Dominant bacteria and influencing factors of early intestinal colonization in very low birth weight infants: A prospective cohort study

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Abstract

Background: The intestine of newborns is colonized by bacteria immediately after birth. This study explored dominant bacteria and influencing factors of early intestinal colonization in the early life of very low birth weight infants (VLBWI).

Methods: We enrolled 81 VLBWI and collected anal swabs at 24 h, 7th, 14th and 21st day after birth. We conducted bacterial culture for anal swabs, then selected the colony with obvious growth advantages in the plate for further culture and identification. Afterward, we analyzed the distribution and influencing factors of intestinal dominant microbiota combined with clinical data.

Results: A total of 300 specimens were collected, of which 62.67% (188/300) had obvious dominant bacteria, including 29.26% (55/188) Gram-positive bacteria and 70.74% (133/188) Gram-negative bacteria. The top five bacteria with the highest detection rates were Klebsiella pneumoniae, Escherichia coli, Enterococcus faecium, Enterococcus faecalis and Serratia marcescens. Meconium-stained amniotic fluid and chorioamnionitis were correlated with intestinal bacterial colonization within 24 h of birth. Mechanical ventilation and antibiotics were independent risk factors affecting colonization. Nosocomial infection of K. pneumoniae and S. marcescens was associated with intestinal colonization. The colonization rates of K. pneumoniae, E. coli, E. faecium, and E. faecalis increased with the birth time.

Conclusions: The colonization rate in the early life of VLBWI increased over time and the predominant bacteria were Gram-negative bacteria. Meconium-stained amniotic fluid and chorioamnionitis affect intestinal colonization in early life. Mechanical ventilation and antibiotics were independent risk factors for intestinal bacterial colonization. The nosocomial infection of some bacteria was significantly related to intestinal colonization.

Keywords
Intestinal bacterial colonization, neonatal intensive care unit, nosocomial infection, very low birth weight infants
1 | INTRODUCTION

Studies have shown that a large number of microorganisms are colonized in the human body, most of which exist in the gut. There are about $10^{12}$ bacteria in the gut, which is the most important, enormous and complex microbiota in the human body. The gut microbiota plays a vital role in maintaining normal metabolism and physiological functions of the human body and is closely related to many diseases such as infectious diseases, digestive system diseases, nervous system diseases, metabolic diseases, autoimmune diseases and so on.

Premature infants refer to newborns whose gestational ages are less than 37 weeks, and those whose birth weights are <1,500 g are very low birth weight infants (VLBWI). There are more clinical problems and higher mortality in preterm infants, especially VLBWI that are the focus of management of preterm infants. In recent years, with the improvement of perinatal medicine and neonatal intensive care treatment, the survival rate of VLBWI has increased significantly. The development of infants’ gut microbiota is related to cognition, metabolism, immune system and nervous system, gradually forming a diversified, interrelated and relatively stable signal network. This dynamic process is influenced by various factors, such as delivery mode, gestational age, feeding practices, environmental microbial exposures, diseases and pharmaceuticals. VLBWI, as a special group of preterm newborns, may have a different intestinal microbiological pattern from normal newborns due to the following complex factors: admission to neonatal intensive care unit (NICU) immediately after birth, isolation from mother, the delayed establishment of intestinal feeding, antibiotics and invasive operations. Once the microbiological imbalance occurs in the vulnerable, it will affect the development of the gut, brain and immune system and even lead to necrotizing enterocolitis (NEC), sepsis, white matter damage, neurodevelopmental disorders, metabolic syndrome and long-term atopic diseases. These complications are related to the changes in gut microbiota. Early life (including embryonic and infant years) is a critical period for the colonization and formation of intestinal flora. For VLBWI, the occurrence of any of the above complications will cause a serious blow to them. Therefore, the study of bacterial colonization and influencing factors is of great importance. We collected four anal swabs from 81 VLBWI at 24 h, 7th, 14th and 21st day after birth and carried out bacterial culture to analyze the predominant colonization bacteria in the early life of VLBWI in NICU combination with clinical data.

2 | METHODS

2.1 | Participants

The VLBWI that met the inclusion criteria from 1 March 2020 to 29 February 2021 in the NICU of Ningbo Women's and Children's Hospital were selected as the research objects. Inclusion criteria: those with birth weight less than 1500g were directly admitted to NICU for treatment after delivery. Exclusion criteria: severe congenital malformation, less than 72 h of hospital stay, giving up treatment or death within 2 weeks of hospitalization, family members refusing to study or quitting during hospitalization. All subjects had informed consent signed by their guardians. This study was approved by the medical ethics committee of Ningbo Women and Children’s Hospital and is in line with the declaration of Helsinki.

