Comparison of gut microbiomes between neonates born by cesarean section and vaginal delivery: prospective observational study

Wipada Laosooksathit[1], Nichapat Pahirah[1], Amarin Narkwichean[1], Malai Taweechotipatr[2], Sivaporn Wannaiampikul[2], and Chinpanee Duang-Udom[3]

[1]Srinakharinwirot University - Ongkharak Campus
[2]Srinakharinwirot University Faculty of Medicine
[3]Srinakharinwirote University Faculty of Medicine - Ongkharak Campus

May 05, 2024

Abstract

Objective The objective of the study was to investigate the differences in the gut microbiomes of neonates delivered via cesarean section compared to those born by vaginal delivery, and to identify the predominant microbial taxa present in each group. **Study design** A prospective observational study. **Setting** At Her Royal Highness Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University, Thailand. **Sample** Fecal sample obtained by 281 healthy neonates born between February 2021 and April 2023. The study population was divided into two groups: 139 neonates born via vaginal delivery and 142 neonates born via cesarean section. **Methods** The microbiota composition of each neonate's fecal sample was identified by using 16S ribosomal ribonucleic acid metagenomic sequencing. **Main Outcome Measures** Neonatal gut microbiome abundancy and diversity was identified according to route of delivery. **Results** Neonates delivered vaginally exhibited a gut microbiome with higher abundance and diversity than those delivered by cesarean delivery. Bifidobacterium was the dominant genus in both groups. *Bifidobacterium breve* was the dominant species and was significantly higher in cesarean-delivered neonates compared to those delivered vaginally (24.0% and 9.2%, respectively) ($p<0.0001$). However, the taxonomy of only 89 (64.0%) and 44 (31.43%) fecal samples could be identified from the vaginal and cesarean delivery groups, respectively. **Conclusions** Route of delivery is associated with neonatal gut microbiome abundancy and diversity. Neonates delivered via vaginal delivery exhibited higher diversity but lower abundancy of the dominant species in the gut microbiome.

Title Page

Comparison of gut microbiomes between neonates born by cesarean section and vaginal delivery: prospective observational study

Short Title

Neonatal gut microbiomes concerning route of delivery

Authors

Nichapat Pahirah, M.D.*, Amarin Narkwichean, M.D., Ph.D.*, Malai Taweechotipatr, Ph.D.**, Sivaporn Wannaiampikul, Ph.D.***, Chinpanee Duang-Udom****, Wipada Laosooksathit, M.D.* *Department of Obstetrics and Gynecology, Faculty of Medicine, Srinakharinwirot University, Nakhon Nayok 26120, Thailand

**Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Thailand

***Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Bangkok, 10110, Thailand

****Faculty of Medicine, Srinakharinwirot University, Nakhon Nayok 26120, Thailand

Corresponding Author: Wipada Laosooksathit, M.D.Assistant Professor, Department of Obstetrics and Gynecology,

Faculty of Medicine, Srinakharinwirot University, Ongkharak, Nakhon Nayok 26120, Thailand *Telephone: 037-395-085 ext. 60811 Email address: Wipada@g.swu.ac.th*

Word count

For the main text 3,056 words

Abstract

Objective

The objective of the study was to investigate the differences in the gut microbiomes of neonates delivered via cesarean section compared to those born by vaginal delivery, and to identify the predominant microbial taxa present in each group.

Study design

A prospective observational study.

Setting

At Her Royal Highness Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University, Thailand.

Sample

Fecal sample obtained by 281 healthy neonates born between February 2021 and April 2023. The study population was divided into two groups: 139 neonates born via vaginal delivery and 142 neonates born via cesarean section.

Methods

The microbiota composition of each neonate's fecal sample was identified by using 16S ribosomal ribonucleic acid metagenomic sequencing.

Main Outcome Measures

Neonatal gut microbiome abundancy and diversity was identified according to route of delivery.

Results

Neonates delivered vaginally exhibited a gut microbiome with higher abundance and diversity than those delivered by cesarean delivery. Bifidobacterium was the dominant genus in both groups. *Bifidobacterium breve* was the dominant species and was significantly higher in cesarean-delivered neonates compared to those delivered vaginally (24.0% and 9.2%, respectively) ($p<0.0001$). However, the taxonomy of only 89 (64.0%) and 44 (31.43%) fecal samples could be identified from the vaginal and cesarean delivery groups, respectively.

