Bifidobacterium animalis subsp. *lactis* BB-12 and infant regurgitation: a real-life experience

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Infant regurgitation is the most common functional gastrointestinal disorder (FGID) worldwide and causes parental concern with relevant direct and indirect costs for families and the healthcare system. *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) is a well-known studied probiotic with evidence in managing another FGID, such as infantile colic. This real-life study evaluated the efficacy of BB-12 in decreasing functional regurgitation symptoms when supplemented in formula-fed infants (partial or absolute). In 17 outpatient services of the Italian National Pediatric Health Care System, formula-fed infants with persisting regurgitation symptoms were randomly (1:1) allocated to receive six drops ($1x10^9$ CFU of BB 12; ABINAT12®) daily (Group A) or any probiotic (Group B) for two months. Regurgitation symptoms were evaluated through the Infant Gastroesophageal Reflux Questionnaire-Revised (I-GERQ-R), performed at the baseline visit (T0) and after 30 (T1) and 60 (T2) days. A positive response was defined as a total score ≥ 16. Nine hundred and sixty infants were randomly allocated to receive BB12 (Group A; 499 subjects) or any probiotic (Group B; 461 subjects). At baseline, 25.8% in Group A and 31.7% in Group B responded positively to the I-GERQ-R questionnaire. At T1, 16% in Group A and 45.8% in Group B ($p<0.001$) had a positive I-GERQ-R. At T2, 14.7% in Group A and 50.7% in Group B ($p<0.001$) had a positive I-GEQ-R. Consistently, the total scores significantly decreased in Group A. In conclusion, this real-life study demonstrated that a two-month BB-12 supplementation (ABINAT12®) significantly reduced regurgitation prevalence and severity in formula-fed infants.

Infant regurgitation is the most common of the functional gastrointestinal disorders (FGIDs) and one of the leading causes of parental concern and anxiety in the first months of life, with relevant direct and indirect costs for families and the healthcare system (1-3). Diagnostic criteria for infant regurgitation include at

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least due episodes of regurgitation per day for at least three weeks in an otherwise healthy infant 3 weeks to 12 months of age without retching, hematemesis, aspiration, apnea, failure to thrive, feeding or swallowing difficulties, or abnormal posturing (4).

Although infant regurgitation must be considered a self-limited manifestation that spontaneously resolves during the first year of life, a low-grade mucosal inflammation, leading to dysfunctional gut motor activities, have been described in infants with persistent regurgitation (5).

Recent evidence underlines the pivotal role of intestinal microbiota in the modulation of intestinal inflammation, and many researchers have hypothesised how using some probiotic strains in infancy could be beneficial in managing functional gastrointestinal disorders (6, 7).

The probiotic strain *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) is the world's most documented probiotic *Bifidobacterium* (8). Indeed, more than 300 scientific publications concerned this probiotic, and more than 130 publications regarded human clinical studies. In addition, the complete genome sequence of BB-12 has been determined and published (9).

Strain characteristics and mechanisms of BB-12 have been established through extensive *in vitro* testing. BB-12 exhibits excellent gastric acid and bile tolerance; it contains bile salt hydrolase and has strong mucus adherence properties, all valuable probiotic characteristics (8). Pathogen inhibition, barrier function enhancement, and immune interactions are mechanisms that all have been demonstrated for BB-12. BB-12 has proven its beneficial health effect in numerous clinical studies within gastrointestinal health and immune function. Clinical studies have demonstrated the survival of BB-12 through the gastrointestinal tract, and BB-12 has been shown to support a healthy gastrointestinal microbiota (8).

Furthermore, BB-12 has been shown to improve bowel function, protect against diarrhoea, and reduce side effects of antibiotic treatment, such as antibiotic-associated diarrhoea. In terms of immune function, clinical studies have shown that BB-12 increases the body's resistance to common respiratory infections and reduces the incidence of acute respiratory tract infections (8). Finally, BB-12 safety has been widely reported (10).

However, it is essential to note that the beneficial effects of a given probiotic are specific to that strain and cannot be regarded as general for other strains of the same species, or other species, of bacteria or yeast.

The current study aimed to evaluate the efficacy of BB-12 supplementation in decreasing regurgitation symptoms in formula-fed infants.

MATERIALS AND METHODS

This was a multicenter, perspective, randomised, and controlled study performed in 17 Italian pediatric outpatient services distributed across the country and assuring comprehensive and complete national coverage. The study was approved by the Ethical Committee of the "Ospedale Policlinico" of Bari, which was allocated to the coordinating centre.

