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Analysis of the gut microbiota using MALDI-TOF and cultural techniques in breastfed infants delivered vaginally and through caesarean section

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ABSTRACT

Objectives: The gastrointestinal tract of newborns is colonised immediately after birth with microbes from the environment, mainly from the mother. According to studies, the early neonatal microbiota is crucial for developing the postnatal immune system. The objective of this study was to investigate the relationship between neonatal gut flora and the mode of delivery during gut microbiota colonisation.

Material and Methods: A total of 30 neonates – 16 born vaginally and 14 by caesarean section – participated in this study of the intestinal bacterial composition at 3 days of age. Stool cultures and matrix-assisted laser desorption ionisation time-of-flight analyses determine aerobic and anaerobic bacterial species.

Results: Neonates delivered by caesarean section appeared to have a less diverse gut microbiota regarding bacterial species than vaginally delivered neonates. *Bifidobacterium* species are absent from the gut microbiota after caesarean delivery. Even though every newborn vaginally born had a unique microbial profile, the most prevalent bacterial species were *Streptococcus* spp., *Veillonella atypica*, *Bacteroides vulgatus* and *Bifidobacterium* spp.

Conclusion: Our results suggest that the mode of birth significantly influences the gut microbiota composition in the 1st year of human life. This study opens the path to further investigations to confirm the link between microbiota composition and enterotypes of the gut microbiome of breastfed neonates. In addition, we underline the importance of MALDI-TOF for species-level identification of organisms within a fraction of a second.

Keywords: Neonates, Vaginal delivery, Caesarean delivery, Gut microbiota

INTRODUCTION

The largest microbiota in the human body is found in the gastrointestinal tract (GIT), with approximately 10^{13}–10^{14} microorganisms. The foetal intestine is sterile and surrounded by amniotic fluid. The gut microbiome is essential for human health and disease.[1] In contrast to the adult gut microbiome, premature infants have a relatively dynamic and unstable microbiome.[1,2] As a source of nutrient influx, GIT is an ideal environment for microbial colonisation.[3] The diversity of the intestinal microbiome could significantly affect human body functions.[4]

Exclusive breastfeeding is essential throughout the first 6 months of life, according to the World Health Organisation (WHO), which makes this claim in its global strategy. The WHO

recommends early breastfeeding initiation within the 1st hours after birth.[5] Researchers using neonatal stool culture in the late 1950s were able to study the development of the gut microbiota after birth.[6] Several studies have isolated probiotic bacteria from human breast milk.[7] Infant formula lacks the diverse bacterial communities found in human breast milk.[8] Breastfed infants have a gut microbiota composed predominantly of *Bifidobacteria*, whereas formula-fed infants have a more heterogeneous composition with a lower proportion of *Bifidobacteria*.[9] Traditionally, *Lactobacillus* and *Bifidobacterium* species are most commonly used as probiotics in humans and are present in breast milk.[10]

Breastfeeding reduces the risk of ear infections, respiratory infections, diarrhoea and sudden infant death syndrome in newborns. Lower risks of diabetes, food allergies and asthma are other benefits.[11] Colonisation of the neonatal microbiota begins in utero.[12] The delivery method and the end of breastfeeding are essential for developing a gut microbiota similar to that of an adult. During the 1st year of life, the microbial composition changes abruptly. In younger and older adults, the gut microbiota changes gradually over time.[12] The gut microbiota influences the growth and differentiation of intestinal epithelial cells and plays a critical role in nutrition, metabolism, immunology and protection.[2]

Using matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry, it is possible to characterise and identify microorganisms quickly, precisely and affordably. The current study, focusing on culture and MALDI-TOF analyses, investigates the effects of the mode of delivery on the gut microbiota of breastfed infants. During vaginal delivery, the newborn is exposed to various maternal microorganisms, some of which colonise the infant's gut. Caesarean sections prevent vertical transmission of intestinal microbes by not exposing the newborn to the mother's microbes.[13]

