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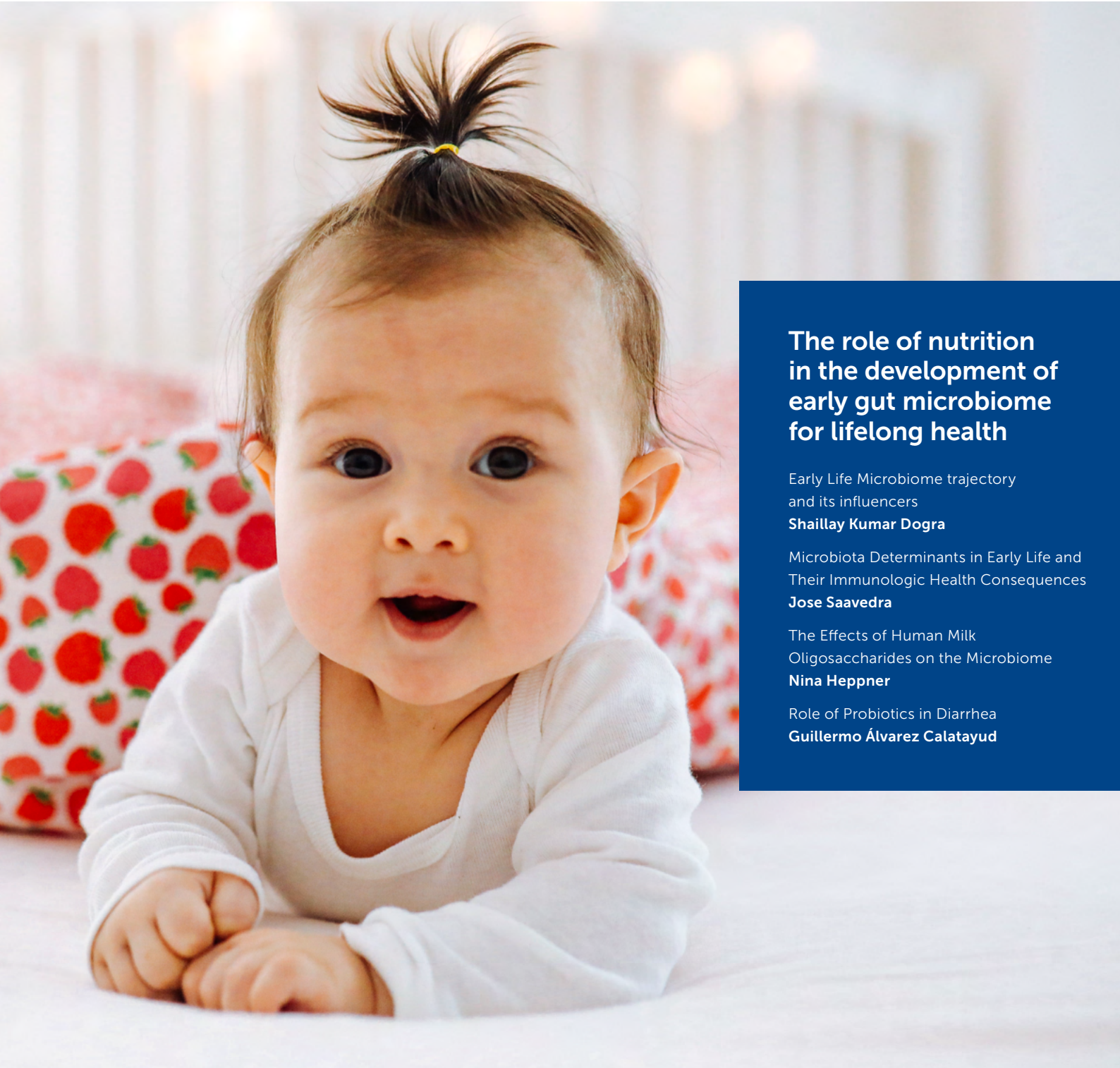

## The role of nutrition in the development of early gut microbiome for lifelong health

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# Early Life Microbiome trajectory and its influencers

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## Key messages:

- The gut microbiome in infants evolves progressively towards an adult-like microbiome.
- Early Life Microbiome (ELM) maturation can be described by different methods that simplify the description of the entire microbiome; here, we focus on the trajectory approach.
- The ELM trajectory is influenced by many factors. Nutrition is a critical modulator of the microbiome.

