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Microbial Biodiversity of Gastrointestinal Microbiome of Preterm Infants during the First Month of Life

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Abstract

The establishment and succession of bacterial communities in infants may have a profound impact on their health, but information about the composition of meconium microbiota and its evolution in hospitalized preterm infants is scarce. In this context, the objective of this work was to characterize the microbiota of meconium and fecal samples obtained during the first 72 hours of life from 181 donors using culture and molecular identification. Culture techniques offer a quantification of cultivable bacteria and allow further study of the isolate, while molecular identification provides deeper information on bacterial diversity. Inter-individual differences were detected in the microbiota profiles, although the meconium microbiota was peculiar and distinct from that of fecal samples. Bacilli and other Firmicutes were the main bacteria groups detected in meconium while Proteobacteria dominated in the fecal samples. The culture technique showed that Enterococcus, together with Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* *Staphylococcus* predominated in meconium. This study highlights that spontaneously-released meconium of preterm neonates contains a specific microbiota that differs from that of feces obtained after the first 72 hours of life.

Keywords

Preterm, Gut, Microbiome, Meconium, Bacteria

1. Introduction

The gut microbiome is the world's largest microbial community, consisting of trillions of bacteria from thousands of species [1]. It has a significant impact on

health and disease. As a complex ecosystem, nutrition, age, and antibiotic use have all been shown to have a major impact on its composition [2]-[5].

Newborns are exposed to pathogens from both their mothers and the environment. The birth process itself has a huge impact on their exposure to their mothers. Infants born vaginally receive germs similar to their mother's vaginal microbiome (mainly Lactobacillus and Prevotella), whereas those born via C-section receive bacteria similar to their skin microbiome (mostly Staphylococcus) [6] [7].

Infants' early exposure to microorganisms at birth is regarded as one of the most important influences on the maturation of microbial populations, particularly in the gut [8]. This critical phase and possibly in utero plays a vital role in immune system development, metabolic programming, neurodevelopment, and future illness risk [9]-[11]. Recent studies have demonstrated that the infant gut microbiome is influenced by a variety of factors, including gestational duration, antibiotic exposure, food, genetics, and delivery method [12] [13]. While various studies have looked at the early development of the newborn gut microbiome [14]-[16], it is unclear how and when bacteria colonize the gut. According to research, neonatal gut colonization begins in pregnancy, contrary to popular perception [17]. However, the sources of bacteria and how they enter the embryonic intestine remain unknown.

The first germs detected in the environment are those prevalent in hospitals and on caretakers' skin. Skin-associated bacteria such as Staphylococcus are more common, whereas beneficial gut microbes take longer to colonize [17].

The conventional concept that a fetus develops in a sterile environment, resulting in a sterile stomach at birth, is currently being challenged [18]. Previously, bacterial colonization was linked to illnesses of the fetal membranes, amniotic fluid, and preterm birth, with little evidence of bacteria in healthy pregnancies [19] [20]. However, recent meconium investigations have found evidence that contradicts the idea of a sterile womb environment. Research of 23 healthy babies found a lower diversity of bacteria in meconium, the first stool after birth, compared to adult stool samples. Notably, there was a decline in bacteriidetes and firmicutes, bacteria that are common in the adult gut microbiome but not in newborns [21]. Meconium, the first stool of a newborn first stool, usually within 24 to 48 hours after birth, is made up of materials such as bile, mucus, hair, bile, and skin cells and typically dark green or black with a thick, tar-like consistency. Meconium is primarily composed of facultative anaerobes, predominately associated with the skin and oral cavity, whereas feces is predominantly composed of obligate anaerobes. Meconium contains a higher concentration of bacteria associated with the skin and oral cavity including, Staphylococcus, Enterococcus, Escherichia coli, Enterobacter, Bifidobacterium, and Lactobacillus The bacterial composition of meconium, which serves as an indicator of the in-utero gut microbiota, did not alter significantly between vaginal and C-section births. This implies that birth mode affects the gut microbiota after delivery, implying that gut colonization occurs before birth, independent of the mode of delivery. Furthermore, gestational

age at birth has a significant effect on the gut flora [22]. Preterm neonates exhibit delayed and atypical patterns of intestinal infection when compared to term births thereby increasing vulnerability to illnesses. Preterm babies' meconium contains a lower bacterial variety, making them more susceptible to diseases like necrotizing enterocolitis and sepsis [23]. Preterm baby meconium and feces microbiomes are less diverse and more varied between individuals. Bacilli and other Firmicutes were found to be more numerous in meconium samples, but Proteobacteria were more prevalent in feces [24].