Healthy individuals can be classified by the differential enrichment of microbial taxa in their gut. These enriched taxa, termed enterotypes, are independent of age, sex, body mass index and region.[14] For Firmicutes/Bacteroidetes and Proteobacteria/Actinobacteria, the ratio of taxa abundance at the strain level was calculated using the relative abundances of taxa at the country level.[14]

The enterotypes 'P-type', 'A-type', 'F-type' and 'B-type', which are relatively abundant in Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, respectively, were found in the overall comparison. Regional variations exist in the makeup of the four enterotypes.[14,15]

There is compelling evidence that the early neonatal microbiota's composition has a significant impact on postnatal immune development. Immediately after birth, microorganisms from the environment, primarily the mother, colonise the neonatal GIT.

The present study investigated the relationship between the neonatal gut microbiota and delivery technique. 16 infants born vaginally and 14 by caesarean section participated in studying intestinal bacterial composition on the 3rd day of life. Aerobic and anaerobic bacteria were differentiated using species-level MALDI-TOF analyses and anaerobic and aerobic cultures. By identifying and assessing the gut microflora of infants, researchers can determine how maternal-infant contact, feeding style and birth affect early microbiota development. This will allow a better understanding of the relationship between the gut microbiota and the type of feeding and delivery. Further analysis will be possible if probiotics and prebiotics influence microbiota development and treat or avert disease.

MATERIAL AND METHODS

A prospective study was conducted in the microbiology research centre laboratory. Samples were collected from hospitalised newborns at Father Muller Medical College Hospital in Kankanady, Mangalore, India. Medical records of infants admitted to the neonatal intensive care unit, paediatric intensive care unit and paediatric wards were reviewed.

The Ethics Committee of FMIEC, Mangalore, approved the study (Reg. No. FMIEC/CCM/755/2022), and the participants were recruited after obtaining written informed consent from the mothers.

The participants were newborns admitted to the neonatal unit of a hospital in Mangalore, India. Newborns who were exclusively breastfed and delivered both vaginally and by caesarean section were included in the study. Critically ill patients, mixed-breastfed infants, neonates who died or were discharged within 24 hours and neonates who received prebiotics and probiotics were excluded.

Data collection and analysis

A structured data collection form was used between December 2022 and February 2023 to collect information from hospitalised neonates and their mothers on their demographic data, clinical and laboratory data, antibiotic treatment and discharge records. For calculating the ratio of taxa abundance at the phylum level (Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria), the country-level relative abundances of the taxa were used.

Sample collection

A fresh stool sample was collected in a sterile, clean container, taken to the laboratory without delay and processed for aerobes and anaerobes.

Sample preparations

The media used to process aerobes were Blood agar (BA) and MacConkey agar (MAC). Samples were inoculated at BA and MAC and then incubated at 37°C for 24–48 h. Isolates were subjected to MALDI-TOF analysis.

For anaerobes, the faeces were inoculated onto anaerobic media such as Robertson's Cooked Meat Medium (RCM), Laked BA, Neomycin BA and *Bacteroides* Bile Esculin agar plates. A metronidazole disc (5 µg) was placed in the plates for the presumptive identification of anaerobes and incubated in an anaerobic jar (BD GasPak jar EZ). An anaerobe pack was used for achieving anaerobiosis, which is a paper sachet, preserved in a foil bag that absorbs O_2 and generates carbon dioxide, immediately in contact with air. The jar was incubated for 48–72 h at 37°C. The jar was opened after incubation, and the colonies that showed a definite zone of inhibition to metronidazole were presumptively identified as anaerobes. The Anaerobic Laboratory Manuals were used to further characterise the isolates based on aerotolerance, Gram stain, colony morphology and biochemical reactions.[16-18] As mentioned, RCM, a supplemental medium, was incubated for 24 hours and subcultured on the anaerobic media. Identification was carried out using MALDI-TOF analysis at the species and genus levels.

