

Gut Microbiota Dysbiosis and Role of Probiotics in Infant Colic

Abstract

Infant colic or excessive crying syndrome is a behavioral syndrome in 1- to 5-month-old infants involving long periods of inconsolable crying and fussing in otherwise healthy infants. Its etiology is unclear, but evidence regarding the implications of the infant gut microbiota is rising in importance.

In this short review, we aim to expose recent findings on alterations in the microbiota of colicky infants which could trigger or facilitate infant colic, and summarize the clinical and mechanistical evidence of several probiotic formulations for the management of infant colic.

The observed increase in the relative abundance of Enterobacteria in colicky babies may facilitate intestinal hypersensitivity due to their endotoxins, while also increasing the amount of intestinal gas, and thus, bloating and digestive discomfort. Also, an association between infant colic and a decrease in the relative abundance of protective bacterial groups such as bifidobacteria or lactobacilli has been pointed out by independent research groups. Therefore, it is not surprising that many clinical trials have explored the effects of supplementing the microbiota of colicky infants with probiotics due to their known effect in modulating gastrointestinal microbiota.

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Introduction

Infant colic or excessive crying syndrome is a behavioral syndrome in 1- to 5-month-old infants involving long periods of crying and hard-to-soothe behavior. The crying bouts occur without obvious cause, so that their unexplained nature is one of the main reasons for caregivers' concerns [1]. Its etiology is unknown and possibly multifactorial [2]. Because it affects otherwise healthy infants without a specific cause, infant colic has a controversial physiopathology. In addition, it represents an auto-limited syndrome that disappears after the fourth or fifth month of life. For all these reasons, infant colic is classified as a Functional Gastrointestinal Disease (FGID) [1]. The incidence of infant colic has been reported to range from 8% to 20% [3], and represents the cause of 10-20% of all pediatrician visits in the first 4 months of life [4].

Infant colic was initially defined in 1954 by Wessel as an excess of crying or paroxysmic irritability without an apparent cause, for more than three hours a day, occurring more than three days a week in three consecutive weeks [5]. However, different concerns were risen regarding this definition, as in daily practice

a noticeable population of infants shows an abnormal and unexplained crying profile despite not perfectly fitting Wessel's criteria. Subjective assessment of crying time was also found to be dependent on the cultural environment of the patient, being subjected to a high variability. For this reason, Wessel's criteria have been abandoned and the international association for FGIDs, known as the Rome Foundation, has reached a consensus definition of colic as "Recurrent and prolonged periods of infant crying, fussing, or irritability reported by caregivers that occur without obvious cause and cannot be prevented or resolved by caregivers" [6]. This new definition is also a consequence of the high interest displayed by the pediatrician community in identifying this pathology, to investigate and provide adequate treatment.

Colicky bouts are more frequent in the late afternoon or evening, starting suddenly and being intense and often high pitched [7]. Moreover, 'colicky infants' are usually very unsettled, fussy or irritable or may show signs of pain (e.g. drawing up knees or arching the back) [8]. Colic is often a serious issue for parents, as excessive crying leads to parental exhaustion and anxiety, with negative consequences such as: difficulties to concentrate, loss

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of patience, feelings of incompetence, fear of harming the child, early cessation of breastfeeding and reduction of face-to-face interaction with their child [8,9].

Etiology of infant colic and role of gut microbiota

A variety of causes for infant colic have been suggested, including abnormal gastrointestinal function, immaturity of the gut, spastic colon, accumulation of gas, allergic problems and lack of sufficient parent-to-child interaction (**Table 1**) [2,6,10]. Despite this unclear and possibly multifactorial etiology, studies by independent research groups have correlated alterations in gut microflora with infant colic [11-16]. This consideration has been growing in importance since the early observations of lower counts of intestinal lactobacilli and bifidobacteria in colicky infants compared to healthy ones [17,18].

