 0.05% and 0.13% to 0.07%, respectively (Fig. 4D).

3.4 LEfSe Analysis of Differential Enrichment of Gut Microbiota

At the phylum level, no significant intergroup differences in bacterial phylum enrichment were observed at D1. In contrast, at D3 (Fig. 5B), the VD group exhibited significant enrichment of Fusobacteriota. Further analysis of temporal shifts within each group revealed dynamic changes. In the CS group (Fig. 5C), Desulfobacterota_I was significantly enriched at D1, whereas Firmicutes_D became significantly enriched by D3. In the VD group (Fig. 5D), enrichment of Fusobacteria was significantly reduced at D3 compared with D1.

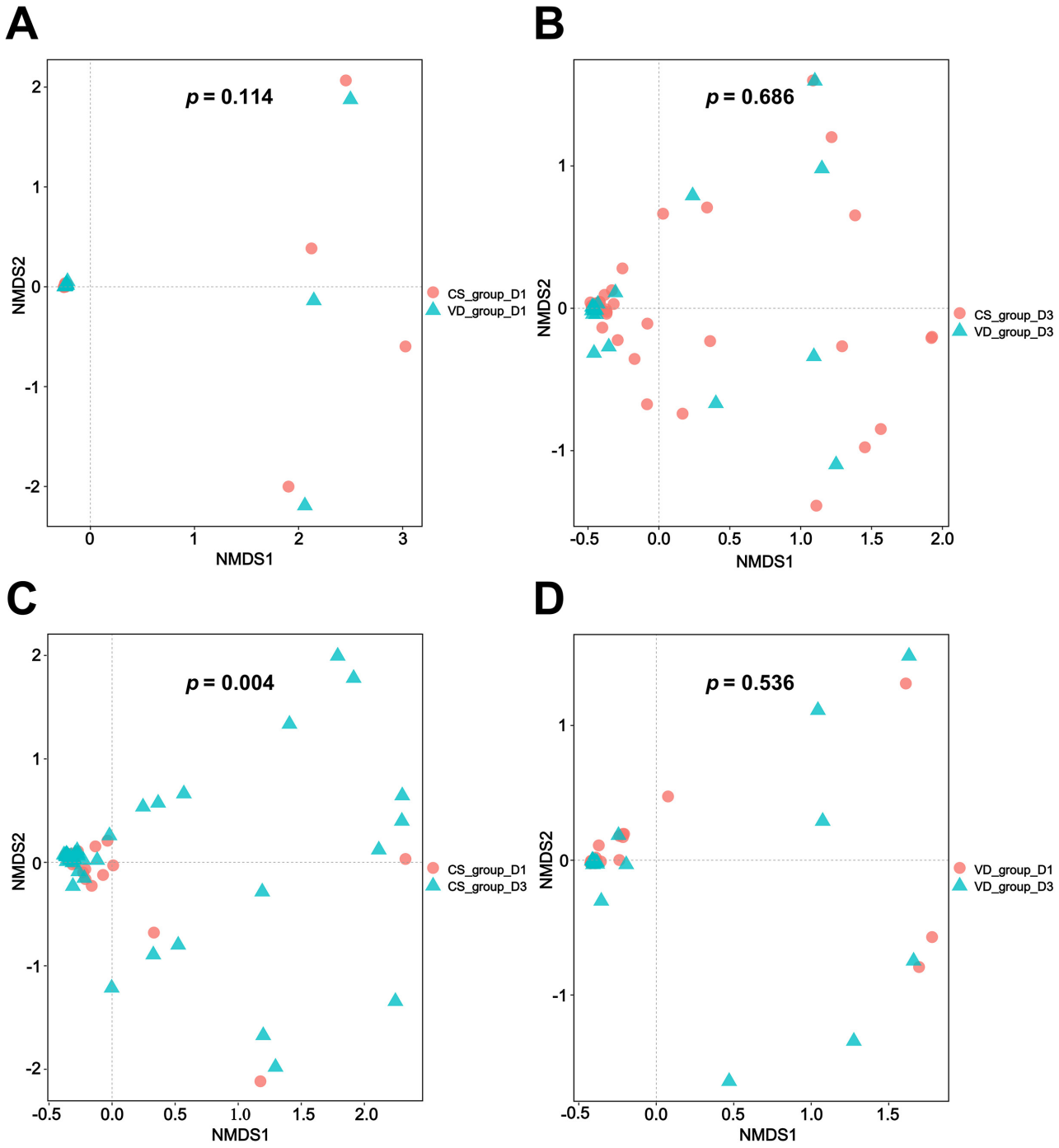

Fig. 3. NMDS of gut microbiota in preterm infants by delivery mode. (A) CS group vs. VD group, D1. (B) CS group vs. VD group, D3. (C) CS group, D1 vs. D3. (D) VD group, D1 vs. D3. NMDS, non-metric multidimensional scaling.

LEfSe analysis at the genus level identified distinct enrichment patterns. At D1 (Fig. 5A), the CS group exhibited significant enrichment of 2 genera (*Muribaculum* and *Cetobacterium_A*), whereas the VD group showed significant enrichment of 17 genera, including *Anaerostipes*, *Sharpea*, and *Pseudorhizobium*. At D3 (Fig. 5B), the CS group displayed significant enrichment of 6 genera, including *Limivicinus*, *Succinivibrio*, and *Sphingomicrobium*, while the VD group showed significant enrichment of 6 genera, including *UBA5232*, *Campylobacter_B*, and *Stenotrophomonas_A*. To assess temporal changes within each group from D1 to D3, additional LEfSe analysis was performed. In the CS group (Fig. 5C), 5 genera were significantly enriched at D1, including *CAG_485*, *Ventrimonas*, and *UBA7173*. At D3, 11 genera were significantly enriched in the CS group, including *Peptococcus*, *Gemella*, and *Angelakisella*. In the VD group (Fig. 5D), 11 genera were significantly enriched at D1, including *Dwaynesavag-*