The meconium of 21 healthy neonates contained species of Staphylococcus and Bifidobacterium that are often seen in the human gut. Furthermore, meconium from 52 preterm and full-term newborns had a low bacterial diversity, dominated by taxa such as Firmicutes and Proteobacteria, indicating a basic gut community [24]. Over half of the bacteria found in the meconium also colonized the amniotic fluid, with little overlap with the vaginal and oral bacterial populations [24]. As a result, the infant intestine may become colonized in utero, before delivery, possibly through the intake of amniotic fluid. However, there is no direct evidence of bacterial colonization in the human embryonic gut, and the mechanisms underlying in-utero gut colonization are unknown. The maternal transfer of microorganisms has been proposed as a global phenomenon. The standard use of antibiotics in newborns to empirically treat early-onset sepsis can adversely affect the neonatal gut microbiome, with potential long-term health impacts. Research into the escalating issue of antimicrobial resistance in preterm infants and antibiotic practices in neonatal intensive care units is limited.

Comprehensive studies have been undertaken on feces; however, there is a scanty exploration of the transition from meconium to feces, especially in preterm newborns.

In this light, the goal of this study was to examine bacterial diversity in the meconium and feces of preterm newborns during their first month of life. For this reason, culture-dependent and culture-independent approaches were chosen since they frequently provide complementary perspectives on biological sample microbial diversity. Comprehending the formation of an advantageous microbiota, especially the initial colonization of bacteria such as Bifidobacterium and Lactobacillus, is essential for preterm infants, as these microbes are vital to immunological development and defense against infections. The practical importance of examining preterm infant microbiota resides in its capacity to discern microbial patterns linked to health and disease, facilitating the creation of targeted interventions, such as probiotic or prebiotic therapy, to enhance healthy microbiota development. Furthermore, examining the microbiota can yield insights into optimizing early microbial colonization to enhance lifetime health.

2. Methods

Patients and Sampling

This prospective study encompassed 181 preterm infants delivered at the Neo-

natal Unit of the Department of Child Health and the Obstetric Department of Korle Bu Teaching Hospital, Accra, from April to November 2022. Informed written consent from parents was secured for each child prior to inclusion. To qualify for enrollment, preterm infants must have been born at a gestational age of under 35 weeks. Infants born preterm to women with HIV, those with congenital deformities, those who were fed, and those with birth asphyxia or hypoxic-ischaemic encephalopathy were excluded from the study. The study employed a systematic random sampling strategy to recruit newborns. Relevant clinical data for each infant, including the duration of antibiotic therapy, parenteral nutrition, nasogastric feeding, pregnancy and delivery history, as well as the infant's gender, birth weight, and gestational age, were collected at delivery using a comprehensive questionnaire, as detailed in Table 2. All infants were nourished with human milk (either donor milk or their own mother's milk) and, on occasion, with preterm formula. An average of three fecal samples was taken from each infant. Using a sterile spoon, the samples were taken from the babies diapers. The initial spontaneously ejected meconium was collected within the first 48 hours post-delivery, before to any feeding of the infant. The second sample was collected between the 3rd and 7th days, while the final sample was obtained from the 8th to the 14th day of life. The second and third stool samples were collected from the infants during postnatal visits or while they were still hospitalized. Stool samples were collected using a spoon affixed to the lid of a sterile container, maintained at 4°C, and delivered to the microbiological laboratory within 24 hours for analysis. The medical personnel of the Neonatal unit at the hospital gathered all fecal specimens. Ethical approval for the study was secured by the Korle Bu Teaching Hospital Ethics Committee (ethical approval reference: KBTH-IRB/0008/2022).