MALDI-TOF extended direct transfer method

After colonies appear on the plate, MALDI-TOF will be performed for species-level identification. An isolated colony was smeared as a thin film directly onto an empty position on the MALDI target plate, overlaid the material with 1.0 µL of 70% formic acid and dried at room temperature within 30 min. After drying at room temperature, the smear was overlayed with 1 µL of matrix solution (α- Cyano-4-hydroxycinnamic acid - HCCA) and dried at room temperature,[18-21] and the sample plate was put into the MALDI-TOF system, the patient's demographic information was entered and the acquisition process began.

Statistical analysis

Statistical data were analysed using a Microsoft Excel spreadsheet. The Chi-square tests and Fisher tests were also performed using the Statistical Package For The Social Sciences (SPSS). Data were collected using a self-designed form that included information on feeding habits, feeding type, frequency, demographics, anthropometric status, diagnosis, drug therapy and other relevant information.

RESULTS

Infants delivered vaginally have a more diversified gut flora than neonates delivered by caesarean. A lack of *Bifidobacterium* species in the gut microbiota following caesarean delivery is remarkable. Even though they displayed individual microbial profiles, vaginally delivered neonates were characterised by prominent species such as *Streptococcus* spp., *Veillonella atypica*, *Bacteroides vulgatus*, *Bifidobacterium* spp. and *Escherichia coli*. The caesarean-delivered neonates show predominant group organisms such as *Streptococcus* spp. and *Clostridium* spp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The intestinal microorganisms of breastfed infants born vaginally and by caesarean section are compared in [Table 1]. Significant differences were observed in *Streptococcus* spp., *Enterococcus* spp. and *Clostridium* spp., but no significant difference in *Staphylococcus* spp., Gram-negative bacilli, *Bifidobacterium* spp., *B. vulgatus* and *V. atypica*.

The gut enterotypes from vaginally and caesarean-born, breastfed infants are shown in [Table 2]. Our study revealed that infants in India predominantly belong to P-type and F-type enterotypes. There is no significant difference between the intestinal enterotypes of vaginally and caesarean-born neonates.

However, *Bifidobacterium* spp., *Streptococcus* spp., *Staphylococcus* spp., *E. coli* and *Klebsiella* species were

Table 1: Comparison of gut microorganisms in vaginally and caesarean-born, breastfed infants.

Categories	Organisms	Vaginal delivery (*n*=16)	Caesarean delivery (*n*=14)	*P*-value
Gram-positive cocci	*Streptococcus* spp. (*parasanguinis*, *anginosus* and *salivarius*)	4	0	**0.04**
	Staphylococcus spp. (*aureus*, *haemolyticus*, *epidermidis, hominis and caprae*)	7	6	0.96
	Enterococcus spp. (*faecium* and *faecalis*)	0	7	**0.002**
Gram-negative bacilli	*Escherichia coli*	9	5	0.26
	Klebsiella pneumoniae	6	7	0.49
	Pseudomonas aeruginosa	1	2	0.46
Anaerobes	*Bifidobacterium* spp.	3	0	0.08
	Veillonella atypica	1	0	0.34
	Clostridium spp.	0	3	**0.05**
	Bacteroides vulgatus	1	0	0.34

Bold values represent statistically significant values.

Table 2: Comparison of gut enterotypes from vaginally and caesarean-born, breastfed infants.

Groups	Organisms	Mode of delivery			
		Vaginal delivery	Enterotypes	Caesarean delivery	Enterotypes
Firmicutes	*Streptococcus* spp. (*parasanguinis, anginosus* and *salivarius*)	**4**	**FFFF**	0	-
Firmicutes	*Staphylococcus* spp. (*aureus, caprae, hominis haemolyticus* and *epidermidis*)	**7**	**FFFFFFF**	6	FFFFFF
Firmicutes	*Enterococcus* spp. (*faecium* and *faecalis*)	0	-	**7**	**FFFFFFF**
Proteobacteria	*Escherichia coli*	**9**	**PPPPPPPPP**	5	PPPPP
Proteobacteria	*Klebsiella pneumoniae*	6	PPPPPP	**7**	**PPPPPPP**
Proteobacteria	*Pseudomonas aeruginosa*	1	P	**2**	**PP**
Actinobacteria	*Bifidobacterium* spp.	**3**	**AAA**	0	-
Firmicutes	*Veillonella atypica*	**1**	**F**	0	-
Firmicutes	*Clostridium* spp.	0	-	**3**	**FFF**
Bacteroidetes	*Bacteroides vulgatus*	**1**	**B**	0	-