Conclusions

Route of delivery is associated with neonatal gut microbiome abundancy and diversity. Neonates delivered via vaginal delivery exhibited higher diversity but lower abundancy of the dominant species in the gut microbiome.

Funding

Funding was provided by Thailand Science Research and Innovation.

Keywords: cesarean section, vaginal delivery, gut microbiome, neonates, 16S rRNA

Funding

The study received full support from Thailand Science Research and Innovation.

Introduction

Human gut microbiomes are the collection of bacteria, viruses, fungi, protozoa, and eukaryotes colonizing the human gastrointestinal tract. These microbiomes have high functional capacity and benefit the host in several ways. The diversity of gut microbiomes is shaped by the host and many environmental factors [1, 2]. Genetics, mode of delivery, diet, population, and location can diversify these gut microbes [1-4]. Loss of gut microbiome diversity and balance can cause diabetes, allergy, autoimmune diseases, and obesity[2, 5].

The changes in gut microbiome diversity during pregnancy increase some risks of pregnancy complications, such as gestational diabetes, preeclampsia, maternal infection, growth restriction, and intrauterine demise [4, 6]. Common bacteria in neonatal gut microbiomes are Actinobacteria (Bifidobacterium) [7], Firmicutes [7, 8], and Lactobacillus[9]. Studies show a relationship between maternal and neonatal gut microbiome diversity [4, 6]. The gut microbes can pass from mothers to babies via many routes, such as via the vagina, the gastrointestinal tract, the skin, breast milk, and subsequently colonize the infant's gut immediately after birth[4, 8]. The infant's immune system is an important system that can be affected by this gut microbiota diversity and potentially affect long-term outcomes [10]. Because the vagina is one of the routes by which mothers pass microbiotas on to their babies, the question of whether the route of delivery can affect the infant's microbiome can be posed.

The cesarean section rate has been increasing over the last few decades. Data from the World Health Organization (WHO) in 2021 showed the cesarean rate was 21%, compared with 7% in 1990[11]. Infants born via cesarean section have different exposures to substances from those born vaginally [5]. There has been conflict regarding whether the mode of delivery can alter a newborn's gut microbiome diversity.

There are many methods widely used to identify gut microbiomes, one of which is metagenomic sequencing. Using a 16S rRNA gene sequencing technique, uncultured microbiomes can be identified. It can provide information on the complete genome in less time and is more accurate than the classic method. This method is widely used today[12, 13].

Attempts to study neonatal outcomes of cesarean delivery related to gut microbiomes in early life have been made and are increasing in many countries. Yet, there have still been no studies carried out in Thailand. Understanding the differences in neonatal gut microbiomes between those delivered via vaginal delivery and those delivered by cesarean section in the Thai population, including the factors that can affect the diversity of gut microbiomes, may help researchers identify risk factors, preventive methods, or interventions to help promote the long-term outcomes of those delivered via cesarean section.

Methods

A prospective study aimed to investigate differences in gut microbiomes between neonates born via vaginal and cesarean delivery. We included term neonates delivered at the Srinakharinwirot University Hospital, Thailand, between February 2021 and April 2023. The study was approved by the Institutional Review Board (SWUEC-M/029/2564E) and registered with the Thai Clinical Trials Registry (TCTR20221024003). The sample size was calculated based on a gut Bifidobacterium prevalence of 36.6% in infants delivered by vaginal delivery compared to one of 48.6% in infants delivered by cesarean delivery [7], which resulted in samples of at least 131 for each group.

All healthy term neonates delivered at the institution were included. We excluded neonates born to mothers with prior infections, those whose mothers received antibiotics for cesarean delivery (except for pre-operative prophylaxis), those unable to provide a fecal specimen within 48 hours post-delivery, and those with neonatal complications or previous antibiotic use. Written consent was obtained from the mothers, and each patient's demographic data, route of delivery, neonatal outcomes, and timing of fecal collection were collected.

The study processes after data collection were fecal collection, genomic DNA extraction, purification, 16S ribosomal ribonucleic acid (rRNA) amplification, 16S rRNA library preparation, and sequencing (Figure 1).