Sample processing, pathogen isolation, and identification

The microbiology laboratory received samples for analysis within 24 hours of collection. The samples were processed step by step. Briefly, feces specimens were separated into two sections, with one preserved for DNA extraction. The second set of stool samples was separated into two portions. One half was immediately inoculated on blood agar (Gram positive organisms), Campylobacter agar and chocolate agar (fastidious organisms), and MacConkey (Gram negative organisms) and incubated at 37˚C for 18 - 24 hours to isolate bacteria. The remaining samples were supplemented with alkaline peptone water and selenite broth before being cultured on Salmonella/Shigella agar and TCBS, respectively. All incubations were performed in a controlled laboratory environment (uniform temperature and humidity), and plates were monitored for development. If no growth was noticed during the initial incubation, plates were incubated for a further 24 hours. Prior to identification and drug sensitivity testing, all separate individual colonies on each agar were purified using nutritional agar. The bacterial and fungal colonies were detected using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS). The manufacturer's instructions for the extended direct transfer technique of identification were followed. A unique spectrum of

the relative abundances of ribosomal proteins was constructed and compared to a reference from the MALDI-TOF database to identify organisms based on similarity score. Microscan was used to determine antimicrobial resistance testing (AST) of all isolated gram-positive and gram-negative organisms, with the Kirby-Bauers method and yeast-specific antifungals. The MoBio Powersoil bacterial DNA isolation kit (MoBio, Carlsbad, California) was used to extract DNA in the Bacteriology Department's Biosafety level 2 laboratory at the Noguchi Memorial Institute for Medical Research, according to manufacturer instructions. The isolated DNA was kept at −20˚C.

Data Handling and Statistical Analysis

Data was entered into the study database using double data entry with correction of any discrepancies based on the record. Statistical analysis was carried out in R statistical software, heat maps were drawn and to ascertain statistical significance and association between groups. Categorical data were summarised with proportions and confidence intervals. Continuous variables were summarised using means with their standard deviations.

3. Results

Descriptive statistics were performed to investigate the association of demographic factors, and clinical characteristics of mothers and preterm babies. microbiome identified were compared across the demographic and clinical characteristics±. Categorical variables are reported as frequencies and percentages. Continuous variables are reported as mean ± and standard deviations (SD) or medians and interquartile range (IQRs). Data was normalized using D'Agostino and Pearson normality alongside Shapiro Wilk to determine the significance and normality of the data. Statistically significant results (P-value < 0.05) were considered for further analysis. Differences between groups were compared using Student's t-test or the Mann-Whitney U test between two groups while ANOVA and Kruskal Wallis were used for groups more than two (depending on the normality of the data) for continuous variables, chi-square (χ^2) or Fisher's exact test for categorical variables.

Table 1 illustrates the distribution of demographic data for mothers from the antenatal visit to the delivery stage. A total of 181 participants were carefully selected based on stool samples from babies received in one of three batches to identify microbiome growth in the stool. The data indicates that mothers aged 20 to 29 comprised the largest group, totalling 106 (58.6%), followed by those aged 30 to 39, who numbered 63 (34.8%), reflecting their presence in the productive stage of life. The majority of the women were married, with 126 (69.6%) participating predominantly in the informal sector of the economy. The proportion of women identified with other conditions during pregnancy was relatively low at 24.3%, in contrast to those without any additional disease or condition during pregnancy. The identified conditions included hypertension, which was the most prevalent, followed by diabetes, hypertension with diabetes, sickle cell disease, malaria, and

other related infections. The medications administered adhered to a similar protocol, comprising anti-hypertensives, metformin, antimalarials, and antibiotics, respectively. In cases of rupture of membranes (ROM) and the onset of Labor, spontaneous ROM occurrences were more prevalent than those resulting from artificial rupture of membranes and induced Labor. Notably, a majority of these cases exhibited a duration of ROM ≤ 8 hours (61.3%) and a duration of Labor ≤ 8 hours (53.0%), respectively. The majority of participants were delivered via caesarean section, accounting for 63.5%.

Table 1. Demographics and clinical characteristics of mother.