F: Firmicutes, B: Bacteroidetes, P: Proteobacteria, A: Actinobacteria, Bold values represent significant enterotypes and highest culture positive results. Numeric values indicate the difference in colonisation in vaginally born and caesarean born babies. *Bifidobacterium* spp., *Streptococcus* spp., primarily colonised in vaginally born neonates but not in delivered by caesarean section.

primarily colonised in vaginally born neonates. *E. coli, Enterococcus* spp., *K. pneumoniae, Staphylococcus* spp. and *Clostridium* spp. were the most prevalent bacteria found in the colons of neonates delivered by caesarean section.

Table 3 shows the characteristics of the study population by birth type and maternal demographics. Compared to female infants (42.8%), most male infants (56.2%) were delivered vaginally. The mean value of the mother undergone vaginal delivery in the study population was 27.6 and caesarean-delivered mothers 26.

DISCUSSION

Mode of delivery and postnatal diet are two of the most significant factors affecting the gut microbiota, according to Adamek *et al*.'s study on the development of the gut microbiota during pregnancy and the early childhood years.[4] The composition of the gut flora is influenced by pre- and probiotic supplementation, antibiotic therapy, gestational age at birth and other environmental factors. The complexity of the flora ensures that its composition is unique to each individual.[4]

The gut microbiome influences immunologic, endocrine and neuronal pathways and plays a vital role in child development. Several factors influence the colonisation of the infant gut microbiome. Different microbial colonisation patterns are related to vaginal and caesarean delivery, antibiotic exposure and infant feeding habits. Infant microbial colonisation patterns can potentially affect long-term physical and neurocognitive development and disease risk due to the broad physiological impact.

Table 3: Characteristics of the mothers and their breastfed infants according to the mode of birth.

Variables	Vaginal delivery	Caesarean delivery
Gender, *n* (%)		
Female	7 (43.75)	**8 (57.14)**
Male	**9 (56.25)**	6 (42.86)
Mother's age (Mean±SD)	27.6±4.71	26±4.47
Gestational age/week (Mean±SD)	**38.2±2.04**	37.5±1.84
Baby's birth weight (Mean±SD)	**3026±543**	2703±736
Primigravida, *n* (%)	7 (43.75)	6 (42.86)
Multigravida, *n* (%)	**9 (56.25)**	**8 (57.14)**
Mothers haemoglobin (Before delivery) (Mean±SD)	11.36±0.72	11.55±0.90
Aerobes, *n* (%)	15/16 (93.75)	14/14 (100)
Anaerobes, *n* (%)	4/16 (25)	3/14 (21.43)
No growth, *n* (%)	1/16 (6.25)	0/14 (00)
Single isolates, *n* (%)	4/16 (25)	3/14 (21.43)
Mixed population, *n* (%)	11/16 (68.75)	11/14 (78.57)

SD: Standard deviation, Bold values represent significant results in comparison with other values.

Understanding these influences will impact neonatal care and parenting. Long-term gut microbiota dysbiosis can have long-lasting functional effects and lead to various diseases. Changes in the microbiota significantly increase the risk of obesity, diabetes, asthma and food allergies. Infants born vaginally had higher concentrations of *Bacteroides, Bifidobacteria* and *Lactobacillus* in the first few days of life and showed more significant microbial variability in subsequent weeks. *Staphylococcus, Streptococcus* and *Clostridium* predominate in the less diverse and similar

microbiome of infants born through caesarean section to that of the mother's skin and hospital environment.