## Early life microbiome development

The infant gut microbiome is a dynamic ecosystem that undergoes changes from birth until two to three years of age, followed by a gradual evolution towards an adult microbiome in later childhood.[1] As per increasing scientific evidence, it is believed that the early life microbiome (ELM) establishment has lasting consequences on lifelong health.[2]

To simplify the depiction and interpretation of the rapidly evolving infant gut microbiome, one approach links the development of the early life microbiome with infant age, and follows a similar statistical framework as the WHO growth curves to inform individual infant microbiome development as compared to the reference trajectory.[3]

First, a set of infants is chosen as "reference" based on certain criteria such as delivery mode, at term birth, exclusive breastfeeding duration, infant growth characteristics, and health/disease status. Then, a machine learning model is applied to predict age using the microbiome data. The microbiome-based predicted age from an infant's fecal sample is compared to the reference for the same age allowing for natural variation. Microbiome development of this infant is considered normal if it falls within the reference trajectory.

## Factors that influence the early life microbiome

Factors such as mode of birth, antibiotic usage and type of feeding impact the ELM profile compared to the reference.[4, 5] Other factors such as geography, rural or urban living, number of siblings and weaning diet may also influence infant microbiome development[4, 6] (Figure 1b). Some of these factors such as mode of birth or antibiotic usage have been directly associated with later health outcomes[7, 8] and it is possible that some mechanisms are mediated through alterations in the microbiome.[9]



Importantly, it is possible to nutritionally intervene in order to modulate the microbiome closer to the reference trajectory.[3, 10] For example, certain species of *Bifidobacterium*, which are important components of the infant gut microbiome in the first few months of life, benefit from Human Milk Oligosaccharides (HMOs).[11] *Faecalibacterium prausnitzii*, a bacterium that establishes in the infant gut from about nine months onwards to become a predominant member of the gut microbiome, utilizes fibers to generate butyrate that is an important health associated metabolite.[12]

In summary, the infant gut microbiome evolves rapidly and is influenced by various factors, some of which are linked to allergy development or poor metabolic health outcomes later in life. The microbiome trajectory description can differentiate several factors influencing the ELM such as mode of birth, breastfeeding practice and nutrition. Importantly, nutritional interventions can modulate the microbiome maturation towards the reference trajectory.[3, 10]

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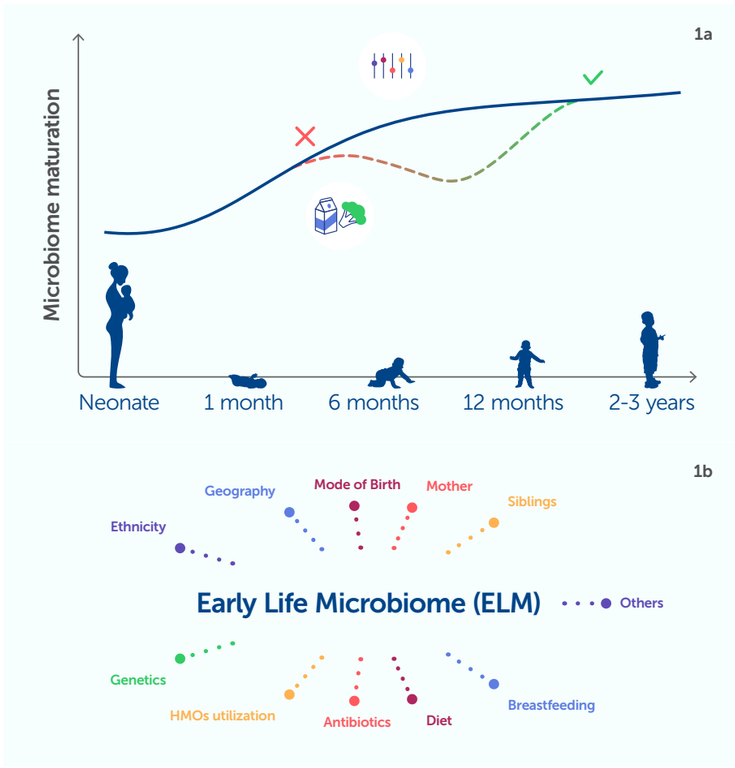