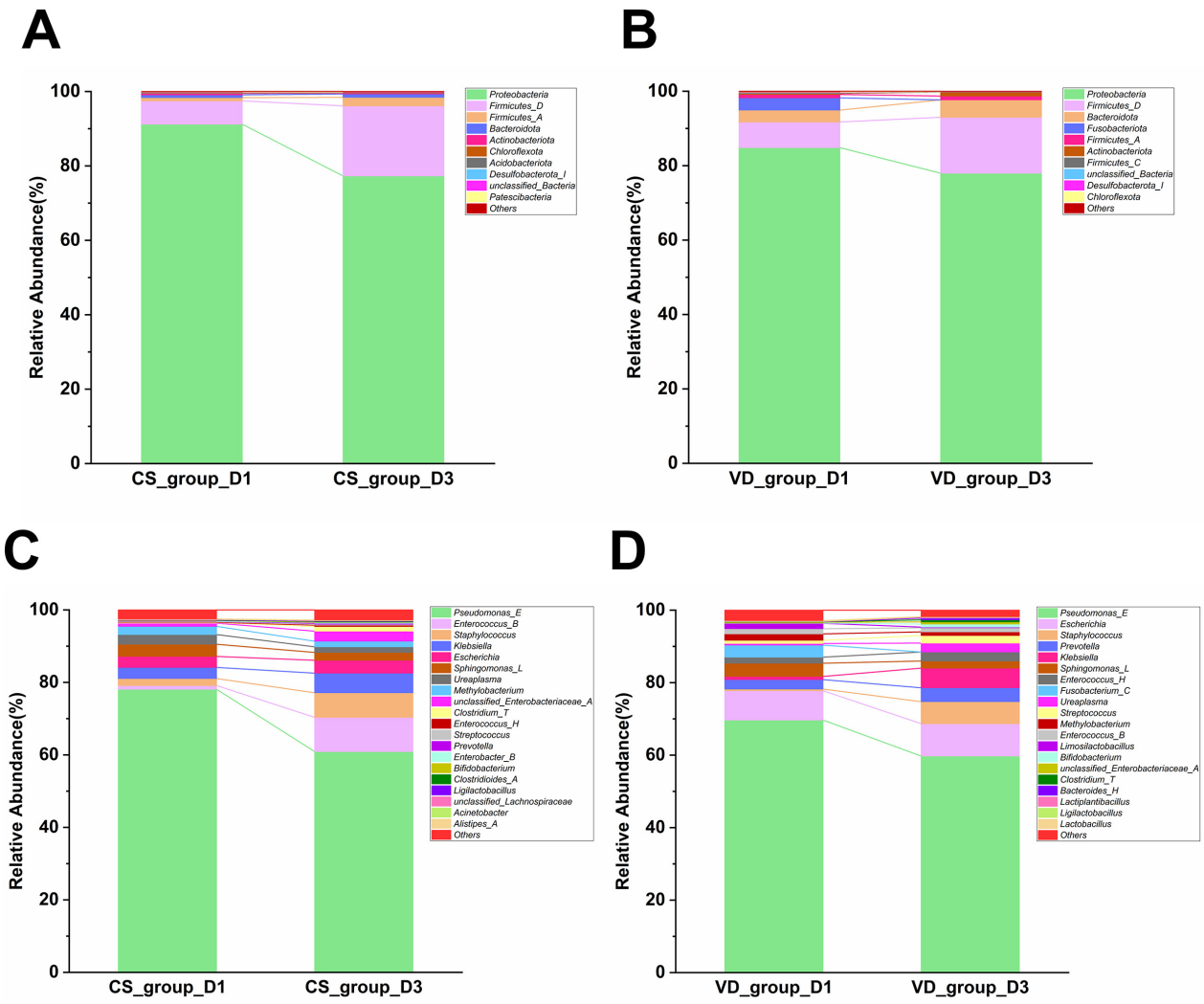

Fig. 4. Distribution of dominant gut bacteria in preterm infants by delivery mode at D1 and D3. (A) Phylum-level relative abundance in the CS group. (B) Phylum-level relative abundance in the VD group. (C) Genus-level relative abundance in the CS group. (D) Genus-level relative abundance in the VD group.

ella, *Alloprevotella*, and *Ligilactobacillus*. At D3, 2 genera, *Aquabacter* and *Cetobacterium_A*, showed significant enrichment.

3.5 Molecular Ecological Network Analysis of Gut Microbiota

Molecular ecological network analysis was performed in both groups (Fig. 6). At D1, the VD group contained 76 nodes, compared with 51 nodes in the CS group, along with more edges (109 vs. 50) and a higher average degree of the microbial network (2.89 vs. 1.96) (Fig. 6A,C). These results indicate that microbial taxa in the VD group exhibited more intensive interactions at D1. At D3, the VD group (85 nodes) retained more nodes than the CS group (61) and had more edges (127 vs. 115) but exhibited a lower average degree of the microbial network (2.98 vs. 3.77) (Fig. 6B,D). These findings indicate that the microbial network in the CS group was more complex at this later time point.

4. Discussion

The colonization and succession of the gut microbiota in preterm infants are complex processes, exhibiting compositional and temporal trajectories that are fundamentally distinct from those of full-term infants [19,29]. Although the effects of delivery mode on the gut microbiota of full-term infants are well characterized, its impact, particularly that of VD, on the gut microbiota of preterm infants remains unclear. Beyond delivery mode, other clinical factors (e.g., duration and type of antibiotic exposure, feeding practices, and disease status) are also known to affect gut microbiome establishment and maturation in preterm infants [30,31]. To minimize potential confounding factors, this study focused on the first 72 hours after birth. During this period, all preterm infants were admitted to the NICU for standardized management, with uniform clinical interventions, including standardized antibiotic prophylaxis and feeding protocols, and relatively consistent environmental exposure. This ap-