History	Indicator	Total		Female		Male	
		n = 181		90 (49.7%)		91 (50.3%)	
Age group	<20	4	2.2%	1	1.1%	3	3.3%
	20 - 29	106	58.6%	55	61.1%	51	56.0%
	30 - 39	63	34.8%	29	32.2%	34	37.4%
	≥40	8	4.4%	5	5.6%	3	3.3%
Marital status	single	37	20.4%	19	21.1%	18	19.8%
	cohabiting	18	9.9%	9	10.0%	9	9.9%
	married	126	69.6%	62	68.9%	64	70.3%
Level of education	none	12	6.6%	5	5.6%	7	7.7%
	primary	38	21.0%	18	20.0%	20	22.0%
	secondary	73	40.3%	37	41.1%	36	39.6%
	tertiary	58	32.0%	30	33.3%	28	30.8%
Occupation	unemployed	22	12.2%	13	14.4%	9	9.9%
	informal	116	64.1%	56	62.2%	60	65.9%
	formal	43	23.8%	21	23.3%	22	24.2%
Condition during preg	No	137	75.7%	65	72.2%	72	79.1%
	Yes	44	24.3%	25	27.8%	19	20.9%
Kind of conditions	None	132	72.9%	63	70.0%	69	75.8%
	Hypertensive	28	15.5%	16	17.8%	12	13.2%
	Diabetes	4	2.2%	3	3.3%	1	1.1%
	Hypertensive & Diabetes	5	2.8%	2	2.2%	3	3.3%
	Sickle cell	5	2.8%	2	2.2%	3	3.3%
	malaria	3	1.7%	2	2.2%	1	1.1%
	infection	4	2.2%	2	2.2%	2	2.2%
Kind of medication	None	150	82.9%	70	77.8%	80	87.9%
	Antihypertensive	25	13.8%	15	16.7%	10	11.0%
	Metformin	2	1.1%	2	2.2%	0	0.0%
	Antihypertensive & Metformin	2	1.1%	1	1.1%	1	1.1%
	Antimalaria	1	0.6%	1	1.1%	0	0.0%
	Antibiotics	1	0.6%	1	1.1%	0	0.0%

Continued

Rupture of membrane	Spontaneous	114	63.0%	56	62.2%	58	63.7%
	Artificial ROM	67	37.0%	34	37.8%	33	36.3%
Duration of ROM	≤ 8hours	111	61.3%	59	65.6%	59	65.6%
	>8 < 12hours	33	18.2%	19	21.1%	19	21.1%
	≥ 12hours	37	20.4%	12	13.3%	12	13.3%
Onset of labour	Spontaneous	112	61.9%	56	62.2%	56	61.5%
	Induced	69	38.1%	34	37.8%	35	38.5%
Duration of labour	≤ 8hours	96	53.0%	49	54.4%	47	51.6%
	>8 < 12hours	78	43.1%	36	40.0%	42	46.2%
	≥ 12hours	7	3.9%	5	5.6%	2	2.2%
Mode of delivery	SVD	66	36.5%	37	41.1%	29	31.9%
	CS	115	63.5%	53	58.9%	62	68.1%
Antibiotics given	No	165	91.2%	82	91.1%	83	91.2%
	Yes	16	8.8%	8	8.9%	8	8.8%

Portion of microorganism vrs babies' sex.

Figure 1 illustrates the quantity of microorganisms identified in the data output, categorized by age and sex of infants. Despite a seemingly equitable distribution between male and female categories, within the two primary age groups, specifically 20 - 29 and 30 - 39, females constitute a higher proportion relative to other age groups, although both genders tend to dominate other groups on relatively equal terms. The diagram illustrates women's active reproductive stages within the childbearing lifecycle, indicating a reproductive peak between the ages of 20 and 29. Subsequently, there is a decline in reproductive capacity as age increases, with downward trends evident from the 30s onward, and a more pronounced decline observed from the 40s and beyond. The analysis examined indicators related to both mothers and infants to assess the significance of the data, employing bivariate measures to enhance understanding of the distributions. The table above presents the demographics and clinical characteristics of the preterm cohort.

Distribution based on mean comparison

Age groups were compared to determine whether the differences among the four categorized age groups were not statistically significant (p = 0.190) (Table 2). marital status was tested to have a statistically significant difference among the three groups (p = 0.033, median = 2.0). test of multiple comparison showed that there is a statistically significant difference between single and married (p = 0.024) but not cohabiting. Level of education with a P value (p = 0.473) was therefore not statistically significant. Even though the informal sector of the economy was higher compared to the other sectors was not statistically significant (p = 0.913).