Fecal collection

All fecal samples were collected within 48 hours using Zymo DNA/RNA Shield™ fecal collection tubes (Zymo Research, U.S.). At least 1 g or 1 ml of neonates' feces were collected, mixed, and then stored at 2-25˚C before being transferred into a -80˚C freezer for storageg. The date and time of fecal sample collection were recorded to determine the neonatal age at the time of sample collection.

Genomic DNA extraction

Genomic DNA (gDNA) was extracted from approximately 100 mg of each fecal sample using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, U.S.), according to the manufacturer's protocol. A NanoDrop™ spectrophotometer was used to quantify and assess the purification of the gDNA. The expected real-time quality score had to be > 7. However, the results obtained using the given protocol showed low DNA concentrations and inadequate purification for amplification. Therefore, we adjusted the protocol to achieve a better yield and more highly purified gDNA (See supplement).

16S rRNA amplification

The full length of 16S rRNA was amplified using 50 ng of gDNA with Q5® High-Fidelity 2X Master Mix (New England Biolabs, MA, USA) and the universal primers 27F (5'-AGRGTTYGATYMTGGCTCAG-3') and 1492R (5'-CGGYTACCTTGTTACGACTT-3') (Lane, 1991). The polymerase chain reaction (PCR) conditions were 98°C for 30 s, 35 cycles at 98°C for 10 s, 55°C for 30 s, 72°C for 1 min, and 72°C for 10 min. The PCR products were purified and concentrated using FavorPrep GEL/PCR Purification Mini Kit (Medibena, Austria). The purified PCR products were quantified and checked for purity by the NanoDrop machine (Thermo Fisher Scientific, USA). The concentration of purified PCR products was adjusted to 200 ng in 10 µl nuclease-free water.

16S rRNA library preparation and sequencing

The 16S rRNA libraries were prepared using a Rapid Barcoding Kit 24 V14 (SQK-RBK114.24) (Oxford Nanopore Technologies, UK) according to the manufacturer's protocol. The 24 barcodes were pooled in equal concentrations. The sequencing was performed using a MinION Flow Cell (R10.4.1) with a GridION sequencer (Oxford Nanopore Technologies, UK).

16S rRNA bioinformatic analysis

The 16S rRNA analysis workflow is shown in Figure 2. The results from the next-generation sequencing were in the FAST5 format. The FastQ files were generated from the results in the FAST5 format by using the Guppy program in MinKNOW (Oxford Nanopore Technologies, UK), in which real-time quality scores of more than 7 were filtered and demultiplexed. The FastQ passing quality was aligned to reference sequences in the NCBI database by using the FASTQ 16S workflow (version 2022.01.07), and the inclusion criteria were a minimum BLAST e-value of 0.01, minimum coverage of more than 30%, and a minimum identity of more than 77%. The data output files were generated as CSV files. Then, the percentage prevalence of each species and genus were calculated, and species and genus were filtered at a level of 0.5% for investigation of relative abundance.

Comparative taxonomy was performed to investigate the relative abundance of species between neonates delivered vaginally and those delivered by cesarean delivery.

Statistical analysis

The baseline characteristics were analyzed using descriptive statistics, i.e., mean ± standard deviation (SD), median with an interquartile range, and percentage, as appropriate. Beta-diversity was used to demonstrate the difference in diversity between groups. Student's T-test was used to compare the statistical differences in gut microbiome between neonates delivered by vaginal delivery and those delivered by cesarean delivery.

Results

A total of 281 neonates were enrolled in the study, consisting of 139 and 142 neonates in the vaginal delivery group (VG) and the cesarean section group (CS), respectively. Three cases from the VG group were excluded due to inability to provide a fecal sample within 48 hours, and two cases from the CS group were excluded due to preterm delivery, after a review of data. The mean fecal collection times were 18.6 (12.1) hours and 21.0 (14.8) hours in the VG and CS groups, respectively.

The sequencing successfully identified the microbial taxonomy of the gut in 89 (64.0%) samples of the VG group and 44 (31.43%) samples of the CS group. We used only these data for statistical analysis. The demographic data of the patients is presented in Table 1. Overall, the mean maternal age was 27.9 (5.1) years old. There were significantly higher levels of income, education status, and underlying maternal disease in the CS group. All mothers consumed all types of food except three cases in the CS group with seafood allergies.