2.2 | Anal swab collection, bacterial culture and identification

The personnel of NICU collected anal swabs 24 h, 7th, 14th and 21st day after admission. When the infant was critically ill or discharged automatically, we would stop the collection on that day. The method of collecting anal swabs is inserting a cotton swab 1–2 cm into the anus, rotating it gently for 2 weeks, then taking it out and putting it into a sterile test tube.

The collected samples were inoculated into the Blood Agar Plate (MacConkey Agar) by the zoned streak plate method (see below). The medium was placed in a 35°C incubator and incubated for 18–24 h. The colonies with the largest number and the best growth, or a single colony with good growth in zone 3 or 4, were selected for purification. The colonies were identified by the automatic microbial analyzer (Vitek2 Compact600, Bio-Meri, France). The whole process shall be operated in strict accordance with the national manual of clinical examination version 3. If there were no dominant bacteria on the culture dish or no single colony in zone 3 and zone 4, we designated it as “negative.” Anerobic culture was not performed.

The method of zoned scribe separation: The experimenter shall first pick out the specimen with the inoculation ring, apply it to zone 1 of the plate (accounting for 1/4 of the total area of the medium) and draw several streaks, and then draw in zones 2, 3 and 4 in turn. After the streaking of one area, the inoculation ring shall be heated and sterilized, and the streaking of the next area shall be carried out after cooling. Each area shall be contacted 1–3 times from the streaking of the previous area.

2.3 | Diagnostic criteria

The diagnosis of chorioamnionitis was a combined clinical picture and histopathology of the placenta and umbilical cord. Nosocomial infection refers to the infection that occurred 48 h after admission. When infants had symptoms or signs of infection, we collected corresponding samples for bacterial cultures, such as sputum, urine and peritoneal drainage. All amniotic fluids were cultured for bacteria. As for the factor of antibiotics, if antibiotics are used on the day of collection, it is considered “yes”; otherwise it is “no.”
2.4 | Statistical analyses

SPSS 20.0 software was used for data analysis. The Chi-square test was used for comparison, while Fisher’s exact probability method was used when the Chi-square test was not applicable. \( p < 0.05 \) was considered statistically significant.

3 | RESULTS

3.1 | Basic characteristics

In total, 81 VLBWI were registered whose information is summarized in Table 1. The length of hospital stay of all VLBWI was 50.40 ± 16.49 days. It should be noted that we did not collect all samples of 81 infants at 4-time points, due to critical illness, automatic discharge or carelessness of the staff and so on. The specific collection process is shown in Figure 1. A total of 300 anal swabs were collected, of which 188 had obvious dominant bacteria after inoculating on the culture dish.

3.2 | Distribution of dominant bacteria

All bacterial culture results at four-time points are shown in Table 2. We conducted a linear-trend Chi-square test for the positive rates \( (\chi^2 = 125.53, p < 0.001) \). It indicated that the probability of colonization of dominant bacteria in the intestine in VLBW infants increased significantly with time (Figure 2). The bacterial distribution of 188 positive anal swabs was summarized in Table 3. A total of 19 genera were identified. The top five were \( K. pneumoniae \), \( E. coli \), \( E. faecium \), \( E. faecalis \) and \( Serratia marcescens \). Gram-positive (G+) bacteria detected included \( E. faecium \), \( E. faecalis \), \( S. epidermidis \), \( S. agalactiae \), \( L. salivarius \), \( Gemella morbillorum \) and \( Staphylococcus haemolyticus \); Gram-negative (G-) bacteria included \( K. pneumoniae \), \( E. coli \), \( S. marcescens \), \( E. cloacae \), \( E. aerogenes \), \( Acinetobacter baumannii \), \( Raoultella planticola \), \( Kirschner Citrobacter \), \( Enterobacter gergoviae \), \( Klebsiella oxytoca \), \( Comamonas testosterone \) and \( Stenotrophomonas maltophil \). G+ bacteria accounted for 29.26% (55/188) and G- bacteria accounted for 70.74% (133/188). The colonization rates of G+ bacteria \( (\chi^2 = 22.97, p < 0.001) \) and G- bacteria \( (\chi^2 = 51.49, p < 0.001) \) increased over time (Table 4). However, there were some differences. Both colonization rates of G+ and G- were low within 24 h of admission, but G- rapidly increased and was significantly higher than G+ after 7 days of birth, and continued to stabilize at a high level, while G+ bacterial colonization rate increased gradually (Figure 3).