As a result of the aforementioned studies, the microbiota profile of colicky infants can start to be delineated. First, omics studies conducted by different means (e.g. microarrays, metagenomic analysis) have reported a reduction in overall microbiota diversity compared to healthy infants [12,19]. Second, particular alterations comprising a reduction in the proportions of *Bifidobacteria* [20,21], *Bacteroides* [21] and lactobacilli [14,15,20-22], and an increase of *Clostridium*, *Staphylococcus* and specially of Enterobacteria such as *Escherichia*, *Shigella*, *Klebsiella* or *Enterobacter* have been found [11-13,15-19,23].

Implication of microbiota disturbances in the symptoms of infant colic

Lower microbiota diversity and stability in the first weeks of life may lead to changes in the metabolome of gut microbiota, the ensemble of gut microbiota by-products which influence human health [24,25]. In turn, changes in bacterial metabolome can alter the speed of intestinal transit, facilitating an accumulation of gas causing general digestive discomfort [11,14,26]. Also, deregulation of motor function could result in spasmotic bowel movements, leading to pain. Additionally, a less diverse gut microbiota has been associated with higher levels of calprotectin, a known biomarker of gut inflammation [19].

As described above, relative abundance of opportunistic and commensal Enterobacterial species seem to increase in infant colic in comparison to healthy infants [12,21]. These bacteria are known to produce gas as a result of their metabolism [27], which could facilitate bloating and digestive discomfort in colicky babies [11]. Moreover, lipopolysaccharide (LPS) molecules (also known as endotoxins) from Enterobacteria are especially pro-inflammatory, compared to LPS from other common gut Gram-negative bacteria such as *Bacteroides* [28]. Inflammatory effect of LPS is mediated by Toll-like receptors, and is known to reduce visceral sensory and pain thresholds (allodynia) both in animal models and in humans [29-31]. By this mechanism, a higher relative abundance of Enterobacteria could facilitate a state of low-grade inflammation and increased visceral sensitivity in colicky infants.

In contrast, evidence suggests that a high proportion of *Bifidobacterium* and *Lactobacillus* in the infants microbiota is protective against colicky crying and fussing [12,20]. This

protective effect might be related with the fact that mucosal *Lactobacillus* are able to induce the expression of anti-inflammatory genes [32], improving gut function and motility and exerting a reduction of visceral pain [33,34]. In addition, lactobacilli and bifidobacteria may protect against colic by modulating immune response [35]. Alterations in gut microbiota of colicky infants are summarized in **Table 2**. Importantly, some factors related to the intestinal microbiota are age-related, which could help explain why infant colic resolves spontaneously around 4 to 6 months of age [2]: First, bile acids represent a key environmental factor modulating gut bacteria at high taxonomic levels, and overall bile acid metabolism is altered during the first months of life (as demonstrated by the fact that cholic to chenodeoxycholic acids ratio in duodenum and bile acid levels in serum are increased during the first months of life). Second, the overall diversity of the intestinal microbiome has been described to increase in an age-related manner during the first months of life, and increased diversity is known to make ecosystems more resistant to perturbations by opportunistic organisms (such as Enterobacteria in the gut).

Treating infant colic with probiotics

Since an effective solution for excessive crying is not available

Table 1 Possible factors implicated in the ethiopathology of infant colic.

Factors intrinsic to the infant	Immaturity of the intestinal tract and of the epithelial barrier
	Microbial imbalance in the gut microbiota
	Immature gastrointestinal function (motility, bile acid mechanisms)
	Accumulation of gas
	Spastic colon
	Alterations in gut hormones
	Transient food intolerance or allergy
	Last stage in the developmental "crying curve" of healthy infants
	Insufficient parent-to-child interaction
Factors related to parents-infant interaction	Family tensions
	Parental anxiety
	First born status
	Maternal smoking
	Increasing maternal age

Table 2 Alterations in gut microbiota of colicky infants that may be implied in the ethiopathology of infant colic.