The 16S rRNA sequencing results revealed higher total gut microbiome reads in the VG group (7,019,452) than in the CS group (3,359,444). Of those reads, 6,897,967 (98.27%) and 3,299,355 (98.21%) were classified as microbiomes in the VG and CS groups, respectively. The diversity of the neonates' gut microbiomes was greater in those born via vaginal delivery than those born via cesarean delivery. Of the species identified in this study, 17 (51.5%) were found exclusively in the VG group, while nine (27.3%) were found exclusively in the CS group. Only seven species (21.2%) were found in both groups (Figure 3).

Bifidobacterium was the dominant genus in both the VG group and the CS group. The proportion of Bifidobacterium was significantly higher in the CS group (Table 2). Among all the Bifidobacterium species identified,*Bifidobacterium breve* was the most dominant, followed by*Bifidobacterium longum.* In the 89 samples of the VG group, 24 bacteria species were found with a relative abundance percentage of more than 0.5%, and in the 44 samples of the CS group, 16 bacteria species were found with a relative abundance percentage of more than 0.5%. (Figure 4). A significantly higher abundance of Bifidobacterium, Enterobacter, and Enterococcus species was found in the CS group compared to the VG group (Table 2).

In the VG group, Clostridium (6.9%), Enterococcus (5.5%), Escherichia (4.5%), and Streptococcus (4.4%) were found to be the second most abundant genera in fecal specimens. Some, such as the genera Fusobacterium, Streptococcus, Bacteroides, Megamonas, and Escherichia, were found exclusively in the VG group. In the CS group, the most abundant genera after Bifidobacterium were Enterobacter (11.2%), Lactobacillus (7.8%), Enterococcus (7.2%), and Klebsiella (4.6%). However, opportunistic pathogens such as Enterococcus, Klebsiella, and Clostridium were found in both groups.

In the CS group, there was no significant difference in neonatal gut microbiome abundancy between those presenting with labor and those presenting without labor before cesarean delivery (Table 3).

Discussion

Main Findings

A lower diversity in gut microbiota was observed in neonates born via cesarean delivery compared to those born through vaginal delivery.*Bifidobacterium spp.* were predominant in both groups, with the abundance higher in the CS group

The diversity of gut microbiomes in neonates, which is significantly influenced by the mode of delivery, is consistent with findings from previous studies [10, 14]. A study conducted by Biasucci et al. in 2008, utilizing 16S rRNA methods and collecting fecal samples on the third day of life, indicated that the intestinal microbiota of neonates delivered by cesarean section appears less diverse regarding bacterial species. A later systematic review by Rutayisire et al. (2016) concluded that low total diversity of the gut microbiota during the first week of life was reported in infants delivered by cesarean section [15]. However, a recent study by Chu et al. in 2017 showed no difference in microbiota community function regardless of the delivery mode [16]. Studies on neonatal gut microbiomes exhibit variations such as timing of stool collection, sample size, and techniques used to identify bacterial genus/species. Furthermore, environmental and genetic factors also play a role in influencing individual gut microbiomes.

A significantly higher abundance of Bifidobacterium found in the CS group is consistent with a study by Azad et al [7]. However, other studies have reported higher levels of Bifidobacterium in vaginal delivery groups [10, 17]. Bifidobacterium is an essential bacterium providing the most common genera in the infant gut microbiome. *Bifidobacterium breve* (*B. breve)* and*Bifidobacterium longum* (*B. longum)* are common species and are more prevalent during infancy than adulthood, especially during the first year of life. Bifidobacterium is involved in various physiological and immunological functions such as the digestion of human milk oligosaccharides, improving gut barrier function, and reducing intestinal permeability [18-20]. Lower levels of*B. longum* may be associated with allergic diseases such as atopic dermatitis [21, 22], but the outcome of such bacterial abundancy has not been well stated. Some studies have shown the benefit of high levels of Bifidobacterium; however, a decrease in Bifidobacterium colonization early in life may increase the risk of neonatal complications [23].

Consistent with previous studies, our study found lower levels of Bacteroides in the CS group [7, 24], and several studies have shown delayed colonization of Bacteroides[22, 24, 25]. Bacteroides is the major genus found in adults, but these babies had lower Bacteroides levels since they lacked exposure to it during cesarean delivery. This may result in a negative impact on infant immune development, maintenance of intestinal homeostasis [26], and, later, food digestion[27].

