O MUNDO DA SAÚDE

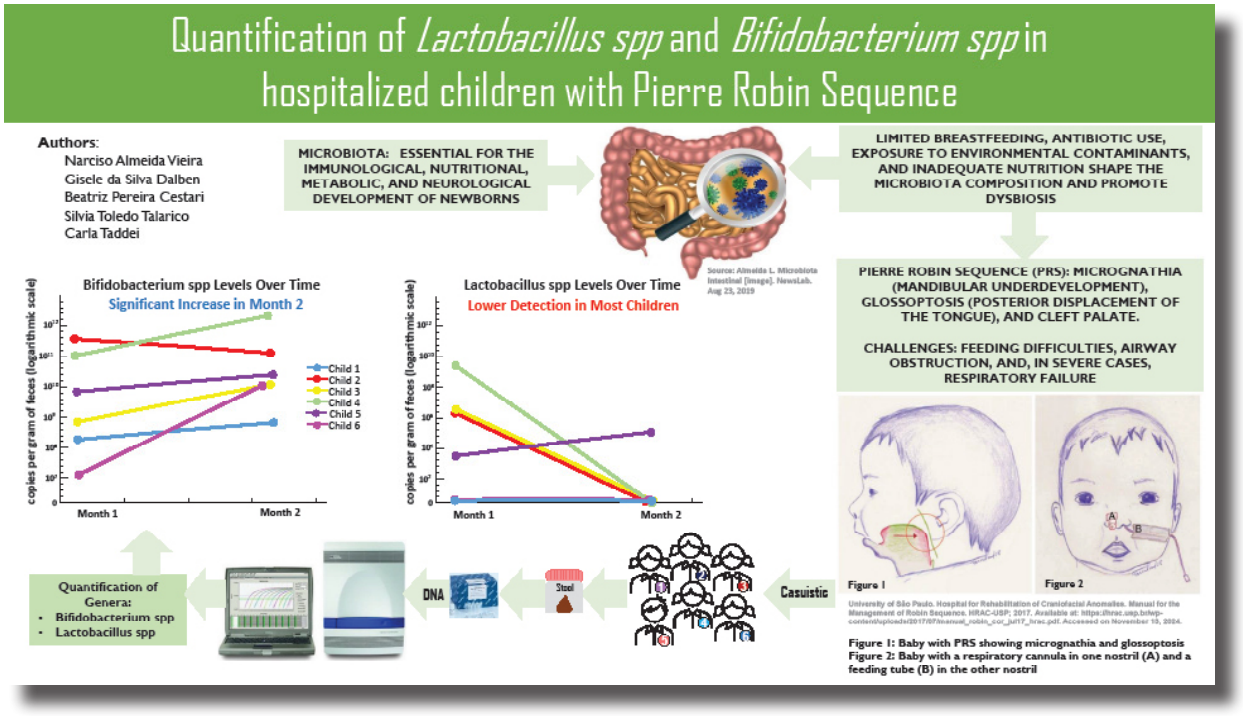
DOI: 10.15343/0104-7809.202448e16202024I

Quantification of *Lactobacillus* spp. and *Bifidobacterium* spp. in hospitalized children with Pierre Robin Sequence

Narciso Almeida Vieira[1] Gisele da Silva Dalben[2] Beatriz Pereira Cestari[3] Silvia Toledo Talarico[4] Carla R. Taddei[5]

[1]Faculdade de Odontologia de Bauru, Universidade de São Paulo - Campus de Bauru – FOB/USP. Bauru/SP, Brasil.
[2]Departamento de Odontopediatria, Hospital de Reabilitação de Anomalias Craniofaciais, Universidade de São Paulo – Campus de Bauru - HRAC/USP. Bauru/SP, Brasil.
[3]Laboratório de Análises Clínicas, Hospital de Reabilitação de Anomalias Craniofaciais, Universidade de São Paulo – Campus de Bauru - HRAC/USP. Bauru/SP, Brasil.
[4]Faculdades Oswaldo Cruz. São Paulo/SP, Brasil.
[5]Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo - FCF/USP. São Paulo/SP, Brasil.
Email: vieirana@usp.br