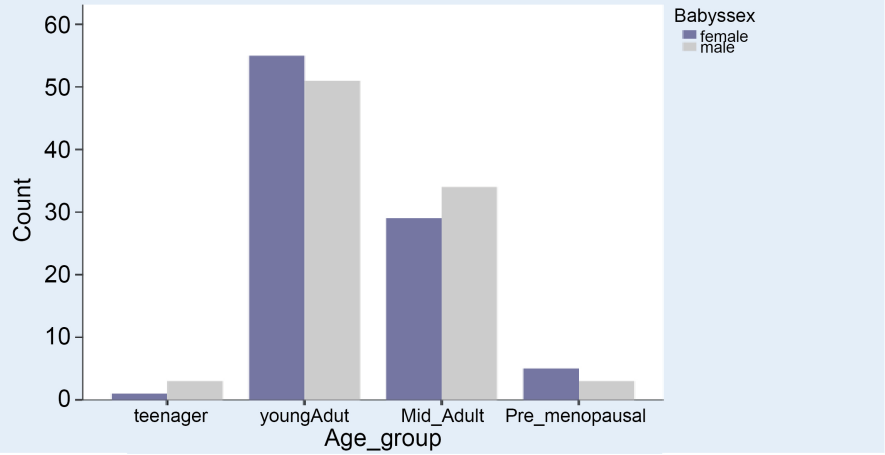

Figure 1. Quantities of microrganisms identified among babies born from mothers in different age groups.

Table 2. Demographics and clinical characteristics of preterm cohort.

History	Indicator	n	%	Sig
Age group	<20	4	2.2%	0.190
	20 - 29	106	58.6%	
	30 - 39	63	34.8%	
	≥40	8	4.4%	
Marital status	single	37	20.4%	0.033
	cohabiting	18	9.9%	
	married	126	69.6%	
Level of education	none	12	6.6%	0.473
	primary	38	21.0%	
	secondary	73	40.3%	
	tertiary	58	32.0%	
Occupation	unemployed	22	12.2%	0.913
	informal	116	64.1%	
	formal	43	23.8%	
Condition during preg	No	137	75.7%	0.453
	Yes	44	24.3%	
Kind of conditions	None	132	72.9%	0.285
	Hypertensive	28	15.5%	
	Diabetes	4	2.2%	
	Hypertensive & Diabetes	5	2.8%	
	Sickle cell	5	2.8%	
	malaria	3	1.7%	
	infection	4	2.2%	

Continued

Kind of medication	None	150	82.9%	0.294
	Antihypertensive	25	13.8%	
	Metformin	2	1.1%	
	Antihypertensive & Metformin	2	1.1%	
	Antimalaria	1	0.6%	
	Antibiotics	1	0.6%	
Rupture of membrane	Spontaneous	114	63.0%	0.568
	Artificial ROM	67	37.0%	
Duration of ROM	≤8 hours	111	61.3%	0.644
	>8 <12 hours	33	18.2%	
	≥12 hours	37	20.4%	
Onset of labour	Spontaneous	112	61.9%	0.828
	Induced	69	38.1%	
Duration of labour	≤8 hours	96	53.0%	0.003
	>8 <12 hours	78	43.1%	
	≥12 hours	7	3.9%	
Mode of delivery	SVD	66	36.5%	0.649
	CS	115	63.5%	
Antibiotics given	No	165	91.2%	0.509
	Yes	16	8.8%	
Baby's sex	female	28	42.4	0.318
	male	38	57.6	
Type of Baby feed	No feeding	2	3.0	0.535
	Breastfeeding only	54	81.8	
	Mixed feeding	10	15.2	
		Mean ±	**SD**	
Baby's weight	Mean, SD	1.67	0.46	

the rest of the demographics including occupation, other conditions during pregnancy, kind of conditions and medication given were not statistically significance.