3.3 | Influence of non-infectious factors

We analyzed the non-infective factors including gender, mode of delivery (vaginal delivery or cesarean section), gestational age, birth weight, feeding mode and mechanical ventilation. The results related to all non-infectious factors are shown in Table 5. From the results, mechanical ventilation is the influencing factor of intestinal bacterial colonization.

| TABLE 1 | Basic information for all infants |
|-------------|--------------------------|----------------|
| Gender (cases) | Male | 46 |
| | Female | 35 |
| Mode of delivery (cases) | Cesarean section | 51 |
| | Vaginal delivery | 30 |
| Birth weight (g) | 1238.35 ± 169.49 |
| Gestational age (weeks) | 29.44 ± 1.77 |
| Feeding mode (cases) | Hydrolyzed cow’s milk | 20 |
| | premature infant formula | 61 |
| Mechanical ventilation (cases) | Yes | 50 |
| | No | 31 |
| Antibiotic (cases) | Yes | 78 |
| | No | 3 |
| Chorioamnionitis (cases) | Yes | 14 |
| | No | 67 |
| Amniotic fluid bacterial culture (cases) | Positive | 5 |
| | Negative | 76 |
| Nosocomial infection (cases) | Yes | 26 |
| | No | 55 |
| Necrotizing enterocolitis (cases) | Yes | 6 |
| | No | 75 |

Note: Birth weight and gestational age are expressed in the form of mean ± standard deviation, while other items represent the number of infants.
3.3.1 | Mode of delivery

We analyzed the impact of different modes of delivery on the colonization of intestinal dominant bacteria four times. There was no statistical difference between the two groups and the specific results are shown in Table 6.

3.3.2 | Feeding mode

It should be explained that all subjects began enteral nutrition on the second day after admission. The feeding regimen is divided into premature infant formula and hydrolyzed cow’s milk. Hydrolyzed cow’s milk has lower osmotic pressure and calories than premature infant formula. Different feeding methods had no effect on the detection rate of intestinal dominant bacteria and the data were shown in Table 7.

3.3.3 | Mechanical Ventilation

The detection rate of intestinal dominant bacteria with mechanical ventilation was 18.75% (9/48) while it without mechanical ventilation was 71.03% (179/252). Mechanical ventilation is one of the factors affecting bacterial colonization in VLBWI ($\chi^2 = 47.11, p < 0.001$) (Table 5). The duration of the mechanical ventilation time was $5.00 \pm 5.64$ days. In addition, we analyzed the relationship between the duration of mechanical ventilation and the colonization of dominant bacteria. The result showed that there was no correlation ($p = 0.435$).

3.4 | Influence of infectious factors

The infection factors we analyzed included meconium-stained amniotic fluid (MSAF), maternal chorioamnionitis, nosocomial infection, NEC and antibiotics. The results are shown in Table 8.

3.4.1 | Meconium-stained amniotic fluid

The total detection rate of dominant bacteria of VLBWI was statistically different from MSAF (Table 8). Then we examined the influence of MSAF at different times. Within 24 h after admission, the detection rate in the MSAF group was 60.00% (3/5), while the non-MSAF group was 4.05% (3/74). MSAF has a significant effect on intestinal colonization in VLBWI within 24 h of birth ($p < 0.05$) (Table 9). There was no statistical significance between the detection of intestinal colonization bacteria on the 7th, 14th and 21st day ($p > 0.05$) (Table 9). MSAF is an independent risk factor affecting early intestinal colonization.

3.4.2 | Chorioamnionitis

Although there was no significant difference in the total detection rate of intestinal bacterial colonization between maternal intrauterine...
Comparisons at different times showed that the detection rate in the maternal chorioamnionitis group was significantly higher within 24 h after admission \( (p < 0.05) \) (Table 10).

Chorioamnionitis is an independent risk factor for early intestinal colonization.