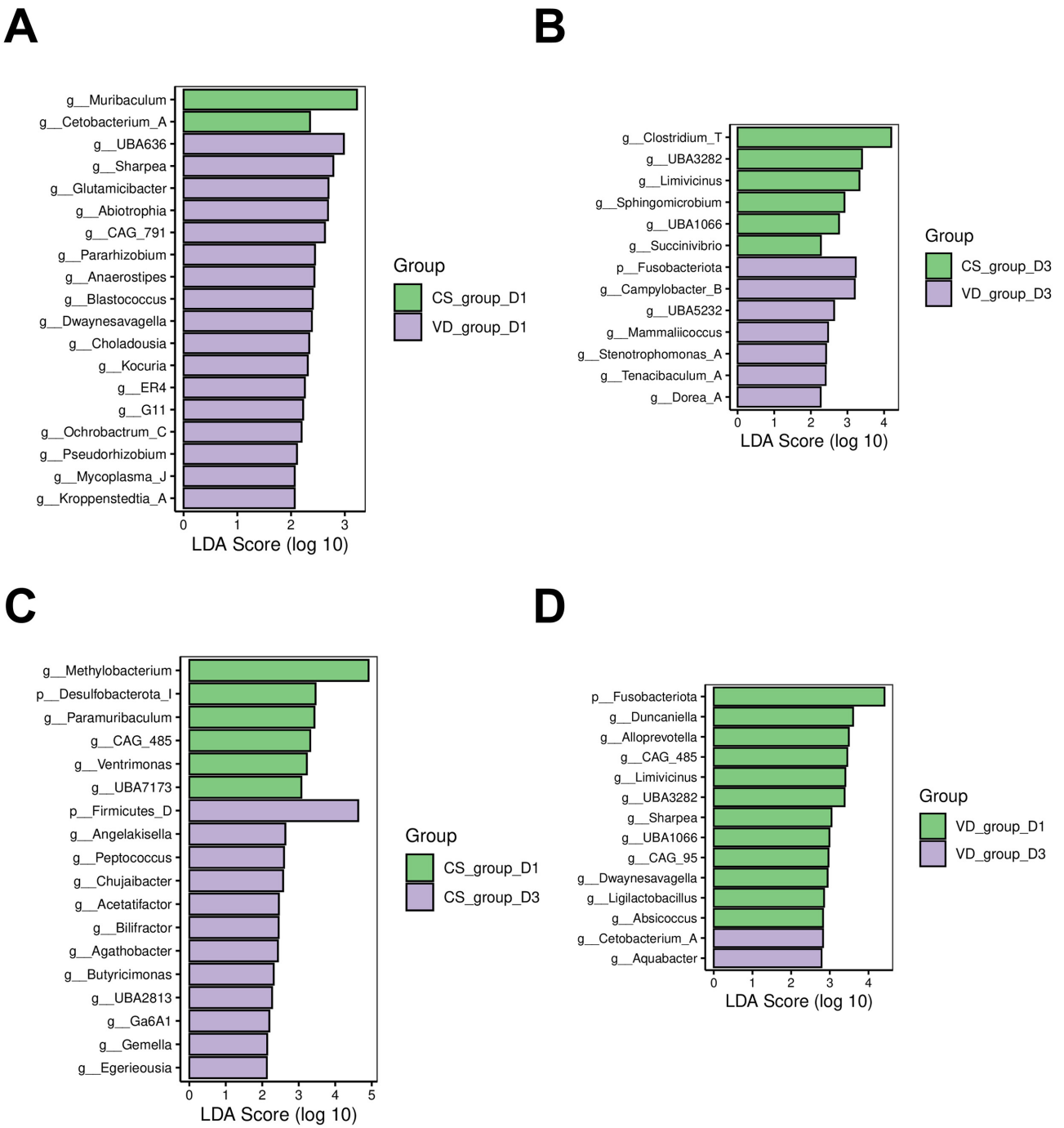

Fig. 5. Gut microbiota taxa at the phylum and genus levels with significant differences in preterm infants by delivery mode, as identified by LEfSe analysis. (A) CS group vs. VD group, D1. (B) CS group vs. VD group, D3. (C) CS group, D1 vs. D3. (D) VD group, D1 vs. D3. LEfSe, linear discriminant analysis effect size.

proach reduced the confounding effects of non-study variables on gut microbiota structure. Furthermore, this period is widely recognized as a "critical window" for initial colonization of the gut microbiota in preterm infants [23,24,25]. During this window, the microbiota is minimally affected by variable clinical interventions and may therefore more accurately reflect delivery mode-mediated colonization characteristics. Leveraging this study design, we conducted a longitudinal dynamic analysis of the gut microbiota in preterm infants and delineated early gut microbial succession patterns in this cohort across different delivery modes.