*Figure 1: Simplified representation of infant gut microbiome development as a maturation trajectory over time (1a). Early life microbiome (ELM) can be influenced by various factors such as mode of birth, feeding, diet, antibiotics or other factors that are linked to microbiome acquisition from mother, siblings, and environment (1b).*

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# Microbiota Determinants in Early Life and Their Immunologic Health Consequences

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## Key messages:

- Birth by cesarean section rather than vaginal delivery, and formula feeding rather than breastfeeding lead to dysbiosis (an altered microbial profile and lower diversity), which may define or influence microbiota composition in later life.
- Both lack of breastfeeding and birth by cesarean section are independently associated with similar immune related disease risks, protective as well as inflammatory. These include infections, allergy, inflammatory bowel disease, leukemia. And these health conditions are also associated with dysbiosis.
- Early life dysbiosis resulting from determining factors such as cesarean section and lack of breastfeeding is a major link, and potentially an etiologic factor, in the risk expression of multiple acute and chronic conditions throughout life.

The gut microbiota plays a major role in the development and function of immune protective mechanisms (e.g., those associated with risk of infectious disease), as well as immunomodulatory functions (e.g., those associated with risk of allergy, inflammatory bowel disease, neoplasia). The development of this complex ecosystem in each infant is driven by multiple determinant factors: genetic, maternal (including prenatal), and environmental. While many environmental factors, including geography, family structure, pets, antibiotics, play a role; by far, the mode of birth (cesarean versus vaginal delivery) and the type of feeding (breast feeding versus other substitutes) appear to be the most significant modulators of the early development of each individual's microbiota.

Breastfeeding promotes microbiota development with significant diversity and predominance of specific species, particularly bifidobacteria and lactobacilli, via multiple mechanisms, including breast milk bacteria composition and human milk oligosaccharides. Non-breastfed infants develop dysbiosis–generally described as altered gut microbiota profiles and changes in diversity, as compared to breastfed. Clinically, breastfed infants are generally protected from a number of health conditions associated with use of breast milk substitutes. These include lower risk from infections, and immunological disease such as asthma, other allergies, type one diabetes, and leukemia, as well as metabolic disease (obesity and type 2 diabetes). Notably, all these health conditions have been associated with dysbiosis later in life.





Similarly, compared with vaginal birth, birth by cesarean section has been associated with a less diverse microbiota, and altered microbial profiles, including lower presence of bifidobacteria[1]. Increasingly, strong associations have been reported between cesarean delivery and disease in infancy and later life.[2] Cesarean delivery increases risk of gastroenteritis, respiratory infections, and otitis media. It has also been shown to be strongly associated with risk of hospitalizations for acute gastroenteritis in the first two years of life, and this risk is exacerbated further for those who are not breastfeed.[3] Cesarean birth also increases risk of obesity[4] and type 2 diabetes. Most notably, it increases risk of allergic disease, including asthma, atopic dermatitis, and particularly food allergy.[5] And the relative risk of leukemia, particularly acute lymphocytic leukemia appears also to be increased.[6] Of note, all these conditions are also associated with dysbiosis.