Microbiota alteration	Implication in infant colic
Low microbiota diversity and stability: changes in metabolome [12,14,19]	Alterations in intestinal transit Spasmodic bowel movements Gas accumulation Higher levels of calprotectin: inflammation
↑Enterobacteriaceae [12,21]	Gas accumulation: bloating and digestive discomfort Pro-inflammatory and hyperalgesia reaction to LPS
↓Bifidobacterium [12,52]	Immune response modulation
↓Lactobacillus [12,52]	Expression of anti-inflammatory genes

as of today, it represents an unmet clinical need and there is a high interest in understanding its physiopathology to provide the best available treatment. Because of the evidence of microbiota changes in colicky infants, some probiotic formulations have

been studied for the management of infant colic, as summarized in **Table 3**. The studies have focused mainly on the reduction of crying time as a primary outcome of baby's discomfort.

In an early double-blind, randomized, placebo-controlled study

Table 3 Comparison of the different effects of probiotics on infant colic described by clinical trials. Since all studies reported good tolerability and absence of side effects, these are not included in the table. FF: Exclusively or predominantly formula-fed; BF: Exclusively or predominantly breast-fed.

Probiotic	Study	Design	Population	Endpoints	Results
<i>Bifidobacterium lactis BB-12 + S. termophilus</i>	Saavedra et al. 2004 [36]	Randomized, double-blind, placebo-controlled study. Prophylactic intervention	180 FF	Crying and fussing behavior (reported by parents)	Significantly lower reported incidence of colic in the probiotic group
<i>Lactobacillus reuteri</i> DSM 17938	Savino et al. 2010 [37]	Randomized, double-blinded, placebo-controlled study	46 BF	Crying time	Reduction in crying time in the probiotic group after 21 days
				Responders rate (50% reduction in crying time)	Significantly higher in the <i>L. reuteri</i> group
				Gut microbiota	Significant increase in fecal lactobacilli Reduction in fecal <i>Escherichia coli</i> and ammonia in the <i>L. reuteri</i> group
				Responders rate (50% reduction in crying time)	Significantly higher in the probiotic group on days 7, 14, 21 and 28
<i>Lactobacillus reuteri</i> DSM 17938	Szajewska et al. 2013 [38]	Randomized, double-blinded, placebo-controlled study	80 BF	Crying time	Significantly reduced in the probiotic group
				Parental perception of colic severity	Significant reduction already on day 7
				Family quality of life	Significant improvement already on day 7
				Responders rate (50% reduction in crying time)	Significantly higher responders rate in the probiotic group
<i>Lactobacillus reuteri</i> DSM 17938	Roos et al. 2013 [21]	Randomized, double-blinded, placebo-controlled study	29 BF	Microbiota composition	Very high inter-individual variability ↑ <i>Bacteroidetes</i> and genus <i>Bacteroides</i> in the responders group on day 21 vs. day 0
				Crying time	Significantly higher reduction in the placebo group, especially fussing in formula-fed infants
				Responders rate (50% reduction in crying time)	No significant differences
				Sleep duration of infants	Significant less sleep in the probiotic group (P=0.04)
				Maternal mental health	No significant differences
				Family functioning	No significant differences
				Parent quality of life	Significant improvement in both groups at 6 months
			167 FF + BF	Fecal microbiota diversity	No significant differences
				Colonization with <i>E. coli</i>	No significant differences
<i>Lactobacillus reuteri</i> DSM 17938	Sung et al. 2014 [42]	Randomized, double-blinded, placebo-controlled study	65	Calprotectin	No significant differences between groups Lower levels in the responders group after 1 month
				Number of responders	Significantly higher in the probiotic group (100% vs. 15.7% in the placebo group)
				Crying time	Significant difference between groups already at day 7 (higher after 4 weeks)
				Parent satisfaction	Significantly higher in the probiotic group since the first week
				Maternal depression	Improved significantly in the probiotic group since the first week
<i>Lactobacillus reuteri</i> DSM 17938	Mi et al. 2015 [40]	Observational randomized, placebo-controlled study	42 BF	Crying time	Significantly shorter in the probiotic group Significant reduction in the probiotic group vs. placebo
				Responders rate (50% reduction in crying time)	Significantly higher in the probiotic group at day 21
<i>Lactobacillus reuteri</i> DSM 17938	Chau et al. 2015 [39]	Randomized, double-blinded, placebo-controlled study	52 BF	Crying time	Significantly shorter in the probiotic group Significant reduction in the probiotic group vs. placebo