Graphical Abstract



DOI: 10.15343/0104-7809.202448e16202024I

Abstract

Objective: To detect and quantify *Bifidobacterium* spp. and *Lactobacillus* spp. in the intestinal microbiota (IM) of children with Pierre Robin sequence (PRS) during the first and second months of life, and to verify possible influences on the definitive establishment of the IM. Design: Longitudinal, descriptive study. Setting: Tertiary cleft care center. Participants: Six children, one male and five females, who were hospitalized for the management of their condition, were evaluated during the first (M1) and second (M2) months of life. Interventions: Fecal samples were collected and analyzed through DNA extraction for the quantification of anaerobic genera *Bifidobacterium* spp. and *Lactobacillus* spp. Socioeconomic and clinical data on the children were also collected. Main outcome measures: The number of copies of bacterial species was calculated for each fecal sample. Results: The genus *Bifidobacterium* was present in all children analyzed, with a significant increase at M2 for five children. The number of copies per gram of feces ranged from 1.8×10^7 to 5.5×10^{11} at M1 and from 4.2×10^8 to 3.0×10^{12} at M2. The genus *Lactobacillus* sp. was not present in all children, being detected in low quantities, with averages ranging from 8.5×10^3 to 9.7×10^8 copies per gram of feces at M1. At M2, only one child presented a quantification of 8.1×10^4, while two other children showed an absence of the microorganism at both M1 and M2. Conclusions: The bacterial colonization of the gastrointestinal tract of children with PRS is influenced by specific clinical and environmental factors. A healthy intestinal microbiota is essential for the development and immunity of newborns, but PRS-associated challenges affect microbiota diversity.

Keywords: Pierre Robin Sequence. Stool Microbiota. Gut Microbiota.

INTRODUCTION

The initial colonization of the gut microbiota is fundamental for the immune, nutritional, metabolic, and neurological development of newborns. The presence of beneficial bacteria, such as those provided by breastfeeding, supports neonatal health by delivering oligosaccharides, immune cells, and other essential components[1]. The early years of life represent a critical window for acquiring and stabilizing a healthy microbial community, which directly influences the immune system. The concept of the 'first 1000 days of life' has been proposed to emphasize the importance of health care from conception through the second year of life[2].

Several factors during childhood, including limited breastfeeding, antibiotic use, exposure to environmental contaminants, and inadequate nutrition, shape microbiota composition by promoting pathogenic bacteria over beneficial symbionts. This imbalance can lead to increased intestinal permeability, disrupted production of short-chain fatty acids (SCFAs), and systemic inflammation, ultimately affecting overall health later in life[3,4]. Such disruptions are associated with increased risks of inflammatory diseases, diabetes, obesity, and allergies.

The human gut microbiota plays a vital role in the host's health and nutrition from its establishment. On average, by two years of age, the diversity of microorganisms is established to interact with the host throughout life. The colony population is essentially composed of anaerobic and aerobic bacteria, reaching 10^{10} to 10^{14} microorganisms per gram of feces[5-13]. Intrinsic and extrinsic factors, such as diet, antibiotic use, age, geographic location, drug therapies, enteric diseases, craniofacial malformations, socioeconomic status, nutrition, type of delivery, and breastfeeding, specifically shape the microbiota of each individual[6, 8-13,14-23].

The microbiota provides protective effects to the host, such as stimulation of the immune system and secretion of anti-inflammatory cytokines, inhibition of the growth of pathogenic microorganisms, improved absorption of essential nutrients, and synthesis of vitamins[7,12]. Conversely, when there is an imbalance, there is a noticeable association with diarrhea, liver damage, carcinogenesis, infections, and bowel putrefaction[24].

It is assumed that the gastrointestinal tract of humans is colonized by more than 500 microorganism species, with culturable bacte-

DOI: 10.15343/0104-7809.202448e16202024I

ria accounting for only 20-30%. Thus, molecular techniques have been applied to better know the microbial composition. The real-time PCR technique (qPCR) is used to determine the unique number of bacteria through primers designed for specific regions of the 16S rRNA gene. This technique is much more sensitive compared to classic PCR, as it can detect bacterial cells per gram of feces at low concentrations[25-29]. Thus, this method was selected to analyze the participation of anaerobic bacteria in the intestinal microbial community of the group of children with Pierre Robin Sequence (PRS)[26].

Few studies have investigated the gut microbiota in newborns with craniofacial malformations, such as Pierre Robin Sequence (PRS), which requires specific and multidisciplinary treatment for full rehabilitation. PRS is characterized by a triad of anomalies: micrognathia (mandibular hypodevelopment), *glossoptosis* (posterior tongue displacement) and cleft palate, which together lead to feeding difficulties, airway obstruction, and, in severe cases, respiratory failure. Studies have suggested a genetic involvement, including regions such as 4q32-qter and SOX9 (17q24.3-q25.1), indicating a complex pathogenesis. At the Hospital for Rehabilitation of Craniofacial Anomalies of the University of São Paulo (HRAC-USP), patients receive multidisciplinary care to support rehabilitation and social integration[30-33].