In terms of delivery characteristics, duration of labour yielded p value ($p = 0.003$) which indicates statistically significance difference among the three classified stages of durations of labour. Further analysis of multiple comparison test was conducted to identify the actual difference. Based on the post hoc analysis those with duration ≤ 8hours and >8 <12 hours had significant differences ($p = 0.010$) but there was not statistical difference for those ≥ 12 hours. Apart from the duration of labor onset, the rest of the characteristics including rupture of membrane, duration of ROM, onset of labour, antibiotics given, and as well as babies' delivery characteristics were not statistically significant.

Cumulative distribution of microbiome signatures identified

The cumulative frequency of the microbiome signatures is indicated in the order of the highest to the lowest (Figure 2). In general, it can be appreciated that eight (8) including E.coli (34.73%), Enterococcus faecium (26.95%), Enterococcus faecalis (17.96%), Klebsiella pneumonia (5.99%), Staphylococccus epidermis (3.29%), Staphylococcus cohnii (2.40%), Staphylococcus haemolyticus (1.50%), Acinetobacter baumanii (1.20%), out of the 25 species were more present in the whole specimen received. The rest of the microorganisms were below count 4, including Enterobacter cloacae, Staphylococcus lugdunensis, Staphylococcus warneri, Burkhoderia cenocepacia, Ochrobactrum intermedium, Stenotrophomonas maltophiia, Enterobacter asburiae, Enterobacter bugandensis, Enterobacter kobei, Enterococcus hirae, Staphylococcus aureus, Proteus mirabiis, Pseudomonas stutzeri, Staphylococcus sciuri, Staphyococcus hominis, Klebsiella variicola and Enterobacter hormaechei.

Moreover, some other organisms were identified in the latter batches as late identified groups including Enterobacter kobei, Enterococcus hirae, Staphylococcus aureus, Proteus mirabiis, Pseudomonas stutzeri, Staphylococcus sciuri, Staphyococcus hominis, Klebsiella variicola and Enterobacter hormaechei.

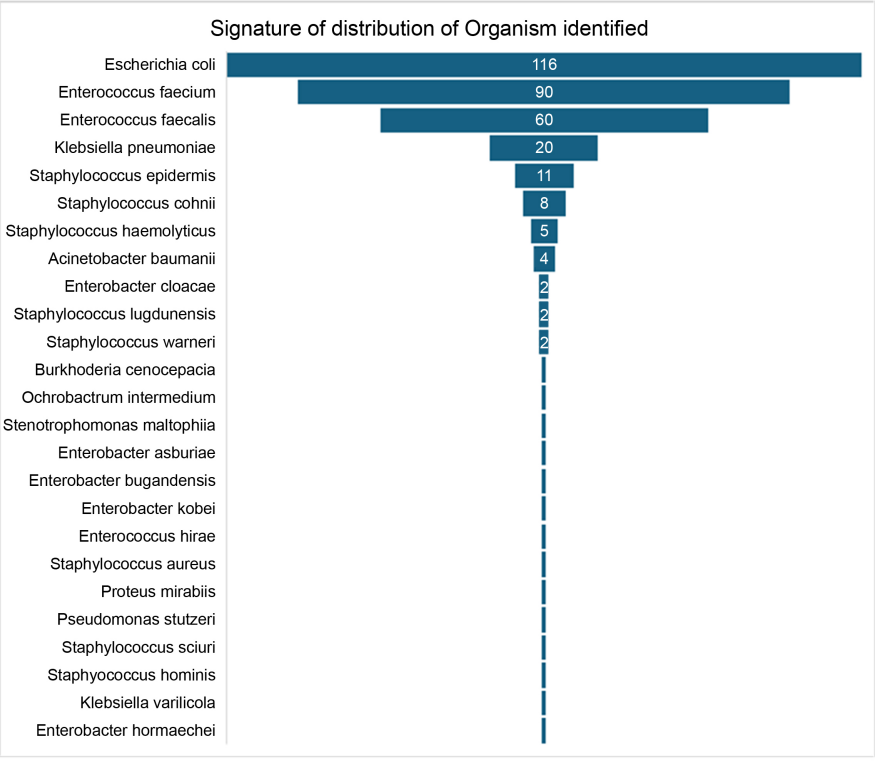

Figure 2. Distribution of microbiome signatures identified.