Probiotic	Study	Design	Population	Endpoints	Results
<i>Lactobacillus rhamnosus</i> GG	Pärtty et al. 2015 [45]	Randomized, double-blinded, placebo controlled study	30 FF + BF	Daily crying (crying diary)	No differences at the end of the study
				Parents subjective report of daily crying	Lower number of crying days in the probiotic group and higher rate of responders
				Fecal calprotectin	No significant change
				Microbiota changes	No statistical differences in microbiota composition Significant increase in different <i>Bifidobacterium</i> species
	Fatheree et al. 2016 [46]	Randomized, double-blinded, placebo-controlled pilot study (LGG+ formula)	20 FF	Crying time	No differences
				Microbiota changes	No differences in diversity (erratic evolution) No differences at the genus level Significant increase in <i>L. rhamnosus</i>
				Fecal calprotectin	No significant differences between groups
				Cytokine levels	No significant differences between groups
<i>Bifidobacterium breve</i> BR03 + <i>B. breve</i> B632	Giglione et al. 2016 [47]	Randomized, double-blinded, placebo-controlled study. Prophylactic intervention	60 FF + BF	Reduction in crying time	No significant effects in the full population of the study
					Significant reduction of crying time vs. placebo in the formula-fed group only, after 3 months of treatment
<i>Pediococcus pentosaceus</i> CECT 8330 + <i>Bifidobacterium longum</i> CECT 7894	Santas et al. 2015 [49]	Randomized, double-blinded, placebo-controlled pilot study	20 FF + BF	Crying time	Trend towards greater reduction in crying time in the probiotic group compared to placebo, after 14 days
	Tintore et al. 2017 [50]	Randomized, double-blind placebo-controlled pilot study	10 FF + BF	Microbiota diversity	Increase in overall microbiota diversity in probiotic (inversely correlated to crying time in the overall study population)
				Changes in genus associated to infant colic	Reduction in relative abundance of <i>Enterobacteria</i> (mostly of <i>Escherichia/ Shigella</i>) and of <i>Staphylococcus</i> , compared to placebo
				Changes in genus associated to a protective effect	Significant increase in relative abundance of <i>Bifidobacterium</i>
<i>Lactobacillus casei</i> , <i>L. rhamnosus</i> , <i>L. bulgaricus</i> , <i>L. acidophilus</i> , <i>Streptococcus thermophilus</i> , <i>Bifidobacterium breve</i> , <i>B. infantis</i> and fructooligosacharides	Kianifar et al. 2014 [51]	Randomized, double-blinded, placebo controlled study	45 BF	Responders rate (50% reduction in crying time)	Significantly higher in the probiotic group at days 7 and 30
				Crying time	Significant reduction in the probiotic group at days 7 and 30
				Symptom resolution	Significantly higher in the probiotic group at day 7, loss of significance at day 30
				Weight gain	No significant changes at the end of the intervention

including 180 healthy infants aged 3-24 months, prophylactic administration of a standard milk-based formula containing a strain of *Bifidobacterium lactis* BB-12 and an unspecified strain of *Streptococcus thermophilus* resulted in a reduction of the incidence of colic, compared to controls [36]. The study represented one of the first proofs of the important role of Bifidobacteria as potential probiotics for preventing infant colic, but to our knowledge, no therapeutic studies have assessed the effectiveness of this combination as a treatment of infant colic after the onset of the inconsolable crying behavior.

Subsequently, *Lactobacillus reuteri* DSM 17938 has been one

of the most studied probiotic strains for treating infant colic. Several small studies in predominantly breast-fed colicky infants have consistently reported a significant effect on infant colic vs. controls [37-40] and, according to meta-analysis, the effects were most evident after 21 days of supplementation [41]. However, a randomized study on a larger population including both breastfed and formula-fed colicky infants reported conflicting results, due to a lack of significant difference in responder rate in the overall population ($P=0.23$), and a significant increase in crying and especially fussing in the subgroup of formula-fed infants (79 minutes on average, $P=0.005$) [42,43]. Following these studies,

the latest World Gastroenterology Organization guidelines on probiotics indicate that *L. reuteri* DSM 17938 may be effective for breastfed infants with colic, but at this stage, the probiotic cannot be recommended for treating infantile colic in formula-fed infants, nor can be routinely used to prevent infantile colic [43].