Considering the important role of the IM in human health and the lack of studies on children with PRS, this study aimed to detect and quantify bacteria of the genera *Bifidobacterium* spp. and *Lactobacillus* spp., which are components of the IM in children with PRS treated at HRAC-USP during their first and second months of life, to investigate possible influences on the definitive establishment of the IM.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of HRAC-USP on June 26th, 2012. The parents or caretakers signed an informed consent form.

Sample collection

A longitudinal descriptive study was conducted on six children with PRS, one male and five females, during the first (M1) and second (M2) months of life, analyzing the IM profile, detecting and quantifying bacteria of genera *Bifidobacterium* sp. and *Lactobacillus* sp., as well as external colonization factors, such as age, geographic location, drug therapies, socioeconomic level, nutritional status, type of delivery, breastfeeding, and antibiotic use. Parents or caretakers were interviewed in private rooms, and after being informed about the study, they signed the informed consent form and received sterile containers for sample storage. Feces collection was carried out at the time of hospitalization, following the interview and parental or guardian authorization. After collection, the samples were immediately sent to the Clinical Laboratory at HRAC-USP and kept refrigerated at -80°C until IM evaluation.

Clinical information

Children in the present study were cared for by professionals of HRAC-USP since the first month of life, with daily information available in the patients' medical records. Thus, it was possible to verify that all children were born via surgical delivery, being five at term and one at 34 weeks of pregnancy, being considered preterm (child 4). Five families were of middle socioeconomic status and one was of lower-middle status. In general, the parents had good educational level and high income; the families lived in their own brick houses, with access to electricity, tap water and proper sewerage system. Most children with PRS were not breastfed due to glossoptosis and

DOI: 10.15343/0104-7809.202448e16202024I

dysphagia; however, two mothers were able to pump and offer breastmilk in the bottle for the children during 3 days (child 4) and 20 days (child 6), alongside formula.

Events such as dysphagia, respiratory and feeding difficulties, utilization of nasopharyngeal and nasogastric tubes, and reflux were observed. Three children received hypercaloric diet with glycose polymers (GP), medium-chain triglycerides (MCT) and infant formula 1 (F1). All six children received drugs for the stomach, reflux and nausea, and only one did not receive ranitidine, a drug that reduces stomach acid production. Child 1 was administered multiple antibiotics: ampicillin (7 days); erythromycin (7 days); Cefepime (9 days); and azithromycin (7 days).

DNA extraction

The DNA of dry-collected samples was obtained using the kit QIAmp DNA Stool Mini (Quiagem) for isolation of bacterial genomic DNA of fecal samples, following the manufacturer's recommendations[28]. To obtain the bacterial DNA used in the standard curve, the growth curve of the quantified bacteria was established. Quantification reactions with primers for the anaerobic genera *Bifidobacterium* spp. and *Lactobacillus* spp. and the standard curve construction were standardized. The concentration of primers was determined to reach an efficiency between 90 and 110% and the the reaction was optimized to a final volume of 20µL, being 2µL of DNA extracted from feces and the remaining volume composed of mix, primers and probes[28].

Aliquots from the reading points of the log stage of growth of the quantified microorganisms were frozen until utilization. From these aliquots, DNA was extracted using the kit WizardGenomic DNA Purification (Promega), following the manufacturer's instructions. Real-time PCR was performed on an equipment 7500 Real-Time PCR System (Applied Biosystems). The anaerobic bacteria (Bifidobacterium animalis subsp. lactis HN019 and *Lactobacillus acidophilus* ATCC 4356) were inoculated in *Lactobacilli* MPRS broth (Difco) and incubated for 24h, in anaerobiosis, at 37°C[28,34].

The *Bifidobacterium* spp. population was quantified using the system TaqMan®, which allowed definition of the concentrations of primers in 300nM, both forward and reverse, and 250nM for the probe with FAM reporter and NFQ-MGB quencher, since in these conditions the efficiency of reaction was 90.516%. A total of five serial decimal dilutions of *Bifidobacterium animalis subsp lactis* HN019 genomic DNA were used for construction of the standard curve. The initial point on the curve had concentration of 5.3ng/µL. The quantification of *Lactobacillus* spp. was performed using the SYBR-Green® system, using the primers and probes described by Furet *et al.*[28], with concentrations of 300nM for forward and reverse initiators, presenting efficiency of 93.237%. The melting curve evidenced the specificity of the reaction. A total of five serial decimal dilutions of genomic DNA of *Lactobacillus acidophilus* ATCC4356 were used for constructing the standard curve, and the initial point had a concentration of 10.1ng/µL.