Microbiomes identified in the preterm

The table (Table 3) represents the distribution of microbiome counts in the three faces of receipt expressed as count. Kruskal-Wallis test showed p = 0.651,

with a statistic = 0.8570, indicated there was not significant difference among the three groups.

Microbiomes identified in the preterm

Table 3. Prevelance of Bacteria identified among samples.

Microorganism	n	%	1st stool	2nd stool	3rd stool
Escherichia coli	116	34.73	49	34	33
Enterococcus faecium	90	26.95	30	32	28
Enterococcus faecalis	60	17.96	23	22	15
Klebsiella pneumoniae	20	5.99	7	9	4
Staphylococcus epidermis	11	3.29	3	1	1
Staphylococcus cohnii	8	2.40	2	0	0
Staphylococcus haemolyticus	5	1.50	2	5	4
Acinetobacter baumanii	4	1.20	2	1	1
Enterobacter cloacae	2	0.60	2	3	3
Staphylococcus lugdunensis	2	0.60	2	0	0
Staphylococcus warneri	2	0.60	1	0	0
Burkhoderia cenocepacia	1	0.30	1	0	0
Ochrobactrum intermedium	1	0.30	1	0	0
Stenotrophomonas maltophiia	1	0.30	1	0	0
Enterobacter asburiae	1	0.30	1	0	0
Enterobacter bugandensis	1	0.30	1	0	0
Enterobacter kobei	1	0.30	0	1	1
Enterococcus hirae	1	0.30	0	1	0
Staphylococcus aureus	1	0.30	0	1	0
Proteus mirabiis	1	0.30	0	1	0
Pseudomonas stutzeri	1	0.30	0	1	0
Staphylococcus sciuri	1	0.30	0	1	0
Staphyococcus hominis	1	0.30	0	0	1
Klebsiella variicola	1	0.30	0	0	1
Enterobacter hormaechei	1	0.30	0	0	1

Figure 3 below represents a cluster barchart of microorganism identified in the three batches examined across the eleven (25) signatures of the microbiomes. Generally the trends showed a decline in the number of organism seen across the groups.

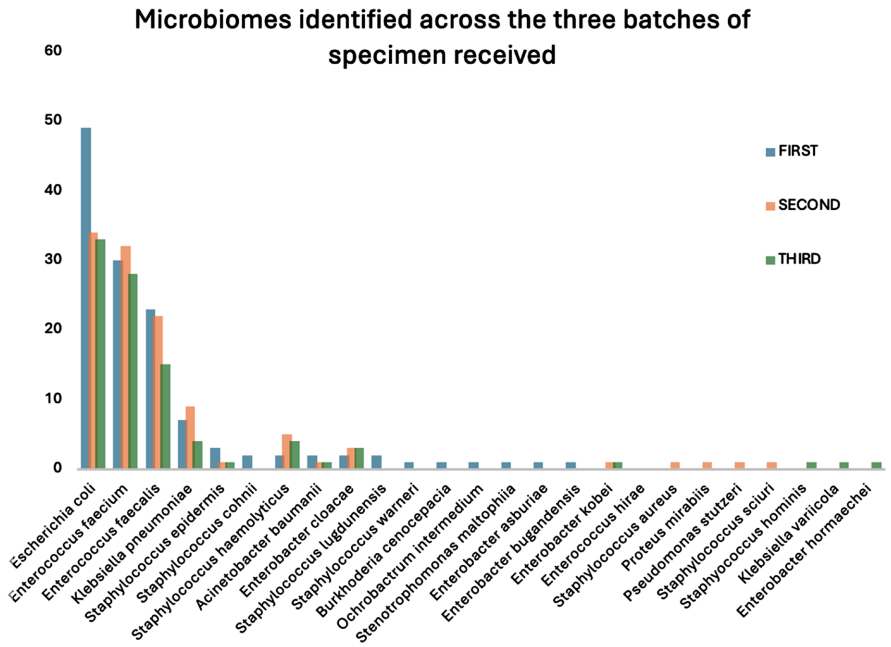

Figure 3. Microorganisms identified across the three collection times.

4. Discussion

This study assessed the succession of bacterial species in the meconium and feces of preterm infants during the first 72 hours of life using culture-based methods.