Most studies so far linking cesarean birth to disease are associative and long-term prospective studies are underway. However, alterations in microbial composition, diversity, and maturity appear to precede some of these manifestations.[7] It is increasingly becoming clear that the development of early life dysbiosis (resulting from determining factors such as cesarean section and lack of breastfeeding) is a major link, and potentially an etiologic factor in the risk expression of multiple acute and chronic conditions.

The use of specific species and strains of bifidobacteria (e.g., *B. infantis*, *B. lactis*) and lactobacilli (*L. rhamnosus, L. reuteri*) as probiotics has been shown to have effects on infectious intestinal and respiratory illness, and have effect on modulating gut barrier function, IgA secretion, and T lymphocytes activity modulation. Thus, probiotics may have the potential for mitigating the dysbiosis associated with cesarean birth of lack of breastfeeding and its longer-term immune related consequences.

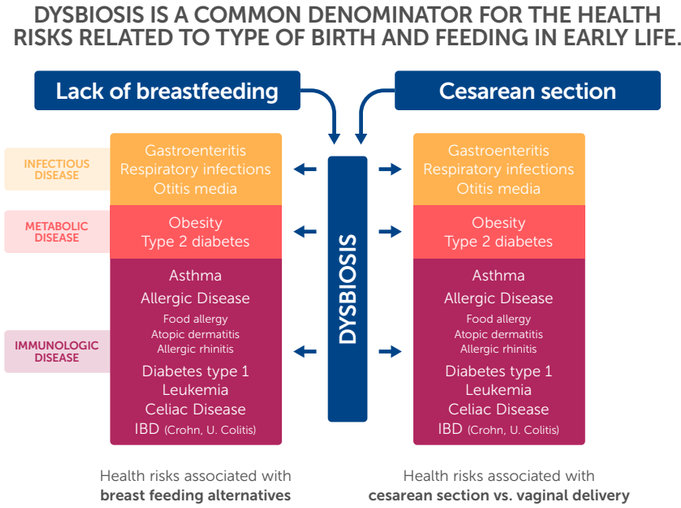


*Figure 1: Dysbiosis is potentially an etiologic factor in the risk expression of multiple acute and chronic conditions.*

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# The effects of Human Milk Oligosaccharides on the Microbiome

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## Key messages:

- Human Milk Oligosaccharides (HMOs) serve as a substrate for bacteria in the gut and thus modulate the developing infant's microbiota.
- The presence or absence of specific HMOs in breast milk can have a direct impact on microbial composition and the risk for developing certain diseases.
- Adding even a few HMOs structurally identical to that in human milk to infant formula has been shown to shift the infant microbiota to a composition that is more similar to that of breastfed babies.

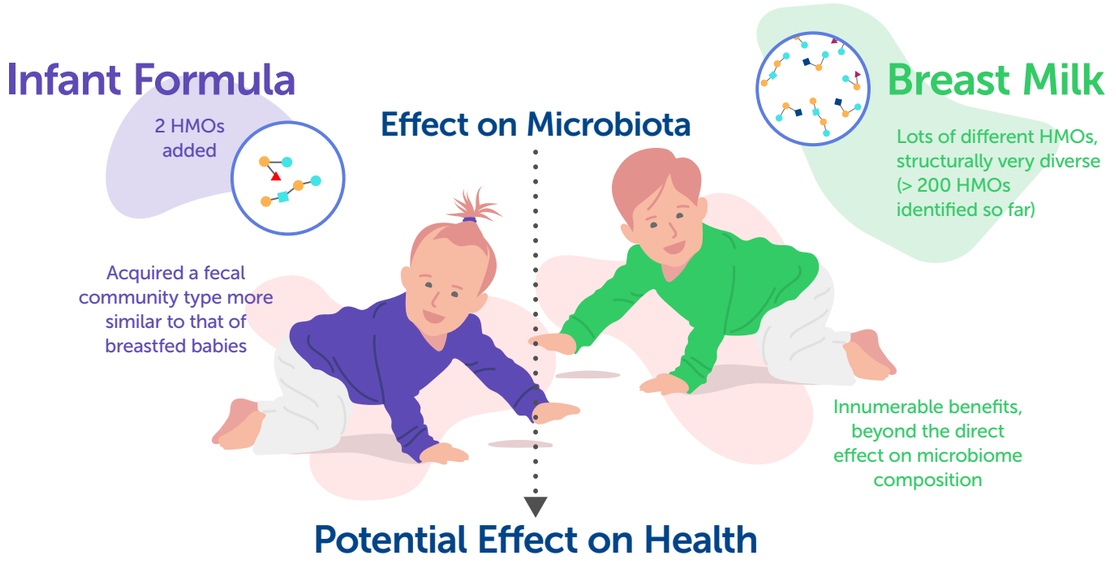
Human breast milk is an evolutionary masterpiece and the recommended way to feed a newborn infant. Despite it being exceptionally dynamic and individualized, the third largest component of maternal milk is always Human Milk Oligosaccharides (HMOs).[1] In the last few years, HMOs became a focus point for research in the field of infant nutrition for their potential to act as prebiotic factors, among other benefits. They have been shown in clinical and preclinical studies to have an impact on the infant's developing gut microbiota, as they serve as a substrate for beneficial bacteria.