The threshold cycle, number of cycles at which the efficiency of fluorescence detection is 100% (region of exponential growth), was monitored[28]. The quantifications of samples were calculated on the Excel software, with the threshold cycle value (Ct) obtained during testing. These values were placed in the equation obtained from the constructed standard curve. The values achieved were corrected according to the sample dilutions[30-33].

The number of copies of bacterial species was calculated for each fecal sample, based on the Ct values, using the constructed standard curves[35-39].

DOI: 10.15343/0104-7809.202448e16202024I

RESULTS

At two months, there was significant presence of the genus *Bifidobacterium* spp. in the microbiota of the children. All children presented great quantity of *Bifidobacterium* spp. and low quantity of *Lactobacillus* spp., which determined each colonization profile.

The genera Lactobacillus and Bifidobacterium were quantified in the first and second months of life of six children with PRS. The genus *Bifidobacterium* was present in all the children analyzed and presented a significant increase in the number of copies/g of feces in the second month for five children. However, one child presented a slight decrease (Table 2), indicating the lack of a pattern in colonization. The number of copies/g of feces ranged from 1.8x107 to 5.5x1011 in M1 and from 4.2x108 to 3.0x1012 copies/g of feces.

The Lactobacillus sp. genus was not present in all children, being detected in low quantity, with an average ranging from 8.5x103 to 9.7x108 copies/g of feces in M1, while in M2 only one child presented a quantification of 8.1x104. Other two children (numbers 1 and 6) showed no presence of the microorganism in both M1 and M2.

The quantification of bacteria according to breastfeeding evidenced that children 1 and 6, who received breastmilk, had an increase in *Bifidobacterium* spp. and a reduction or total absence of *Lactobacillus* spp., alike the other children. Each child presented a fecal microbiological profile.

Table 1 - Clinical information of hospitalized children with Pierre Robin Sequence, including gender, origin, use of antacid drugs, socioeconomic level, diet, and breastfeeding, Bauru, SP.

Child N.	Gender	Origin	Use of Ranitidine	Socioeconomic Level	Delivery	Hypercaloric Diet	Breastfeeding	TFFA	NPT	NGT
1	F	GO	Yes	Middle	Surgical	Yes	No	Yes	Yes	Yes
2	F	SP	Yes	Middle	Surgical	No	No	Yes	Yes	Yes
3	F	SC	No	Middle	Surgical	No	No	Yes	Yes	Yes
4	F	SP	Yes	Lower-middle	Surgical	Yes	Yes	No	No	Yes
5	M	AL	Yes	Middle	Surgical	Yes	No	Yes	Yes	Yes
6	F	RJ	Yes	Middle	Surgical	No	Yes	No	Yes	Yes

Captions: AL = Alagoas; GO = Goiás; RJ = Rio de Janeiro; SC = Santa Catarina; SP = São Paulo.
FFT – Feeding-facilitating techniques
NPT – Nasopharyngeal tube
NGT – Nasogastric tube

DOI: 10.15343/0104-7809.202448e16202024I

Table 2 - Quantification of number of copies of DNA/g of feces, Bauru, SP.

SYSTEM TARGET	*Bifidobacterium* spp.		*Lactobacillus* spp.	
	TaqMan® 300/300/250nM Vol 20µL		SYBR® Green I 300/300nM Vol 20µL	
Child nº.	M1	M2	M1	M2
1	1.5E+08	4.2E+08	0.0	0.0
2	5.5E+11	1.4E+11	1.6E+06	0.0
3	9.7E+08	2.4E+10	2.4E+06	0.0
4	1.1E+11	3.0E+12	9.7E+08	0.0
5	8.8E+09	2.9E+10	8.5E+03	8.1E+04
6	1.8E+07	8.2E+09	0.0	0.0

M1 = first month of life
M2 = second month of life

DISCUSSION

The microbiota is composed of several microorganisms that colonize the host from birth and stabilize around the age of two. The development of interactions between microorganisms and host gives rise to a healthy environment that becomes important to human health. These microorganisms act as a source of nonspecific antigens and immunomodulators, stimulating local and systemic immune responses and influencing the number and distribution of the lymphoid tissue cell population associated with the gut, and also acting in the defense against invading pathogens[7,8,12]. However, several factors influence the microbiota establishment and stability, including diet[6,9-11,13,21], use of antibiotics[12,15], age[8,15], geographic location[14,17,18], drug therapies[19,20], enteric diseases[40], craniofacial malformation[23] and hormonal status[31].