In this study, *Staphylococcus aureus* (*S. aureus*) was higher in the CS group, consistent with the study of Shao et al.[24]. The proposed source of *S. aureus*colonization was maternal skin flora [28]. Intestinal colonization with *Staphylococcus aureus* is associated with higher levels of inflammation, which contribute to the development of inflammatory diseases such as asthma and allergies, including atopic dermatitis, or atopic eczema [29], and food allergies[30].

We identified higher levels of *C. perfringens* in the VG group, comprising 5.08% of all identified species, consistent with a previous study [7]. This may be attributed to initial gut colonization from maternal vaginal and fecal microbiota. Association with a higher risk of developing necrotizing enterocolitis, especially in preterm infants [31, 32], and prolonged diarrhea[33] has been found.

Strengths and Limitations

Our study had several strengths. Firstly, it was the first to report data on the differences in gut microbiome between neonates delivered via vaginal delivery and those delivered by cesarean delivery in Thailand. Secondly, we collected all fecal samples from healthy neonates within 48 hours, and neonates whose mothers had used antibiotics were not included in the study, except when prophylactic antibiotics had been used for cesarean delivery. Thirdly, the adjusted DNA extraction protocol can be used as an alternative technique in subsequent studies to create the proper meconium-specific protocol for analyzing a neonate's gut microbiome.

The limitation of our study was the potential confounding effect of breastfeeding practices on a neonate's gut microbiome. Thus, collection of the first passing of meconium may represent an opportunity to access the neonatal gut microbiome directly after delivery[34]. Second, we have not stated the details of cesarean delivery, such as presenting with membrane rupture before cesarean delivery. These variations may result in different neonatal gut microbiomes.

Interpretation

There were many explanations for this lower diversity. Firstly, there was a lack of transmission of gut microbiome from mother to child through the vaginal route. Secondly, there was a lack of or delayed colonization with Bacteroides [8, 35], which can be persistent [24] or delayed by up to 1 year in some infants [8]. And finally, empirical antibiotics were used in cesarean deliveries [36].

The reasons behind elevated Bifidobacterium levels in the gut microbiomes of cesarean-delivered infants in some studies are not yet fully understood [7, 10]. Although most studies associate vaginal delivery with higher Bifidobacterium levels, some studies present different findings [8, 27]. Potential explanations include the use of antibiotic prophylaxis for cesarean delivery, which could lead to a decrease in overall gut microbial diversity or selectively eliminate certain bacterial species[7, 8]. For instance, Bifidobacterium species may be

sensitive only to penicillin administration, not cephalosporins[8, 27]. Nonetheless, conflicting results in other studies were observed. For instance, Jakobsen et al.[8] did not observe a significant difference in infant gut microbiome between mothers who had received prophylactic antibiotics and those who did not. Yassour et al. demonstrated a lower level of Bifidobacterium species in infants born via cesarean section and exposed to antibiotics, including cefazolin, compared to infants not exposed to antibiotics [36]. Our institutional protocol is to administer cefazolin before making a skin incision on the mother in the CS group. Cephalosporin does not affect maternal microbiomes[37]; however, there has been limited research on the specific effects of cefazolin on Bifidobacterium levels in the infant gut microbiome. Therefore, we cannot draw a definite conclusion regarding the influence of empirical antibiotic use on the abundance of Bifidobacterium in our study.

Another hypothesis suggests that early breastfeeding may influence Bifidobacterium levels in infants, as breastfed infants are more likely to have higher levels due to the utilization of oligosaccharides as a food source [1]. Cesarean-delivered infants are more likely to experience delays in breastfeeding initiation, potentially affecting Bifidobacterium abundance [38, 39]. However, in our hospital, there is a policy of early breastfeeding for all mothers, making it uncertain whether this could confound our study. One of the challenges in obtaining information about breastfeeding practices was the reliance on subjective self-reporting by mothers, which could introduce informative bias on aspects such as timing, frequency, and the amount of breast milk.

In the future, exploring the impact of antibiotic prophylaxis on the gut microbiome of infants born through cesarean delivery and its long-term health effects is interesting. Moreover, considering that gut microbiota diversity tends to diminish after six months of life[15], efforts should be directed toward identifying factors that restore healthy gut microbiota.