### 3.4.3 Nosocomial infection

We performed statistics of VLBWI intestinal bacterial colonization with nosocomial infection, and the results showed no statistical difference \( (p > 0.05) \) (Table 8). Therefore, we analyzed the relationship between the common four nosocomial infection bacteria and the detection of intestinal dominant bacteria, including *K. pneumoniae*, *S. marcescens*, *E. coli* and *E. cloacae* (Table 11). The detection rate of *K. pneumoniae* was 100% (5/5) in the *K. pneumoniae*-infected group while 43.42% (33/76) in the non-*K. pneumoniae*-infected group. The probability of intestinal colonization of *K. pneumoniae* in the *K. pneumoniae*-infected group was significantly higher than that in the non-*K. pneumoniae*-infected group \( (p < 0.05) \) (Table 11).

The colonization rate of *S. marcescens* in VLBWI infected *S. marcescens* was 100% (3/3), and non-infected was 7.69% (6/78). The colonization rate of VLBWI infected *S. marcescens* was significantly higher than the non-infected group \( (p < 0.05) \) (Table 11).

### TABLE 3 Distribution of dominant bacteria in intestinal colonization

| Dominant bacteria                  | 24h | D7  | D14 | D21 | Total | Detection rate (%) | Composition ratio (%) |
|------------------------------------|-----|-----|-----|-----|-------|--------------------|----------------------|
| *Klebsiella pneumoniae*            | 1   | 20  | 20  | 23  | 64    | 21.33 (64/300)     | 34.04 (64/188)       |
| *Escherichia coli*                 | 2   | 6   | 14  | 14  | 36    | 12.00 (36/300)     | 19.15 (36/188)       |
| *Enterococcus faecium*             | 0   | 3   | 11  | 12  | 26    | 8.67 (26/300)      | 13.83 (26/188)       |
| *Enterococcus faecalis*            | 0   | 4   | 7   | 8   | 19    | 6.33 (19/300)      | 10.11 (19/188)       |
| *Serratia marcescens*              | 0   | 5   | 5   | 0   | 10    | 3.33 (10/300)      | 5.32 (10/188)        |
| *Enterobacter cloacae*             | 0   | 6   | 1   | 2   | 9     | 3.00 (9/300)       | 4.79 (9/188)         |
| *Staphylococcus epidermidis*       | 1   | 2   | 0   | 2   | 5     | 1.67 (5/300)       | 2.66 (5/188)         |
| *Kirschner citrobacter*            | 0   | 0   | 2   | 1   | 3     | 1.00 (3/300)       | 1.60 (3/188)         |
| *Enterobacter aerogenes*           | 0   | 0   | 1   | 2   | 3     | 1.00 (3/300)       | 1.60 (3/188)         |
| *Acinetobacter baumannii*          | 0   | 1   | 0   | 1   | 2     | 0.67 (2/300)       | 1.06 (2/188)         |
| *Raoultella planticola*            | 0   | 0   | 2   | 0   | 2     | 0.67 (2/300)       | 1.06 (2/188)         |
| *Streptococcus agalactiae*         | 2   | 0   | 0   | 0   | 2     | 0.67 (2/300)       | 1.06 (2/188)         |
| *Lactobacillus salivarius*         | 0   | 1   | 0   | 0   | 1     | 0.33 (1/300)       | 0.53 (1/188)         |
| *Gemella morbillorum*              | 0   | 0   | 1   | 0   | 1     | 0.33 (1/300)       | 0.53 (1/188)         |
| *Enterobacter gergoviae*           | 0   | 0   | 1   | 0   | 1     | 0.33 (1/300)       | 0.53 (1/188)         |
| *Klebsiella oxytoca*               | 0   | 0   | 0   | 1   | 1     | 0.33 (1/300)       | 0.53 (1/188)         |
| *Staphylococcus haemolyticus*      | 0   | 0   | 0   | 1   | 1     | 0.33 (1/300)       | 0.53 (1/188)         |
| *Comamonas testosterone*           | 0   | 1   | 0   | 0   | 1     | 0.33 (1/300)       | 0.53 (1/188)         |
| *Stenotrophomonas maltophilia*     | 0   | 0   | 1   | 0   | 1     | 0.33 (1/300)       | 0.53 (1/188)         |
| Total                              | 6   | 49  | 66  | 67  | 188   | 62.67 (188/300)    | 100.00 (188/188)     |

### TABLE 4 Distribution of G+ and G− bacteria

| Result                | 24h | D7  | D14 | D21 | \( \chi^2 \) | \( p \) Value |
|-----------------------|-----|-----|-----|-----|-------------|--------------|
| G+                    | 3   | 10  | 19  | 23  | 22.97       | <0.001       |
| Non-G+                | 76  | 64  | 55  | 50  |             |              |
| G−                    | 3   | 39  | 47  | 44  | 51.49       | <0.001       |
| Non-G−                | 76  | 35  | 27  | 29  |             |              |

**Abbreviations:** G+: Gram-positive bacteria, non-G+: non-Gram-positive bacteria, G−: Gram-negative bacteria, non-G−: non-Gram-negative bacteria.