This study demonstrated that both groups were dominated by the phylum Proteobacteria at D1 and D3, consistent with previously reported gut microbial ecological profiles of preterm infants [32]. At D3, both groups showed

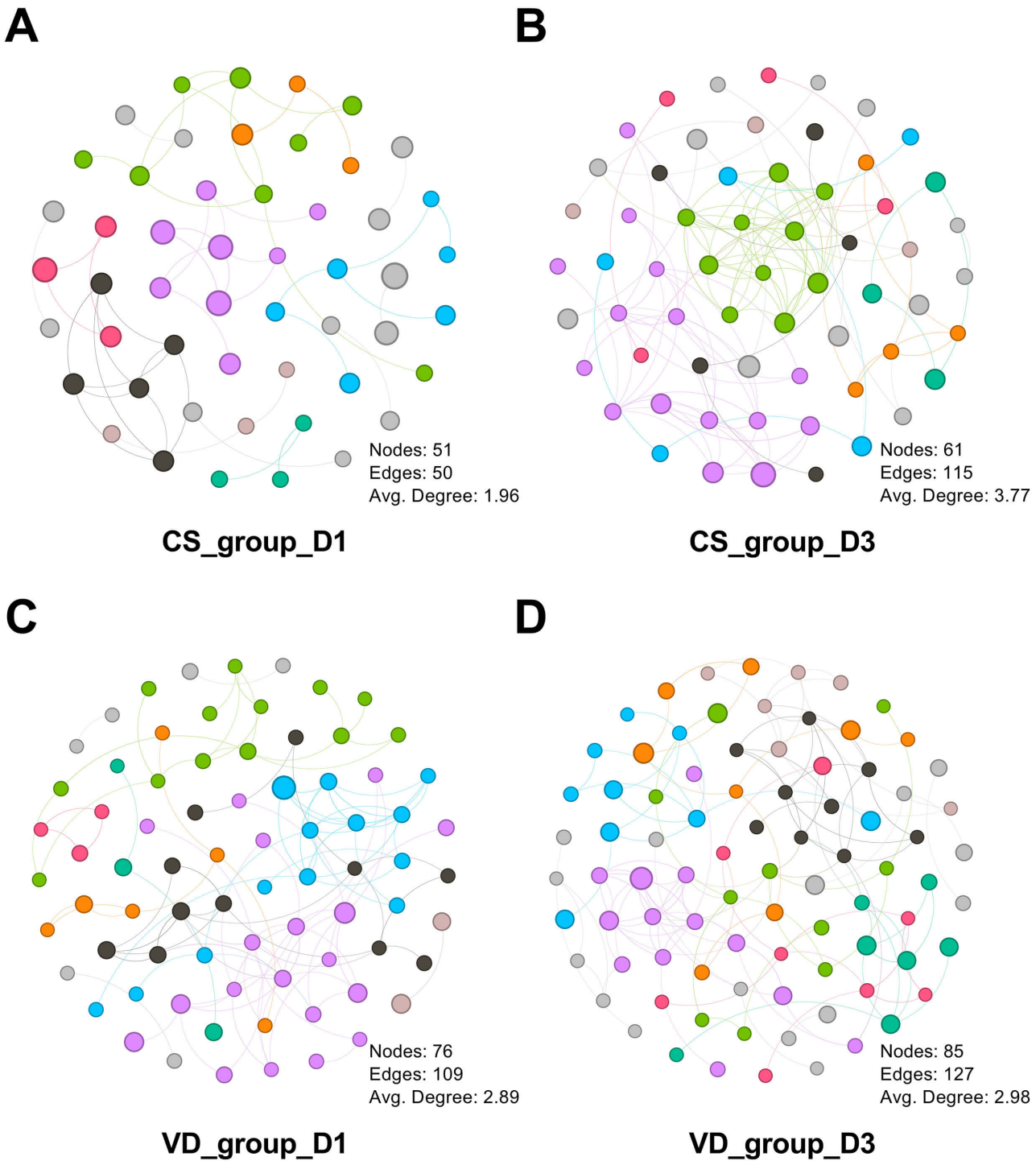

Fig. 6. Molecular ecological network analysis of gut microbiota in preterm infants by delivery mode, showing interactions and network complexity. (A) CS group, D1. (B) CS group, D3. (C) VD group, D1. (D) VD group, D3. Different colored circles stand for different network modules.

high relative abundances of *Pseudomonas_E*, *Escherichia*, *Staphylococcus*, and *Klebsiella*, which underscores the pervasive gut microbiota dysbiosis in preterm infants exposed to antibiotic stress [10]. However, within this shared context, delivery mode drove distinct microbial succession trajectories. The gut bacterial community structure of CS preterm infants underwent a significant structural shift from D1 to D3, with opportunistic pathogenic bacteria such as *Enterococcus_B* exhibiting a trend of enrichment. In contrast, the abundance of *Bifidobacterium* decreased from 0.22% to 0.06%, whereas *Lactobacillus* and *Bacteroides* remained at extremely low levels (≤0.09%). In addition,

LEfSe analysis further showed a notable increase in enriched genera in the CS group over time, from 5 to 11. These findings are somewhat consistent with observations in CS-delivered term infants, which are typically characterized by unstable colonization by beneficial bacteria and expansion of opportunistic pathogens [12]. Compared with CS preterm infants, the VD group exhibited distinct temporal dynamics. At D1, the VD group not only showed higher α-diversity, community stability, and a larger number of enriched genera (17 vs. 2 in the CS group) but also had higher relative abundances of the phyla Fusobacteriota and Bacteroidota than the CS group. The enrichment of *Fusobacterium_C* and *Prevotella* in the VD group is