Our study has limitations, with 47 (34.6%) and 96 (68.6%) samples in the VG and CS groups being unsuccessfully sequenced. This may be attributed to sterility or very low bacterial abundance, particularly in the CS group, where the tar-like meconium posed extraction difficulties due to its low biomass microbiome and high PCR inhibitor concentration[40]. Meconium analysis lacks standardized sequencing techniques for microbial assessment of the gut. We adjusted our extraction protocol to enhance DNA yield, yet uninterpreted samples suggest a potentially minimal bacterial presence. Future research should employ additional techniques to achieve clearer conclusions.

Conclusions

Neonates born via vaginal delivery exhibited higher diversity but lower dominant bacterial abundancy in the gut microbiome compared to those born via cesarean delivery. The dominant genus in neonates' gut microbiomes was Bifidobacterium, with a statistically significant higher abundance observed in infants born via cesarean delivery. Many evidence suggest that gut microbiome can influence future health, this highlights the careful consideration of route of delivery that may affect individual well-being in the future. Moreover, our findings are fundamental to future investigation on replenishing neonatal gut microbiome in neonates born by cesarean section.

Acknowledgment

The authors would like to thank Dr.Chanon Kunasol for research consultation and assistance with the laboratory process, the Thailand Science Research and Innovation (TSRI) for providing funding (Fundamental Fund) for the research, and the Faculty of Medicine, Srinakharinwirot University, for affording the opportunity to work on this project.

Authors Contributions

Nichapat Pahirah Conceptualization, Investigation, Methodology, Writing

Amarin Narkwichean Conceptualization, Project administration, Funding acquisition

Malai Taweechotipatr Conceptualization, Formal analysis, Project administration, Funding acquisition

Sivaporn Wannaiampikum Data curation, Formal analysis

Chinphanee Duang-Udom Data curation, Formal analysis

Wipada Laosooksathit Conceptualization, Investigation, Methodology, Writing

Ethics Approval

The study was approved by the Institutional Review Board (SWUEC-M/029/2564E), Faculty of medicine, Srinakharinwirot University, Thailand. Registered was performed with the Thai Clinical Trials Registry (TCTR20221024003).

Funding

Funding was provided by Thailand Science Research and Innovation.

Conflicts of Interest

The authors declare that there is no conflict of interest.

References

1. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474(11):1823-36.

2. Hasan N, Yang H. Factors affecting the composition of the gut microbiota, and its modulation. PeerJ. 2019;7:e7502.

3. Mahnic A, Rupnik M. Different host factors are associated with patterns in bacterial and fungal gut microbiota in Slovenian healthy cohort. PLOS ONE. 2018;13(12):e0209209.

4. Yao Y, Cai X, Chen C, Fang H, Zhao Y, Fei W, et al. The Role of Microbiomes in Pregnant Women and Offspring: Research Progress of Recent Years. Frontiers in Pharmacology. 2020;11.

5. Sandall J, Tribe RM, Avery L, Mola G, Visser GHA, Homer CSE, et al. Short-term and long-term effects of caesarean section on the health of women and children. The Lancet. 2018;392(10155):1349-57.

6. Amir M, Brown JA, Rager SL, Sanidad KZ, Ananthanarayanan A, Zeng MY. Maternal Microbiome and Infections in Pregnancy. Microorganisms. 2020;8(12).

7. Meghan BA, Theodore K, Heather M, David SG, Catherine JF, Radha SC, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. Canadian Medical Association Journal. 2013;185(5):385.

8. Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut. 2014;63(4):559-66.

9. Mitsou EK, Kirtzalidou E, Oikonomou I, Liosis G, Kyriacou A. Fecal microflora of Greek healthy neonates. Anaerobe. 2008;14(2):94-101.

10. Biasucci G, Benenati B, Morelli L, Bessi E, Boehm G. Cesarean Delivery May Affect the Early Biodiversity of Intestinal Bacteria1,2. The Journal of Nutrition. 2008;138(9):1796S-800S.

11. Ana Pilar B, Jiangfeng Y, Ann-Beth M, João Paulo S, Jun Z. Trends and projections of caesarean section rates: global and regional estimates. BMJ Global Health. 2021;6(6):e005671.

12. Allaband C, McDonald D, Vázquez-Baeza Y, Minich JJ, Tripathi A, Brenner DA, et al. Microbiome 101: Studying, Analyzing, and Interpreting Gut Microbiome Data for Clinicians. Clinical Gastroenterology and Hepatology. 2019;17(2):218-30.