**Figure 3** The detection rate of G+ and G− bacteria at different time

**Table 4** Distribution of G+ and G− bacteria
Nosocomial infections such as *K. pneumoniae* and *S. marcescens* were significantly associated with intestinal colonization in VLBWI. However, the infection of *E. coli* and *E. cloacae* were not significantly associated with intestinal colonization (*p* > 0.05). Therefore, we believed that bacterial colonization is a potential risk factor for bacterial infection.

### 3.4.4 | Necrotizing Enterocolitis

The detection rate of intestinal dominant bacteria was 63.63% (14/22) in the NEC group and 62.59% (174/278) in the non-NEC group. NEC had no obvious correlation with dominant colonization bacteria (*p* = 0.92).

### 3.4.5 | Antibiotic

The detection rate of intestinal colonization bacteria was 27.82% (37/133) during antibiotic use, while 92.07% (151/164) during non-using of antibiotics. The detection rate in the period without antibiotics was significantly higher than that in the period with antibiotics ($\chi^2 = 124.01$, *p* < 0.01) (Table 8). Antibiotics are a strong factor affecting the colonization of intestinal bacteria.

### 3.5 | Distribution of main colonization bacteria detected at different time

We compared the colonization detection rate of the top five dominant colonizing bacteria at different times (Figure 4). It was obvious that the colonization rate of *K. pneumoniae* increased over time. *K. pneumoniae* always maintained a high detection rate within 7–21 days. In addition, we found that the colonization rates of *E. coli*, *Enterococcus faecium* and *E. faecalis* also increased over time. However, the detection rate of *S. marcescens* did not increase linearly with time (Table 12).

### 4 | DISCUSSION

Our results showed that Gram− bacteria were the main colonization bacteria in the early stage of VLBWI. The colonization rate of intestinal bacteria increased over time. In addition, the colonization rate of G− bacteria increased rapidly on the 7th day after birth and maintained a high level. However, Gram+ bacteria increased slowly and were significantly lower than G− bacteria. Korpela found that the total abundance of intestinal microorganisms in preterm infants increased with age at birth. Our results are consistent with the research that the intestinal microflora of premature infants less than 3 weeks old are characterized by G+ cocci such as *Streptococcus* and *Staphylococcus*, but are rapidly replaced by G− bacteria (Proteus and *Enterobacteriaceae*).
TABLE 6  Results of intestinal dominant bacteria culture related to delivery mode

| Result                     | 24 h | D7 | D14 | D21 |
|----------------------------|------|----|-----|-----|
| Positive (cases)           | 24   | 41 | 41  | 41  |
| Negative (cases)           | 2    | 15 | 6   | 5   |
| $\chi^2$                   | -    | -  | -   | -   |
| $p$ value                  | 0.61 | 0.70 | 0.40 | -   |

Note: Fisher's exact probability method.

TABLE 7  Results of intestinal dominant bacteria culture related to feeding mode

| Result                     | 24 h | D7 | D14 | D21 |
|----------------------------|------|----|-----|-----|
| Positive (cases)           | 2    | 14 | 14  | 14  |
| Negative (cases)           | 18   | 55 | 6   | 52  |
| $\chi^2$                   | -    | 0.18 | - | - |
| $p$ Value                  | 0.64 | 0.68 | 1 | 1.00 |

Note: *subjects were fasting at 24 h.
Fisher's exact probability method.

TABLE 8  Detection results related to infectious factors

| Infectious factors | MSAF | Chorioamnionitis | Nosocomial infection | NEC | Antibiotic |
|--------------------|------|------------------|----------------------|-----|------------|
| Positive (cases)   | 14   | 31               | 129                  | 14  | 37         |
| Negative (cases)   | 2    | 110              | 75                   | 8   | 104        |
| $\chi^2$           | 4.46 | 0.26             | 0.09                 | 0.01| 124.01     |
| $p$ Value          | 0.03 | 0.61             | 0.77                 | 0.92| <0.001     |

Abbreviations: MSAF, meconium-stained amniotic fluid, NEC, necrotizing enterocolitis.