This study identified significant differences in the IM composition in a small group of children with PRS by quantifying the bacterial genera *Bifidobacterium* sp. and *Lactobacillus* sp.

Bacteria of the genus *Bifidobacterium* are part of the microbiota of the human lower gastrointestinal tract and have no pathogenicity. Some of their known benefits include improvement of the nutritional value of some foods, control of intestinal infections in children, anticarcinogenic activity, activation of the immune system and protection against infections by pathogenic microorganisms. *Lactobacillus* spp. are used in nutritional supplementation, since they act as an effective treatment in preventing and combating various diseases, without the risk of attacking or poisoning the body. Since they protect the intestinal microbiota, therapeutic supplementation with *Lactobacillus* is recommended during and after antibiotic therapy[41,42].

These two bacterial genera are known as probiotic organisms and are defined as live microorganisms used as food supplements to improve the host's health when administered at the correct dosage. They are therapeutic agents that acidify the intestinal microbiota, thus preventing the development of pa-

DOI: 10.15343/0104-7809.202448e16202024I

thogenic microorganisms. They also play an antibacterial and anti-inflammatory role and can alter the microbial composition[42].

Most children with PRS in this study are females. This agrees with the reports of Meyer *et al.*[43] and Pinheiro Neto *et al.*[44].

One factor that may influence the newborn microbiota is the type of delivery. At this moment, mainly during normal delivery, the baby has contact with bacteria from the mother's vaginal and fecal microbiota or bacteria from the environment and, during surgical delivery, there is likely contact with surgical devices, along with the mother's presence with signs of affection, like kisses. In this research, it was found that all children were born by surgical delivery, in agreement with data from Vieira and Pereira[45].

According to the results (Table 1), most children were not breastfed. With breastfeeding and oxygen consumption from the intestinal lumen, bacterial genera may appear, such as *Bifidobacterium* spp., which can vary in quantity depending on the time of breastfeeding, since human milk presents growth factors for this respective genus, along with *Lactobacillus* spp., *Bacteroides* spp., *Eubacterium* spp., among others. All children were colonized by *Bifidobacterium* spp., present in greater quantities than *Lactobacillus* spp. during the first sixty days of life. This result was similar to the studies conducted by Talarico[29].

Breastfeeding, along with the introduction of supplementary formulas and solid foods, is related to the development of strictly anaerobic populations[42]. This favors an increase in the quantity of bacteria of genera *Bifidobacterium* spp. and *Lactobacillus* spp., as described by Vael and Desager[46]. The genus *Lactobacillus* spp. was not found in all children, differing from the studies by Talarico[29], but similar to those by Jost *et al.*[47], which quantified this genus in a sample of seven children.

At birth, due to contact with the mother's microbiota, the environment and other external factors, newborns will have a diverse population of bacteria that stabilizes after the second year of life[5]. The importance of these microorganisms is so relevant that one of the main colonizing genera, such as *Bifidobacterium* spp., is used for the treatment and prevention of gastrointestinal diseases[48].

According to Vandenplas *et al.*[49], the balance of the microbiota can also be altered by the use of drugs, changing its composition, opening the way for pathogenic microorganisms. Vieira *et al.*[45] showed that the quantity of some genera, such as *Bifidobacterium* spp. and *Lactobacillus* spp., is significantly decreased after 24 hours of use of cefazolin in palatal repair surgeries, which can generate negative complications for the host. The present study revealed that the majority of children used ranitidine (antacid). The effects of antibiotics on the composition of IM in this group of children demonstrated heterogeneity during the study period. It was possible to observe change in the microbiota profile in some children according to the group and period. According to some authors, the antibiotic treatment causes disturbances in the expected patterns of *Bifidobacterium* spp. and excessive growth of enterobacteria[17]. The present study revealed that the use of antimicrobial drugs interferes with the individual variability of the intestinal microbiota.

Molecular techniques to assess diversity, individual variability and complexity of the microbiota have been applied in the most recent studies, regardless of microbiological culture. qPCR is a procedure used to detect and quantify specific bacteria, using primers designed to recognize specific regions of rRNA. This enables the detection of bacterial cells per gram of fecal sample at lower concentrations compared to the conventional technique[25,26-28].