Inclusion criteria were: 1) at least two episodes of regurgitation per day for 3 or more weeks, according to Rome IV criteria (4); 2) gestational age > 34 weeks; 3) age at enrollment ≤ 4 weeks; and 4) fed with exclusively or predominantly not-thickened formula. Exclusion criteria were: 1) acute gastrointestinal diseases; 2) current infections; 3) chronic diseases, and 4) malformations.

All parents of eligible infants answered the Infant Gastroesophageal Reflux Questionnaire-Revised (I-GERQ-R), a validated questionnaire to evaluate GER and GERD-related symptoms in infants (11,12). We considered persistent regurgitation when I-GERQ-R total score was above the cut-off limit, such as ≥16, as previously reported (13). Infants with I-GERQ-R ≥16 were enrolled and randomly allocated (ratio 1:1) into two groups: Group A was supplemented with a probiotic (BB-12), Group B did not receive any supplementation and was considered the control group. The probiotic (ABINAT12®) was administered once daily (six drops = $1x10^9$ CFU). I-GERQ-R has been repeated at follow-up visits: after 30 (T1) and 60 (T2) days during probiotic supplementation.

The Wilcoxon and Mann U Whitney tests were used. Analyses were performed using GraphPad Prism software, GraphPad Software Inc, CA, USA.

RESULTS

The present study included 960 infants who completed the treatment period: 499 (52%) in Group

A and 461 (48%) in Group B. The mean gestational age in Group A was 38.2 weeks and 38.1 weeks in Group B. The mean birth weight was 3.2 Kg in both Group A and Group B. The two groups were well matched at baseline.

Table I reports the clinical data concerning the anthropometric measures and breastfeeding details. At baseline, 129 (25.8%) infants of Group A and 146 (31.7%) of Group B had an I-GERQ-R total score ≥ 16 (Table II). The mean total score was 22.3 (SD 5.3) in Group A and 23 (SD 5.3) in Group B (Table III). At T1, 16% of infants of Group A and 45.8% of Group B had an I-GERQ-R total score ≥ 16 ($p<0.001$). The mean total score was consistently 14.6 (SD 5.2) in Group A and 22.3 (SD 7.8) in Group B. At T2, 14.7% of infants of Group A and 50.7% of Group B had an I-GERQ-R total score ≥ 16 ($p<0.001$). The mean total score was consistently 11 (SD 4.5) in Group A and 21 (SD 7.6) in Group B.

Figures 1 and 2 show the over-time changes of infants with a positive response to I-GERQ-R and severity of regurgitation, such as the total score. The oral probiotic was safe and well-tolerated.

DISCUSSION

Regurgitation is a common medical problem in infants with well-defined and standardised diagnostic criteria (14-15). In the present study, I-GERQ-R was used to consider the relevance of regurgitation symptoms when the total score was equal to or more than 16 (16).

Table I. *Clinical data in the groups of infants*

	Group A	**Group B**	**p-value**
	N=499	N=461	
Weight, kg, mean (SD)	3.1 (0.5)	3.1 (0.5)	0.675
Height, cm, mean (SD)	49.7 (3.7)	49.5 (3.4)	0.127
Cranial circumference, cm, mean (SD)	34.3 (1.8)	34.2 (1.8)	0.238
Type of breastfeeding - formula alone - mixed	 134 (26.9%) 365 (73.1%)	 112 (24.3%) 349 (75.7%)	 0.364
Number of daily suckings, mean (SD)	6.4 (2.9)	6.6 (3.3)	0.642
Quantity of milk for each sucking, mL, mean (SD)	41.4 (26.8)	42.4 (27.0)	0.584

Table II. *Percentages of infants with positive I-GERQ-R in both groups at different visits*

Visits	**Group A**	**Group B**	**p-value**
T0	25.8%	31.7%	0.046
T1	16%	45.8%	<0.001
T2	14.7%	50.7%	<0.001

Table III. *Mean (+ Standard Deviation) total scores of I-GERQ-R in both groups at different visits*

Visits	**Group A**	**Group B**	**p-value**
T0	22.3 (5.3)	23 (5.3)	0.204
T1	14.6 (5.2)	22.3 (7.8)	<0.001
T2	11 (4.5)	21 (7.6)	<0.001

According to our results, the prevalence of relevant regurgitation symptoms at baseline was about 25-30% in an unselected population of formula-fed infants. This data was consistent with others, although the prevalence of regurgitation has a wide range in literature, according to the different populations studied (1, 17, 18).

The current guideline does not recommend pharmacological treatment but only reassurance and behavioral approach as a first steps strategy (19). However, we speculated that nutritional supplementation with probiotics could improve microbiota colonisation and represent a strategy to reduce the prevalence of relevant regurgitation symptoms in formula-fed infants.