13. Winand R, Bogaerts B, Hoffman S, Lefevre L, Delvoye M, Van Braekel J, et al. Targeting the 16S rRNA Gene for Bacterial Identification in Complex Mixed Samples: Comparative Evaluation of Second (Illumina) and Third (Oxford Nanopore Technologies) Generation Sequencing Technologies. International Journal of Molecular Sciences [Internet]. 2020; 21(1).

14. Grönlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. J Pediatr Gastroenterol Nutr. 1999;28(1):19-25.

15. Rutayisire E, Huang K, Liu Y, Tao F. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. BMC Gastroenterol. 2016;16(1):86.

16. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat Med. 2017;23(3):314-26.

17. Huurre A, Kalliomäki M, Rautava S, Rinne M, Salminen S, Isolauri E. Mode of Delivery – Effects on Gut Microbiota and Humoral Immunity. Neonatology. 2007;93(4):236-40.

18. Wong CB, Iwabuchi N, Xiao J-z. Exploring the Science behind Bifidobacterium breve M-16V in Infant Health. Nutrients [Internet]. 2019; 11(8).

19. Ewaschuk JB, Diaz H, Meddings L, Diederichs B, Dmytrash A, Backer J, et al. Secreted bioactive factors from Bifidobacterium infantis enhance epithelial cell barrier function. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2008;295(5):G1025-G34.

20. Sela DA, Mills DA. Nursing our microbiota: molecular linkages between bifidobacteria and milk oligosaccharides. Trends in Microbiology. 2010;18(7):298-307.

21. Fang Z, Pan T, Li L, Wang H, Zhu J, Zhang H, et al. Bifidobacterium longum mediated tryptophan metabolism to improve atopic dermatitis via the gut-skin axis. Gut Microbes. 2022;14(1):2044723.

22. Kim J, Kim BE, Leung DYM. Pathophysiology of atopic dermatitis: Clinical implications. Allergy and Asthma Proceedings. 2019;40(2):84-92.

23. Saturio S, Nogacka AM, Alvarado-Jasso GM, Salazar N, de los Reyes-Gavilán CG, Gueimonde M, et al. Role of Bifidobacteria on Infant Health. Microorganisms [Internet]. 2021; 9(12).

24. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, Simpson N, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. Nature. 2019;574(7776):117-21.

25. Long G, Hu Y, Tao E, Chen B, Shu X, Zheng W, et al. The Influence of Cesarean Section on the Composition and Development of Gut Microbiota During the First 3 Months of Life. Frontiers in Microbiology. 2021;12.

26. Zafar H, Saier MH. Gut Bacteroides species in health and disease. Gut Microbes. 2021;13(1):1848158.

27. Zhang C, Li L, Jin B, Xu X, Zuo X, Li Y, et al. The Effects of Delivery Mode on the Gut Microbiota and Health: State of Art. Frontiers in Microbiology. 2021;12.

28. Lindberg E, Adlerberth I, Hesselmar B, Saalman R, Strannegård I-L, Åberg N, et al. High Rate of Transfer of Staphylococcus aureus from Parental Skin to Infant Gut Flora. Journal of Clinical Microbiology. 2004;42(2):530-4.

29. Alghamdi HA, Behieldin A, Edris S. Gut microbiome skin axis in the development of atopic dermatitis. J Pak Med Assoc. 2021;71(4):1221-7.

30. Acton DS, Tempelmans Plat-Sinnige MJ, van Wamel W, de Groot N, van Belkum A. Intestinal carriage of Staphylococcus aureus: how does its frequency compare with that of nasal carriage and what is its clinical impact? European Journal of Clinical Microbiology & Infectious Diseases. 2009;28(2):115-27.

31. Kaplina A, Kononova S, Zaikova E, Pervunina T, Petrova N, Sitkin S. Necrotizing Enterocolitis: The Role of Hypoxia, Gut Microbiome, and Microbial Metabolites. International Journal of Molecular Sciences [Internet]. 2023; 24(3).

32. Fallani M, Rigottier-Gois L, Aguilera M, Bridonneau C, Collignon A, Edwards CA, et al. Clostridium difficile and Clostridium perfringens species detected in infant faecal microbiota using 16S rRNA targeted probes. Journal of Microbiological Methods. 2006;67(1):150-61.