TABLE 9  Effect of MSAF on the detection of intestinal dominant bacteria in VLBWI at different time

| MSAF | 24 h | D7 | D14 | D21 |
|------|------|----|-----|-----|
| Positive (cases) | 3    | 4  | 4   | 3   |
| Negative (cases)  | 2    | 71 | 0   | 0   |
| $p$ Value         | 0.002| 0.29| 1.00| 1.00|

Fisher's exact probability method.

TABLE 10  Influence of maternal chorioamnionitis at different time

| Intrauterine infection | 24 h | D7 | D14 | D21 |
|------------------------|------|----|-----|-----|
| Positive (cases)       | 4    | 5  | 12  | 11  |
| Negative (cases)       | 10   | 62 | 4   | 1   |
| $\chi^2$               | 5.49 | 0.12| 0.66| -   |
| $p$                    | 0.02 | 0.73| 0.42| 1.00|

Fisher's exact probability method.
Afterward, we analyzed the non-infectious factors that may affect the colonization of intestinal dominant bacteria on VLBWI, including gender, mode of delivery, gestational age, birth weight, mechanical ventilation and feeding mode. It is worth noting that the detection rate of intestinal dominant colonization bacteria is only 7.59% (6/79) when VLBWI are fasting within 24 h of birth. This does not mean that the rectal swab within 24 h is completely sterile, but there is no obvious vigorous growth of bacteria. This may be related to the low bacterial diversity in meconium of VLBWI. It has long been considered that the fetus grows in a sterile environment and bacterial colonization begins at rupturing of the fetal membrane. More recent careful research have shown that the amniotic fluid is not sterile, suggesting that colonization of the gut begins in utero. The analysis of placenta obtained during full-term cesarean section without rupture of membranes showed that the microbiota of placenta, amniotic fluid and meconium were similar. The development of the intestinal microbiota of newborns is usually driven by the colostrum. However, VLBWI admitted to NICU immediately after birth are usually fed with hydrolyzed milk and formula, which seem to have little effect on the colonization of intestinal dominant bacteria. The study has shown that diet has a lesser impact on the gut microbiota in extremely preterm infants than is seen in term infants. In addition, the detection rate of intestinal bacteria without mechanical ventilation was significantly higher than that on VLBWI with mechanical ventilation (Table 5), which may be due to the severe condition, large dosage and frequent use of antibiotics in VLBWI with mechanical ventilation affected the normal colonization of intestinal bacteria.

The analysis of intestinal flora of preterm infants based on 16S pyrophosphate sequencing found that gestational age and mode of delivery were important influencing factors. However, these differences were not found in this study. The possible reason is that our method based on bacterial culture has no way to compare its flora diversity and composition. From the dominant bacteria identification, gestational age and delivery mode have no effect on the colonization of intestinal dominant bacteria.

Among the infectious factors, we found MSAF and maternal chorioamnionitis were closely related to the bacterial colonization in the early stage of VLBWI, and the influence became smaller with time.
MSAF and maternal chorioamnionitis may be one of the causes of premature birth. Early maternal exposure to inflammation can lead to a series of events, including prostaglandin increase, promoting uterine contraction, degradation of chorioamniotic extracellular matrix and cervical ripening. Fetuses may be exposed to pathogenic microorganisms, resulting in a decrease in the diversity of intestinal microbiota and a relatively large number of pathogenic bacteria in infants. It is well known that infection during pregnancy can change the vaginal flora of bacterial vaginosis, or increase the susceptibility of other infections with altered maternal and infant microbiota, thus interrupting normal pregnancy. In this study, NEC and intestinal colonization bacteria detection on VLBWI had no obvious correlation. NEC in preterm infants is not only associated with a single pathogen. There may be a reason for insufficient sample size, and its correlation may need larger samples and more scientific research program to confirm.

There is no doubt that antibiotic is a strong influencing factor. We found that the rate was only 27.82% (37/133) during the use of antibiotics, while it was as high as 92.07% (151/164) during the non-use of antibiotics. The use of antibiotics markedly reduced the colonization rate of intestinal bacteria. VLBWI are almost universally treated with antibiotics while in NICU. Arboleya found a decrease in fecal diversity among premature infants who received long-term antibiotic treatment. Vangay proposed four biological disorders induced by antibiotics: the loss of keystone taxa, the loss of diversity, the changes of metabolic capacity and the proliferation of pathogens.