The analysis of meconium and feces from preterm infants typically revealed low species diversity and considerable inter-individual variability, aligning with earlier research [19] [25] [26]. The findings indicate that first-pass meconium likely contains a unique microbiota. Distinct microbiomes were identified in the meconium samples, which were consistent with previous research findings. This indicates that meconium reflects the initial stages of perinatal gut colonization. The results of the bacterial culture in this study indicate that the majority of meconium samples produced positive cultures. Meconium samples contained bacteria recognized as primary gut colonizers, including Escherichia, Enterococcus, Staphylococcus, and Klebsiella pneumoniae.

Premature birth generally results in a delayed and unusual qualitative pattern of gut colonization, often deemed aberrant in comparison to that of healthy term infants [27] [28]. This factor influences infant health and acts as a risk factor for gastrointestinal infections, such as necrotizing enterocolitis [29].

A recent study examined meconium and fecal samples from six preterm infants through 16S rRNA high-throughput pyrosequencing, revealing distinct patterns in gut colonization [30]. Patients with sepsis demonstrate an increased prevalence of Proteobacteria and Firmicutes, particularly Staphylococcus. Healthy individuals administered limited antibiotics without developing sepsis exhibited an increase in the relative abundance of anaerobes, resembling more 'mature' microbial communities, such as Clostridium, Klebsiella, and Veillonella [30]. The fecal microbiota of preterm infants is predominantly composed of cultivable bacteria

commonly found in antibiotic-rich hospital environments, particularly in neonatal intensive care units (NICUs). This study identified a significant correlation between the presence of Klebsiella and several hospital-related factors, such as antibiotic therapy and mechanical ventilation.

The fear of infections often results in the premature and widespread administration of broad-spectrum antibiotics in NICUs, increasing the risk of colonization by resistant bacterial strains [31]. The notable environmental impact aligns with previous studies that identified a tendency for uniformity in the bacterial populations of preterm infants in the NICU [16]. Recent findings indicate that the NICU significantly influences clostridia colonization in preterm newborns, with antibiotic treatment affecting colonization levels [32]. An extensive pyrosequencing study examined the gut-associated microbiome of 11 extremely low birth weight infants during the initial month post-birth. This study revealed that Enterobacteriales, Staphylococcus, and Enterococcus were prevalent bacterial taxa within a low-diversity community characterized by pathogens associated with invasive illnesses in newborns [12]. In preterm newborns, the colonization by strict anaerobes is notably delayed. Clostridia colonization exhibits significant variability among infants regarding the timing of initial appearance; however, Bacteroides and bifidobacteria are infrequently detected in the feces of these infants [33].

Sampling time is a crucial variable in research concerning the meconium microbiome. Meconium is defined as the first stool produced within 48 hours following birth. The neonatal microbiota undergoes rapid diversification following birth, indicating that prolonged sampling periods could lead to the incorporation of bacteria acquired post-delivery. A prior study found that sampling times of 24 hours or less after birth did not affect the bacterial load of the samples. Therefore, it is unlikely that the sampling time affected the results of this study.

The first-pass meconium represents the initial sample available for gut microbiome research following birth and may signify the beginning of true bacterial colonization in the gut. The distinctions in gut microbiome development between newborns delivered vaginally and those born via C-section are observable in the initial stool after birth. The proposed interventions designed to modify gut colonization in newborns born via C-section, such as fecal transplants from the mother's first milk, may prove ineffective if the early stages of the colonization process are crucial for later health outcomes. The clinical importance of the meconium microbiome is not yet fully comprehended.

5. Limitations

This study had some limitations. As a subset of a bigger study, this study had limited data. Further analysis will be done to explore the relationship between early microbiota composition and clinical outcomes like necrotizing enterocolitis or late-onset sepsis as this will help to identify predictive microbial signatures and inform targeted interventions to mitigate these severe preterm infant morbidities.

In conclusion, this study presents a hypothesis connecting bacteria found in

meconium to preterm birth. The immunoreactivity of the fetal intestine, along with the presence of specific bacteria, indicates that some instances of spontaneous preterm labor may be attributed to fetal intestinal bacteria. The identification and characterization of these organisms, along with their interactions with the host, may lead to the development of novel therapeutics for preventing many instances of preterm birth.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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