DOI: 10.15343/0104-7809.202448e16202024I

CONCLUSION

This study demonstrated that the establishment of the gut microbiota in children with PRS is influenced by clinical and environmental factors, with significant variability in bacterial composition between individuals. A healthy gut microbiota is vital for the immunological and developmental health of newborns; however, children with PRS face unique challenges that hinder the establishment of a balanced microbiota. These findings highlight the importance of tailored care strategies for PRS patients, including early intervention to promote breastfeeding and cautious use of antibiotics. Additionally, future research should focus on comparing PRS patients with healthy controls to understand the deviations from the expected norm and their potential implications.

CRediT author statement

Conceptualization: Vieira, NA; Neves, CTC. Methodology: Vieira, NA; Neves, CTC; Talarico, ST. Validation: Vieira, NA; Neves, CTC; Dalben, GS. Statistical analysis: Vieira, NA. Formal analysis: Vieira, NA; Neves, CTC; Talarico, ST; Cestari, BP. Investigation: Vieira, NA; Neves, CTC; Talarico, ST; Cestari, BP. Resources: Vieira, NA; Neves, CTC. Writing-original draft preparation: Vieira, NA; Neves, CTC; Dalben, GS. Writing-review and editing: Vieira, NA; Neves, CTC; Dalben, GS. Visualization: Vieira, NA; Neves, CTC; Dalben, GS; Cestari, BP. Supervision: Vieira, NA; Neves, CTC. Project administration: Vieira, NA; Neves, CTC.

All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Carr LE, Virmani MD, Rosa F, Munblit D, Matazel KS, Elolimy AA, et al. Role of human milk bioactives on infants' gut and immune health. Front Immunol. 2021;12:604080. doi: 10.3389/fimmu.2021.604080.
2. Robertson RC, Manges AR, Finlay BB, Prendergast AJ. The human microbiome and child growth - first 1000 days and beyond. Trends Microbiol. 2019;27(2):131-47. doi: 10.1016/j.tim.2018.09.008.
3. Davis EC, Castagna VP, Sela DA, Hillard MA, Lindberg S, Mantis NJ, et al. Gut microbiome and breast-feeding: implications for early immune development. J Allergy Clin Immunol. 2022;150(3):523-34. doi: 10.1016/j.jaci.2022.07.014.
4. Enav H, Bäckhed F, Ley RE. The developing infant gut microbiome: a strain-level view. Cell Host Microbe. 2022;30(5):627-38. doi: 10.1016/j.chom.2022.04.009.
5. Bullen CL, Tearle PV, Willis AT. Bifidobacteria in the intestinal tract of infants: an in vivo study. J Med Microbiol. 1976;9:325-33. doi: 10.1099/00222615-9-3-325.
6. Hentges DJ. Fecal flora of volunteers on controlled diets. Am J Clin Nutr. 1978;31(10 Suppl). doi: 10.1093/ajcn/31.10.S123.
7. Van der Waaij D. Colonization resistance of the digestive tract: Clinical consequences and implications. J Antimicrob Chemother. 1982;10(4):263-70. doi: 10.1093/jac/10.4.263.
8. Moreau MC, Coste M. Immune responses to dietary protein antigens. World Rev Nutr Diet. 1993;74:22-57. doi: 10.1159/000422601.
9. Gibson GR, Wang X. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. J Appl Bacteriol. 1994;77(4):412-20. doi: 10.1111/j.1365-2672.1994.tb03443.x.
10. Falk PG, Hooper LV, Midtvedt T, Gordon JI. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. Microbiol Mol Biol Rev. 1998;62(4):1157-70. doi: 10.1128/mmbr.62.4.1157-1170.1998.
11. Tannock GW. Analysis of the intestinal microflora: a renaissance. Antonie Van Leeuwenhoek. 1999;76(1-4):265-78. doi: 10.1023/A:1002038308506.
12. Monreal MTFD, Pereira PCM, Lopes CAM. Intestinal microbiota of patients with bacterial infection of the respiratory tract treated with amoxicillin. Braz J Infect Dis. 2005;9(4):292-300. doi: 10.1590/S1413-86702005000400005.
13. Aldeberth I, Word AE. Establishment of the gut microbiota in Western infants. Acta Paediatr. 2009;98(2):229-38. doi: 10.1111/j.1651-2227.2008.01060.x.
14. Finegold SM. Interaction of antimicrobial therapy and intestinal microflora. Am J Clin Nutr. 1970;23(11):1466-71. doi: 10.1093/ajcn/23.11.1466.
15. Haenel H. Human normal and abnormal gastrointestinal flora. Am J Clin Nutr. 1970;23(11):1433-9. doi: 10.1093/ajcn/23.11.1433.
16. Bhat P, Shantakumari S, Rajan D, Mathan VI, Kapadia CR, Swarnabai C, et al. Bacterial flora of the gastrointestinal tract in southern Indian control subjects and patients with tropical sprue. Gastroenterology. 1971;62:11-21.