Newborns receive a wider variety of colonising microorganisms that help build and prime the immune system from the maternal vaginal microbiota. Vaginal birth is the ideal birth route, and a caesarean section should be performed if there are medical indications. In our study, we compared the faecal microbiota of 30 breastfed infants depending on the mode of delivery. *Enterococcus faecium, Enterococcus faecalis* and *Clostridium* spp. were higher in infants born through caesarean section. Infants born vaginally had higher levels of *Streptococcus parasanguinis, Streptococcus anginosus, Streptococcus salivarius* and *Bifidobacterium bifidum.*

Our results suggest that the delivery mode strongly influences newborns' gut bacteria during the first 3 days of life. Infants delivered vaginally and by caesarean section appear to have different gut flora.

According to Layuk *et al*.,[5] breast milk contains various prebiotics passed from mother to child through breastfeeding. The most prevalent prebiotics vary depending on the type of breast milk and include different amounts of *Lactobacillus* spp., *Bifidobacterium* spp. and *Staphylococcus* spp.[5] *Bifidobacterium* spp. is also a component of the breast milk microbiota but was found only in the faecal microbiota of vaginally delivered infants in the present study. Human milk, in addition to the birth canal, is a significant source of *Bifidobacterium* and contributes to the establishment of this genus in the gut microbiota of infants.

By global comparison, mainly four different enterotypes, referred to as 'P-type', 'A-type', 'F-type' and 'B-type', which were relatively abundant in Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, respectively. The compositions of the four enterotypes differ geographically. Some studies have reported that there were no significant differences in the faecal relative abundance of *Bifidobacterium* between caesarean-delivered and vaginally delivered infants; however, the prevalence of the species *Bifidobacterium longum* was lower in caesarean. *Akkermansia* and *Kluyvera* were more abundant in the faeces of caesarean newborns, whereas *Bacteroides* was similarly less abundant.[20-24] However, in our study, we noticed a significant difference in the abundance of *Bifidobacterium* between caesarean-delivered and vaginally delivered infants. All infants delivered by both routes had *E. coli, K. pneumonia* and *Staphylococcus* spp. in their faeces.

A study by Shao *et al*. reported the disrupted transmission of maternal *Bacteroides* strains and high-level colonisation by opportunistic pathogens associated with the hospital environment, including *Enterococcus*, *Enterobacter* and *Klebsiella* species in babies delivered by caesarean section.[24] In our study, beneficial organisms such as *Bifidobacterium*, *B. vulgatus*, *Streptococcus* and *Veillonella* species are found in infants delivered by the vaginal route, and pathogenic bacteria such as *Klebsiella, Enterococcus* and *Clostridium* species were colonised in babies delivered by caesarean section.

CONCLUSION

In summary, these studies show that delivery technique significantly affects the gut microbiota composition during the neonatal period, which may affect the gut biota of early childhood. The beneficial effects of the gut microbiota have long been known; it also plays an essential role in immunity against pathogenic microbes. Our findings indicate that the manner of delivery significantly impacts the gut microbiota's composition during the neonatal period, which may impact the gut microbiota of young children. In addition, our findings demonstrate that probiotic producers such as *Bifidobacterium* spp. and *Veillonella* spp. are more prevalent in the gut microbiota of infants born vaginally than in the gut microbiota of infants born through caesarean section. Healthcare providers could use this information to develop strategies to promote healthy gut microbiota in newborns, such as promoting vaginal birth and breastfeeding.

The study could be improved by including a larger sample and a more diverse population to provide more comprehensive conclusions about the association between mode of delivery, breastfeeding and gut microbiota.

Ethical approval

Ethics approval Reg No: FMIEC/CCM/755/2022.

Declaration of patient consent

Patient's consent not required as the patients' identity is not disclosed or compromised.