33. Ramamurthy T, Kumari S, Ghosh A. Chapter Six - Diarrheal disease and gut microbiome. In: Das B, Singh V, editors. Progress in Molecular Biology and Translational Science. 192: Academic Press; 2022. p. 149-77.

34. Turunen J, Tejesvi MV, Paalanne N, Pokka T, Amatya SB, Mishra S, et al. Investigating prenatal and perinatal factors on meconium microbiota: a systematic review and cohort study. Pediatric Research. 2023.

35. Arrieta M-C, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The Intestinal Microbiome in Early Life: Health and Disease. Frontiers in Immunology. 2014;5.

36. Yassour M, Vatanen T, Siljander H, Hämäläinen A-M, Härkönen T, Ryhänen SJ, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Science Translational Medicine. 2016;8(343):343ra81-ra81.

37. Se Jin S, Maria Gloria D-B, Rob K. How delivery mode and feeding can shape the bacterial community in the infant gut. Canadian Medical Association Journal. 2013;185(5):373.

38. Hobbs AJ, Mannion CA, McDonald SW, Brockway M, Tough SC. The impact of caesarean section on breastfeeding initiation, duration and difficulties in the first four months postpartum. BMC Pregnancy and Childbirth. 2016;16(1):90.

39. Li L, Wan W, Zhu C. Breastfeeding after a cesarean section: A literature review. Midwifery. 2021;103:103117.

40. Stinson LF, Keelan JA, Payne MS. Comparison of Meconium DNA Extraction Methods for Use in Microbiome Studies. Frontiers in Microbiology. 2018;9.

Figure legends

Figure 1: Steps of the process performed.

Figure 2: Data analysis of next-generation sequencing workflow.

Figure 3. Genus of gut microbiome profiles generated by 16s rRNA full length gene using Oxford nanopore sequencer. 89 samples of VG group represented 16 bacteria genera with percentage of relative abundance more than 0.5% and 44 samples of CS group represented 10 bacteria genera with percentage of relative abundance more than 0.5%.

Figure 4. Species of gut microbiome profiles generated by 16s rRNA full length gene using Oxford nanopore sequencer.

Table legends

Table 1. Patient's demographic data.

Table 2 Comparison the abundancy of taxon species between neonate's gut microbiome delivered via vaginal delivery and cesarean delivery.

Table 3 Comparison of the abundancy of taxon species between neonates' gut microbiome delivered via cesarean delivery with and without labor.

Hosted file

`Figure 1.docx` available at https://authorea.com/users/778168/articles/912797-comparison-of-gut-microbiomes-between-neonates-born-by-cesarean-section-and-vaginal-delivery-prospective-observational-study

Hosted file

Figure 2.docx available at https://authorea.com/users/778168/articles/912797-comparison-of-gut-microbiomes-between-neonates-born-by-cesarean-section-and-vaginal-delivery-prospective-observational-study

Hosted file

Figure 3.docx available at https://authorea.com/users/778168/articles/912797-comparison-of-gut-microbiomes-between-neonates-born-by-cesarean-section-and-vaginal-delivery-prospective-observational-study

Hosted file

Figure 4.docx available at https://authorea.com/users/778168/articles/912797-comparison-of-gut-microbiomes-between-neonates-born-by-cesarean-section-and-vaginal-delivery-prospective-observational-study

Hosted file

Table 1.docx available at https://authorea.com/users/778168/articles/912797-comparison-of-gut-microbiomes-between-neonates-born-by-cesarean-section-and-vaginal-delivery-prospective-observational-study

Hosted file

Table 2.docx available at https://authorea.com/users/778168/articles/912797-comparison-of-gut-microbiomes-between-neonates-born-by-cesarean-section-and-vaginal-delivery-prospective-observational-study

Hosted file

Table 3.docx available at https://authorea.com/users/778168/articles/912797-comparison-of-gut-microbiomes-between-neonates-born-by-cesarean-section-and-vaginal-delivery-prospective-observational-study

Hosted file

Supplementation.docx available at https://authorea.com/users/778168/articles/912797-comparison-of-gut-microbiomes-between-neonates-born-by-cesarean-section-and-vaginal-delivery-prospective-observational-study