17. Mata LJ, Jejicanos ML, Jiménez F. Studies on the indigenous gastrointestinal flora of Guatemalan children. Am J Clin Nutr. 1972;25(12):1380-90. doi: 10.1093/ajcn/25.12.1380.
18. Moore WEC, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl Environ Microbiol. 1974;27(5):961-79. doi: 10.1128/am.27.5.961-979.1974.
19. Nordenvall B, Hallberg D, Larsson L, Nord CE. The effect of clindamycin on the intestinal flora in patients with enteric hyperoxaluria. Scand J Gastroenterol. 1983;18(2):177-81. doi: 10.3109/00365528309181580.
20. Kurpad AV, Shetty PS. Effects of antimicrobial therapy on fecal bulking. Gut. 1986;27(1):55-8. doi: 10.1136/gut.27.1.55.
21. Marteau P, Pochart P, Flourié B, Pellier P, Santos L, Desjeux JF, et al. Effect of chronic ingestion of fermented dairy product containing Lactobacillus acidophilus and Bifidobacterium bifidum on metabolic activities of the colonic flora in humans. Am J Clin Nutr. 1990;52(4):685-8. doi: 10.1093/ajcn/52.4.685.
22. Salminen S, Isolauri E, Onnela T. Gut flora in normal and disordered states. Chemotherapy. 1995;41(Suppl 1):5-15. doi: 10.1159/000239391.
23. Mitchell DK, Lidsky ME, Sampson DE, Lander TA, Liu M, Sidman JD. Prospective study of toxigenic Clostridium difficile in children given amoxicillin/clavulanate for otitis media. Pediatr Infect Dis J. 1996;15(6):514-9. doi: 10.1097/00006454-199606000-00008.
24. Gronlund MM, Gueimonde M, Laitinen K, Kociubinski G, Grönroos T, Salminen S, et al. Maternal breast-milk and intestinal bifidobacteria guide the compositional development of the Bifidobacterium microbiota in infants at risk. Clin Exp Allergy. 2007;37(12):1764-72. doi: 10.1111/j.1365-2222.2007.02849.x.
25. Matsuki T, Watanabe K, Fujimoto J, Kado Y, Takada T, Matsumoto K, et al. Quantitative PCR with 16S rRNA-Gene-Targeted Species-Specific primers for analysis of human intestinal bifidobacteria. Appl Environ Microbiol. 2004;70(1):167-73. doi: 10.1128/AEM.70.1.167-173.2004.
26. Furrie E. A molecular revolution in the study of intestinal microflora. Gut. 2006;55(2):141-3. doi: 10.1136/gut.2005.081695.
27. Mathys S, Lacroix C, Mini R, Meile L. PCR and real-time PCR primers developed for identification of Bifidobacterium thermophilum in faeces. BMC Microbiol. 2008;8:179. doi: 10.1186/1471-2180-8-179.
28. Furet JP, Firmesse O, Gourmelon M, Bridonneau C, Tap J, Mondot S, et al. Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. FEMS Microbiol Ecol. 2009;68(3):351-62. doi: 10.1111/j.1574-6941.2009.00671.x.
29. Talarico ST, Santos FE, Brandt KG, Martinez MB, Taddei CR. Anaerobic bacteria in the intestinal microbiota of Brazilian children. Clinics (Sao Paulo). 2017;72(3):154-60. doi: 10.6061/clinics/2017(03)05.
30. Silva KCP, Messias TS, Dalben GS, Vieira NA. Male individuals with Robin Sequence: emerging significant association with ABO and RhD blood group phenotypes. Hematol Transfus Cell Ther. 2018;40(4):354-7. doi: 10.1016/j.htct.2018.03.009.
31. Marques IL, Sousa TV, Carneiro AF, Peres SPBA, Barbieri MA, Bettiol H. Sequência de Robin: protocolo único de tratamento. J Pediatr. 2005;81(1):14-22. doi: 10.1590/S0021-75572005000100005.
32. Mondini CCSD, Fontes CMB, Trettene AS, Cianciarullo TI, Lazarini IM. Applicability of Orem: training of caregiver of infant with Robin Sequence. Rev Bras Enferm. 2018;71(suppl 3):1469-73. doi: 10.1590/0034-7167-2016-0562.
33. Jakobsen LP, Knudsen MA, Lespinasse J, García AC, Ramos C, Fryns JP, et al. A base genética da Seqüência de Pierre Robin. Cleft Palate Craniofac J. 2006;43(2):155-9.
34. Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol. 2004;97(6):1166-77. doi: 10.1111/j.1365-2672.2004.02409.x.
35. Bolotin A, Wincker P, Mauger S, Jaillon O, Malarme K, Weissenbach J, et al. The complete genome sequence of the lactic acid bacterium Lactococcus lactis ssp. lactis IL1403. Genome Res. 2001;11:731-53. doi: 10.1101/gr.gr-1697r.
36. Altermann E, Russell WM, Azcarate-Peril MA, Barrangou R, Buck BL, McAuliffe O, et al. Complete genome sequence of the probiotic lactic acid bacterium Lactobacillus acidophilus NCIM. Proc Natl Acad Sci U S A. 2005;102(11):3906-12. doi: 10.1073/pnas.0409188102.
37. Durfee T, Nelson R, Baldwin S, Plunkett G 3rd, Burland V, Mau B, et al. The complete genome sequence of Escherichia coli DH10B: insights into the biology of a laboratory workhorse. J Bacteriol. 2008;190(7):2597-606. doi: 10.1128/JB.01695-07.
38. Barrangou R, Briczinski EP, Traeger LL, Loquasto JR, Richards M, Horvath P, et al. Comparison of the complete genome sequences of Bifidobacterium animalis subsp. lactis DSM 10140 and Bl-04. J Bacteriol. 2009;191(13):4144-51. doi: 10.1128/JB.00155-09.
39. Zhang T, Shao MF, Fang HHP. A qRT-PCR-based method for the measurement of rrn operon copy number. Lett Appl Microbiol. 2009;49:26-30. doi: 10.1111/j.1472-765X.2009.02613.x.
40. Hasan N, Yang H. Factors affecting the composition of the gut microbiota, and its modulation. PeerJ. 2019;7. doi: 10.7717/peerj.7502.
41. Okoniewski A, Dobrzy□ska M, Kusyk P, Dziedzic K, Przys□awski J, Drzyma□a-Czy□ S. The Role of Fermented Dairy Products on Gut Microbiota Composition. Fermentation. 2023;9(3):231. doi: 10.3390/fermentation9030231.
42. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr. 1999;69(suppl):1035S-45S. doi: 10.1093/ajcn/69.5.1035s.
43. Meyer AC, Ottenhof S, Moroi MK, Limberger I, Furtado FM, Antunes NL. Airway interventions in children with Pierre Robin Sequence. Otolaryngol Head Neck Surg. 2008;138(6):782-7. doi: 10.1016/j.otohns.2008.03.002.
44. Pinheiro Neto CD, Alonso N, Sennes LU, Goldenberg DC, Santoro PP. Avaliação polissonográfica e de videoendoscopia da deglutição de pacientes portadores da sequência de Pierre-Robin. Braz J Otorhinolaryngol. 2009;75(6):852-6. doi: 10.1590/S1808-86942009000600014.
45. Vieira NA, Borgo HC, Dalben GS, Bachega MI, Pereira PCM. Evaluation of fecal microorganisms of children with cleft palate before and after palatoplasty. Braz J Microbiol. 2013;44(3):835-8. doi: 10.1590/S1517-83822013000300026.
46. Vael C, Desager K. The importance of the development of the intestinal microbiota in infancy. Curr Opin Pediatr. 2009;21(6):794-800. doi: 10.1097/MOP.0b013e328332351b.
47. Jost T, Lacroix C, Braegger CP, Chassard C. New insights in gut microbiota establishment in healthy breastfed neonates.

DOI: 10.15343/0104-7809.202448e16202024I

DOI: 10.15343/0104-7809.202448e16202024I

PLoS One. 2012;7(8). doi: 10.1371/journal.pone.0044595.
48. Langendijk PS, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MH, et al. Quantitative fluorescence in situ hybridization of Bifidobacterium spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. Appl Environ Microbiol. 1995;61(8):3069-75. doi: 10.1128/aem.61.8.3069-3075.1995.
49. Vandenplas Y, Veereman-Wauters G, De Greef E, Peeters S, Casteels A, Mahler T, et al. Probiotics and prebiotics in prevention and treatment of diseases in infants and children. J Pediatr (Rio J). 2011;87(4):292-300. doi: 10.2223/JPED.2103.

Received: 06 may 2024.
Accepted: 28 october2024.
Published: 22 november 2024.