The well-known probiotic strain *Lactobacillus rhamnosus* GG, while being strongly supported for the treatment of acute diarrhea in children [44], has not reached positive results for the treatment of baby colic in clinical trials. In a first study, diary-verified crying time did not reach a statistical difference upon *L. rhamnosus* GG supplementation, despite parental subjective assessment of crying suggesting some effectiveness [45]. Of note, this study replicated the finding that Bifidobacteria relative abundance was higher in healthy controls than in colicky infants. A more recent study also reported a lack of efficacy of LGG-supplemented formula with respect to the control one, and little impact on the microbiota composition and on inflammation was observed [46]. These results add to the growing evidence that probiotic effects are strain-specific and condition-specific, as suggested by WGO guidelines [43].

More recently, a combination of Bifidobacteria – *B. breve* BR03 and *B. breve* B632 – has shown a significant reduction in crying time in a subgroup of formula-fed infants after 3 months of prophylactic treatment [47]. However, no significant differences were observed for the full population including breastfed infants. As observed in the study with *B. lactis* BB-12 and *S. thermophilus* [36], these results suggest Bifidobacteria may be useful for infantile colic and support the preventive use of the specific strains studied against infant colic in formula-fed infants, but are not sufficient to confirm their effectiveness as a treatment for colicky babies.

Pediococci are round-shaped bacteria belonging to the *Lactobacillus* group [48]. A probiotic formula including strains *Pediococcus pentosaceus* CECT 8330 and *Bifidobacterium longum* CECT 7894 showed a significant trend towards a greater reduction in daily crying time in just 14 days, in a pilot RCT comparing to placebo ($p=0.083$). The study included both breastfed and formula-fed colicky infants, and in vitro data suggest that the mechanism of action for the probiotic formula may be ascribed to an antagonistic effect against Enterobacteria and a capacity to counteract the pro-inflammatory effect of their endotoxin

on the immune cells [49]. Clinical studies with larger sample sizes are required to validate these findings. However, findings are further supported by an additional study on the observed changes in the microbiome after the supplementation with said probiotic strains compared to placebo [50]. More precisely, this study reports an increase in α -diversity in the probiotic group, which correlated with a reduction in the total crying time in the full study population. Also, an increase in *Bifidobacterium* and a decrease in species belonging to the *Enterobacteriaceae* family was observed in probiotic-treated infants compared to placebo-treated ones. Noteworthy, these results are in agreement with the in vitro studies on the antagonistic effects of the probiotic strains used [49], as well as with observational evidence on the relationship between infant colic and microbiome diversity, Enterobacterial abundance and Bifidobacterial abundance [12,19].

Finally, symbiotic formulas have also been assessed in the management of infant colic, showing promising results. A mixture of fructooligosaccharides (FOS) and seven unspecified probiotic strains of the species *Lactobacillus casei*, *L. rhamnosus*, *L. bulgaricus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, and *B. infantis* significantly improved symptoms in colicky babies in comparison to placebo [51,52]. Positive effects were observed already on day 7; however, the positive effect of the probiotic vs. placebo had disappeared after 30 days of treatment. In addition, the fact that the probiotic strains were not specified hinders the subsequent replication of this study.

Conclusions

Effective management of infant colic, a condition that severely impacts family quality of life, represents an unmet medical need. Specific changes in gut microbiome have been described in colicky babies, affecting overall diversity and relative abundance of specific bacterial groups such as Enterobacteria, Bifidobacteria and Lactobacilli. In this regard, several clinical trials report evidence of the usefulness of some probiotics to treat this condition. Importantly, not all probiotics studied appear to be effective, and some seem to depend on the infant's feeding (breastfed vs. formula-fed) for their effectiveness. Further evidence about validated mechanisms of action and randomized clinical studies with larger sample sizes are needed to confirm the benefits of this approach.

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