There are well-described differences in the gut microbiota of babies that are breastfed compared to those that are bottle-fed. The majority of studies show an increase in the abundance of Bifidobacteria in breastfed babies in combination with an overall less diverse microbial community.[2] The establishment of the infant gut microbiota, especially in the first few months of life, is believed to be heavily influenced by components of maternal milk, such as HMOs. Through their modulation of the microbiota composition, these milk components can thus have an impact on host health. The establishment of an optimal microbial community immediately after birth and the maintenance of a balanced intestinal microbiota are important factors in the development of the immune system.

Human Milk Oligosaccharides are structurally very diverse, yet not every mother can produce all types of HMOs[3]. Genetic disposition of the lactating mother, such as being a "secretor" (and thus having a functional α1-2-fucoslyltransferase FUT2), influences the HMOs composition in the maternal milk. This in turn can then directly influence the bacterial species present in the infant gut.[4]

Prematurely born infants are especially at risk for developing diseases like Necrotizing entercolitis (NEC), a severe and often fatal intestinal disorder. Whilst breastmilk has been shown to have a protective effect compared to formula-feeding when it comes to the risk for developing NEC, some premature babies still develop NEC despite being predominantly breastfed.[5]






A prospective cohort study showed that breast milk of babies that developed NEC differed from that of the healthy babies by having a significantly lower concentration of a specific Human Milk Oligosaccharide called disialyllacto-N-tetraose (DSLNT).[6] This is an example how HMOs can potentially modulate the microbiota and in turn affect the health status of the infant. Different HMOs are currently being studied in this field.

Thanks to recent technological advances, it is now possible to synthesize a small subset of HMOs for use in infant formula. The hope is to transfer some of the observed benefits of human milk oligosaccharides to children who are bottle-fed. 2-fucosyllactose (2-FL) and lacto-N-neotetraose (LNnT) have been shown in a double-blind, randomized clinical trial to significantly increase the relative abundance of Bifidobacteria and steer the microbial composition more towards the microbiota of breastfed infants at month 3.[7] The formula-fed babies that had acquired a fecal community type similar to that of breastfed babies in the HMOs supplementation group were less likely to require antibiotic treatment up to the age of 12 months, compared to babies with other fecal community types.[7]

These results paint a positive outlook for the future where infant formula can be further optimized to minimize differences in gut microbiota composition, which could therefore result in potential health benefits.

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# Role of Probiotics in Diarrhea


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### Key messages:

- The use of antibiotics in early life changes the gut microbiota with possible long-term health implications.
- Dysbiosis in infectious diarrhea or antibiotic-associated diarrhea can be prevented and treated with the use of probiotics.
- The strain that has the greatest efficacy in both disorders is *Lacticaseibacillus* (L) *rhamnosus* GG (LGG) (previously known as *Lactobacillus rhamnosus* GG*) as confirmed by major clinical practice guidelines.