According to the current data, the percentage of relevant regurgitation symptoms significantly decreased over the time when a probiotic supplementation with BB-12 was scheduled.

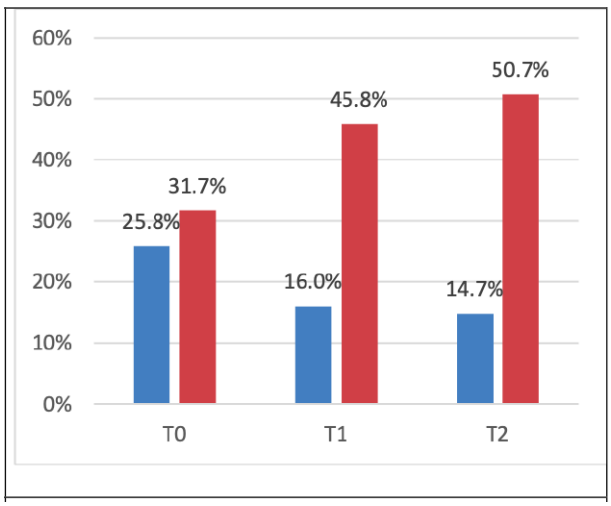

Fig. 1. *Changes over time for prevalence of persistent regurgitation (total score > 16 for I-GERQ-R) in Group A (blue) and Group B (red).*

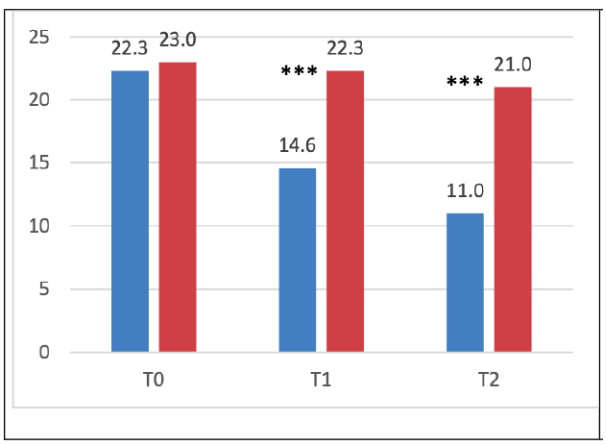

Fig. 2. *Changes over time for the total score of I-GERQ-R questionnaire in Group A (blue) and Group B (red).*

These results were consistent with some previous studies investigating the role of probiotics in managing infants with regurgitation. The first placebo-controlled study explored the effects of *Lactobacillus reuteri* DSM 17938 on gastric emptying in infants with regurgitation (20). Thirty-day probiotic supplementation with *L reuteri* significantly reduced the fasting antral area, increased the Δ gastric emptying rate, and, consequently, reduced the regurgitation episodes. A further randomised study, conducted by the same researchers, investigated the prophylactic use of *Lactobacillus reuteri* DSM 17938 during the first 3 months of life for reducing the onset of colic, gastroesophageal reflux, and constipation in term newborns (21). The probiotic supplementation significantly reduced the duration of crying time, regurgitation episodes, and evacuation number. Another study demonstrated that the early administration of *Lactobacillus reuteri* DSM 17938 for 4 weeks in full-term breastfed infants significantly reduced daily regurgitation (22).

The previous study investigated the effect of a partially hydrolysed whey infant formula supplemented with starch (as prebiotic) and the probiotic *Lactobacillus reuteri* DSM 17938 on regurgitation and gastric motility in infants with functional regurgitation (23). The formula containing the probiotic significantly decreased the regurgitation frequency and improved the gastric emptying rate. Therefore, this body of evidence underscores the potential benefit of probiotics in managing infants with functional digestive disorders. Namely, the present study confirmed previous positive outcomes in infants supplemented with oral probiotics.

In addition, BB-12 supplementation exerted positive effects in colic infants (24,25), increased the production of intestinal short-chain fatty acids in infants (26), improved the microbiota composition in preterm infants (27), and reduced the gastrointestinal infections in early childhood (28). Consequently, the outcomes obtained in the current study conducted in infants with regurgitation confirmed the multifaceted efficacy of BB-12 in pediatric disorders.

However, the present study had limitations, including the open design, the lack of microbiota assessment, and biomarkers measurement. Nevertheless, the strength of this experience was the relevant number of infants who participated and the clinical setting, such as neonatal clinics. Thus, the findings could faithfully reflect what occurs in daily practice.

In conclusion, this study demonstrated that persistent regurgitation is a relevant medical problem in infants. In addition, a two-month BB-12 supplementation (ABINAT12®) reduced the prevalence and severity of persistent regurgitation in formula-fed infants significantly.

Conflict of interest:

All authors state that there is no conflict of interest.

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