In the VLBWI enrolled in this study, the hospital infection rate was as high as 32.10% (26/81) which is much higher than usual. However, this data are authentic. Generally, the nosocomial infection rate of the neonatal department is 4.5%–5.0%. The vulnerability of VLBWI, long hospitalization and many invasive procedures may lead to this result. Of course, we can not rule out the effect of cross infection. We analyzed the influence of common bacteria in a nosocomial infection on VLBWI’s intestinal colonization. Five children infected K. pneumoniae and three children with S. marcescens had corresponding bacterial colonization. The detection rates of K. pneumoniae and S. marcescens ranked first and fifth with a high colonization rate in this study. It is worth noting that late-onset sepsemia caused by Gram-negative bacteria is one of the main causes of incidence rate and mortality in the neonatal intensive care unit, and it is especially significant for premature infants with small gestational age. Zaidi et al. pointed out that K. pneumoniae is one of the most common pathogens of nosocomial respiratory tract infection, and is also the second-largest factor causing nosocomial sepsis and urinary tract infection. In this study, the detection rate of K. pneumoniae was as high as 21.33% (64/300) and increased over time. Epidemiological studies have suggested that K. pneumoniae host-to-host transmission requires close contact and usually occurs through the fecal-oral route. Moles et al. indicated that the presence of S. marcescens was strongly related to a higher degree of immaturity and hospital-related parameters, including antibiotic and mechanical ventilation. Although S. marcescens displays relatively low virulence, it can lead to nosocomial infections and outbreaks in immunocompromised or critically ill patients, particularly in settings such as NICU. However, the contaminated hands of medical staff are considered to be a major medium of transmission. In NICU, colonized or infected newborns are the main potential source of pathogens, especially in the respiratory apparatus, but also in the gastrointestinal tract. To curb the spread of infection, it is necessary to identify colonized or infected patients as soon as possible and prompt implement infection control measures, particularly strict hand hygiene and contact precautions.

Garcia Hernandez pointed out that E. coli is the most common pathogen of nosocomial and community-acquired infections. E. cloacae is responsible for nosocomial outbreaks in susceptible patients in NICU. In this study, we did not find a significant correlation between intestinal colonization of E. coli and E. cloacae and nosocomial infection. The reasons may be that the sample size is insufficient or the two bacteria are not identified as the most dominant bacteria. This could be due to insufficient sample size or the failure of these two bacteria to be identified as the most dominant bacteria.

In this study, we adopted an anal swab bacterial culture to select the most dominant bacteria, and then analyzed the distribution and influencing factors of intestinal colonization from this perspective. Although flora sequencing can usually obtain more bacterial composition and diversity, it cannot distinguish living bacteria from dead.

### TABLE 12 Distribution of main colonization bacteria detected at different time

| Bacterium                  | 24 h | D7  | D14 | D21 | \( \chi^2 \) | p Value |
|----------------------------|------|-----|-----|-----|-------------|---------|
| *Klebsiella pneumoniae*    | Positive | 1   | 20  | 20  | 23          | 19.12   | <0.001  |
|                            | Negative | 78  | 54  | 54  | 50          |         |        |
| *Escherichia coli*         | Positive | 2   | 6   | 14  | 14          | 13.28   | <0.001  |
|                            | Negative | 77  | 68  | 60  | 59          |         |        |
| *Enterococcus faecium*     | Positive | 0   | 3   | 11  | 12          | 17.25   | <0.001  |
|                            | Negative | 79  | 71  | 63  | 61          |         |        |
| *Enterococcus faecium*     | Positive | 0   | 4   | 7   | 8           | 8.78    | 0.003   |
|                            | Negative | 79  | 70  | 67  | 65          |         |        |
| *Serratia marcescens*      | Positive | 0   | 5   | 5   | 0           | 0.007   | 0.932   |
|                            | Negative | 79  | 69  | 69  | 73          |         |        |
bacteria, let alone judge the vitality of bacteria. Of course, our study has some limitations. Firstly, we did not carry out anaerobic culture. Secondly, we did not set up a control group. For further research, we have collected feces from VLBWIs and term infants. After that, we will perform 16S pyrophosphate sequencing to obtain more information about the gut microbiota of VLBWI.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

CONSENT
All subjects had informed consent signed by their guardians.

DATA AVAILABILITY STATEMENT
The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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