## Infections and antibiotic use in early life: Effects on the microbiome.

Infections are the most frequent reason for consultation in early life in both emergency and primary care. Although most infections, usually respiratory and gastrointestinal, are viral, many children are treated with antibiotics and their consumption is three times higher than in adults. Antibiotics can change the resistance to colonization of the gut microbiota, resulting in diarrhea in up to 40% of cases. Generally, all antibiotics can trigger it, although it is more frequent with those of wide spectrum.

However, the main problem with the consumption of antibiotics is that they can condition the development of our microbiota and their use during the first six months of life increases the risk of suffering from different diseases. Thus, their use has been associated with a greater risk of obesity, diabetes or autoimmune diseases, such as celiac disease.

## How is the intestinal microbiota recovered?

The alteration of the gut microbiota or dysbiosis that is observed in many diseases can be corrected through the use of probiotics. The greatest evidence of their effectiveness has been described in the treatment of acute infectious diarrhea, especially in childhood, being more significant in diarrhea caused by rotavirus. The beneficial effects are greater the earlier probiotics are administered and their efficacy is characterized by shortening the duration of the episode by approximately one day and decreasing the number of stools. A reduction in the rate of hospitalizations has also been observed, a frequent cause in children under 3 years of age. When combined with antibiotics, they reduce the risk of antibiotic-associated diarrhea, although their administration should be done at the beginning of the treatment and not when the condition has developed.



## Benefits of *L. rhamnosus* GG

But not all probiotics are the same and only some strains, mainly *Lacticaseibacillus* (L) *rhamnosus* GG (LGG) (previously known as *Lactobacillus rhamnosus* GG*) and *Saccharomyces boulardii* CNCM I-745 have proven to be effective in acute infectious diarrhea and antibiotic-associated diarrhea. Evidence shows that, in general, LGG reduces both the duration of diarrhea and hospitalization in these patients, although the effective dose has yet to be defined since beneficial effects have been mostly observed with ≥ $10^{10}$ CFU/day.[1-3]

The randomized clinical trials are included in the different clinical practice guidelines that demonstrate that LGG is the strain that has more scientific evidence as shown in the attached table based on the World Gastroenterology Organisation (WGO) guideline.[4] This strain is also recommended in the latest ESPGHAN review, although the level of evidence is lower as more methodological rigour is applied.[5] In short, we can conclude that LGG has beneficial effects on the infant by promoting a balanced or healthy microbiota.

| Disorder | Probiotic strain | Recomended dose | Evidence level** | Comments |
|---|---|---|---|---|
| Treatment of acute gastroenteritis | LGG | ≥ $10^{10}$ CFU/day (typically 5-7 days) | 1 | ESPGHAN/ESPID recommendations 2014 [6] |
| | *S. boulardii* CNCM I-745 | 250-750 mg/day (typically 5-7 days) | 1 | ESPGAH Working Group on Probiotics 2014 [7]<br>Meta-analysis of RCTs |
| Prevention of antibiotic-associated diarrhea (AAD) | LGG | 1-2 x $10^{10}$ CFU | 1 | ESPGHAH Working Group on Probiotics 2016[8] |
| | *S. boulardii* CNCM I-745 | 250-500 mg | 1 | |
| Prevention of nosocomial diarrhea | LGG | $10^{10}$-$10^{11}$ CFU Twice daily | 1 | Meta-analysis of RCTs[9] |
| Infections in children attending day-care centers | LGG | 1-2 x $10^{10}$ CFU | 1 | Prevention of AAD[10] |

*Figure 1: ** Systematic review of randomized trials or n-of-1 trials "2011 level of evidence" Oxford Centre for Evidence-Based Medicine*


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* Due to reclassification of *Lactobacillus genus* into groups of closely related species, *Lactobacillus rhamnosus* GG is renamed to *Lacticaseibacillus rhamnosus* GG.




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