consistent with a previous report [13], clearly reflecting the vertical transmission of maternal microbiota via VD. However, this early advantage was not sustained at D3. Notably, colonization levels of beneficial bacteria (e.g., *Lactobacillus*, *Bifidobacterium*) in the VD group were lower than reference values reported for full-term infants [33]. The relative abundance of *Lactobacillus* and *Ligilactobacillus* decreased from D1 to D3, whereas the abundances of opportunistic pathogens (e.g., *Staphylococcus*, *Klebsiella*) increased concurrently. LEfSe analysis showed a marked decline in the number of enriched genera in the VD group over time (from 11 to 2), with key taxa (e.g., *Ligilactobacillus*, which was significantly enriched at D1) dropping markedly by D3. This finding contrasts with the pattern of persistent dominance of beneficial bacteria typically observed in full-term VD infants [12,13].

Molecular ecological network analysis further revealed distinct differences in interaction patterns between the CS and VD bacterial communities. At D1, the CS group network structure was relatively simple (51 nodes, 50 edges) with an average degree of 1.96, consistent with its limited initial bacterial colonization profile. In contrast, the VD group exhibited a more complex network structure at D1 (76 nodes, 109 edges, average degree of 2.89), reflecting its acquisition of a more diverse initial bacterial community via maternal vertical transmission. By D3, this pattern shifted: the average degree of the CS group network increased substantially, which indicates that its colonizing bacterial genera (e.g., *Enterococcus_B* and *Staphylococcus*) may have developed enhanced synergistic interactions to occupy ecological niches [10]. In contrast, although the VD group maintained some network complexity, it failed to achieve beneficial community restructuring under antibiotic pressure, thus failing to sustain its initially established mutualistic advantage.