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Conflicts of interest

Sanjeev B. Rai is the member of the editorial board of the journal.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Wang Z, Neupane A, Vo R, White J, Wang X, Marzano SY. Comparing gut microbiome in mothers' own breast milk-and formula-fed moderate-late preterm infants. Front Microbiol 2020;11:891.
2. Guaraldi F, Salvatori G. Effect of breast and formula feeding on gut microbiota shaping in newborns. Front Cell Infect Microbiol 2012;2:94.
3. Senn V, Bassler D, Choudhury R, Scholkmann F, Righini-Grunder F, Vuille-dit-Bille RN, *et al*. Microbial colonization from the fetus to early childhood-a comprehensive review. Front Cell Infect Microbiol 2020;10:637.
4. Adamek K, Skonieczna-Żydecka K, Wegrzyn D, Loniewska B. Prenatal and early childhood development of gut microbiota. Eur Rev Med Pharmacol Sci 2019;23:9667-80.
5. Layuk N, Sinrang AW, Asad S. Early initiation of breastfeeding and gut microbiota of neonates: A literature review. Med Clin Pract 2021;4:100222.
6. Kabeerdoss J, Ferdous S, Balamurugan R, Mechenro J, Vidya R, Santhanam S, *et al*. Development of the gut microbiota in southern Indian infants from birth to 6 months: A molecular analysis. J Nutr Sci 2013;3:e18.
7. Bharadia L, Agrawal N, Joshi N. Development and functions of the infant gut microflora: Western vs. Indian infants. Int J Pediatr 2020;2020:7586264.
8. Lyons KE, Ryan CA, Dempsey EM, Ross RP, Stanton C. Breast milk, a source of beneficial microbes and associated benefits for infant health. Nutrients 2020;12:1039.
9. Hascoët JM, Hubert C, Rochat F, Legagneur H, Gaga S, Emady-Azar S, *et al*. Effect of formula composition on the development of infant gut microbiota. J Pediatr Gastroenterol Nutr 2011;52:756-62.
10. Koletzko B, Bhatia J, Bhutta ZA, Cooper P, Makrides M, Uauy R, *et al*., editors. Pediatric nutrition in practice. Switzerland: Karger Medical and Scientific Publishers; 2015.
11. Granger CL, Embleton ND, Palmer JM, Lamb CA, Berrington JE, Stewart CJ. Maternal breast milk, infant gut microbiome and the impact on preterm infant health. Acta Paediatr 2021;110:450-7.
12. Tang Q, Jin G, Wang G, Liu T, Liu X, Wang B, *et al*. Current sampling methods for gut microbiota: A call for more precise devices. Front Cell Infect Microbiol 2020;10:151.
13. Korpela K. Impact of delivery mode on infant gut microbiota. Ann Nutr Metab 2021;77:11-9.
14. Mobeen F, Sharma V, Tulika P. Enterotype variations of the healthy human gut microbiome in different geographical regions. Bioinformation 2018;14:560.
15. Kuang YS, Li SH, Guo Y, Lu JH, He JR, Luo BJ, *et al*. Composition of gut microbiota in infants in China and global comparison. Sci Rep 2016;6:36666.
16. Willis AT. Anaerobic bacteriology: Clinical and laboratory practice. 3rd ed. London, Boston: Butterworths; 1977.
17. Wadsworth KT. In: Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM, editors. Anaerobic bacteriology manual. 6th ed. Belmont, California: Star Publishing Company; 2002.
18. Mellmann A, Cloud J, Maier T, Keckevoet U, Ramminger I, Iwen P, *et al*. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria. J Clin Microbiol 2008;46:1946-54.
19. Dubois D, Leyssene D, Chacornac JP, Kostrzewa M, Schmit PO, Talon R, *et al*. Identification of a variety of *Staphylococcus* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010;48:941-5.
20. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. Front Microbiol 2015;6:791.
21. Saffert RT, Cunningham SA, Ihde SM, Kristine E, Monson J, Patel R, *et al*. Comparison of Bruker biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometer to BD phoenix automated microbiology system for identification of gram-negative bacilli. J Clin Microbiol 2011;49:887-92.
22. Tonon KM, Morais TB, Taddei CR, Araújo-Filho HB, Abrão AC, Miranda A, *et al*. Gut microbiota comparison of vaginally and cesarean born infants exclusively breastfed by mothers secreting α1-2 fucosylated oligosaccharides in breast milk. PLoS One 2021;16:e0246839.
23. Heavey PM, Rowland IR. The gut microflora of the developing infant: Microbiology and metabolism. Microb Ecol Health Dis 1999;11:75-83.
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;574:117-21.

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