This study provides preliminary evidence that, in the context of antibiotic exposure, delivery mode shapes dynamic changes in the gut microbiota within 72 hours after birth in preterm infants, but its underlying mechanisms and phenotypic manifestations likely differ from those in full-term infants. In addition to the inherent physiological immaturity and underdeveloped immune system of preterm infants, "constraints on maternal microbial transmission" and "antibiotic-mediated selection pressure" are proposed as two key factors that further modulate the unique microbial colonization trajectory. Specifically, in CS preterm infants, gut colonization initiates in the absence of maternally derived beneficial bacteria, with the initial microbiota primarily sourced from the infant's skin and the hospital environment, a pattern analogous to that in CS full-term infants [12,13,14]. Against a physiological backdrop of impaired gut barrier function and compromised immune surveillance, antibiotics eliminate sensitive bacteria, create ecological niches for inherently resistant environmental bacteria or opportunistic pathogens [10], and thereby enable the development of a succession pattern characterized by the prevalence of these taxa. For VD preterm infants, the maternally transmitted microbial "seed bank" exhibits suboptimal quality [18,20,21,22]. This limitation, together with the immature gut microenvironment and broad postnatal antibiotic exposure, further diminishes the already constrained colonization potential of maternally derived beneficial bacteria. This ultimately hinders the development of the ideal "maternal transmission-stable colonization" trajectory observed in full-term infants [17,18]. Based on these findings, we propose targeted strategies. For CS preterm infants, in addition to probiotic supplementation, we recommend prioritizing dynamic monitoring of opportunistic pathogens and optimization of antibiotic regimens [34,35]. For VD preterm infants, we suggest prioritizing early probiotic supplementation to offset the insufficiency of beneficial bacteria, thereby suppressing proliferation of opportunistic pathogens and preserving gut barrier integrity [36]. It is critical to emphasize, however, that these interventional strategies are derived exclusively from the preliminary findings of the present study, and their clinical efficacy and safety warrant further validation via large-scale prospective trials.

Limitations

While this study provides valuable insights, several limitations warrant consideration. First, due to the single-center design and limited sample size, the study does not support meaningful subgroup analysis (e.g., by CS category or finer strata of gestational age), and the generalizability of the findings requires validation in more heterogeneous cohorts. Second, this study did not characterize the functional profiles of microbial ARGs, nor did it assess associations between microbial characteristics and clinical outcomes. Third, the presented data are cross-sectional and capture only the initial 72-hour period after birth. Although a longitudinal follow-up of this cohort is planned to track microbial dynamics over the first 2 months of life, the current analysis does not inform longer-term succession. Nevertheless, these limitations delineate clear directions for future research. Expansion of the sample size through multicenter collaboration, along with integration of metage-

nomic sequencing, ARG profiling, and long-term clinical follow-up, will support a more systematic evaluation of the impact of delivery mode on gut microbiota colonization dynamics and associated health outcomes in preterm infants.

5. Conclusions

This study demonstrates that, under antibiotic exposure, delivery mode drives distinct gut microbial succession trajectories in preterm infants within the first 72 hours of life. CS preterm infants exhibited a sequential pattern marked by high colonization by opportunistic pathogens, whereas VD preterm infants, despite initial acquisition of maternally derived beneficial bacteria, underwent depletion of these taxa and did not sustain their initial colonization advantage. This insight establishes a theoretical basis for understanding the mechanisms underlying early gut microbial establishment in preterm infants and informs the development of targeted microbial interventions.

Availability of Data and Materials

The sequence data generated and analysed during the current study are available in the NCBI Sequence Read Archive (SRA) repository (BioProject ID: PRJNA1406820). The data that support the findings of this study are available from the corresponding author on reasonable request.

Author Contributions

CY, HZ and CZ designed the research study. YX, QL, LL and HY performed the research. CY and HZ analyzed the data. CY, QL and CZ supported the research process and contributed to funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Ethics Committee of Women and Children's Hospital of Ningbo University (Ethic Approval Number: EC2019-024). Written informed consent was obtained from the parents or legal guardians of all enrolled premature infants.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used DeepSeek in order to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG49063.

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