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ORIGINAL ARTICLE

Use of a probiotic mixture containing *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Enterococcus faecium* L3 as prophylaxis to reduce the incidence of acute gastroenteritis and upper respiratory tract infections in children

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ABSTRACT

BACKGROUND: For healthy children, attending communities such as nurseries, kindergartens or schools, exposes them to the risk of acute gastroenteritis (AGE) and/or upper respiratory tract infections (URTIs). We therefore evaluated whether the use of a well-documented probiotic formula could act as prophylaxis for AGE and URTIs, reducing the risk of occurrence.

METHODS: In a randomized study, we tested a probiotic mixture containing *Bifidobacterium animalis* subspecies *lactis* BB-12 and *Enterococcus faecium* L3 on 94 healthy children, comparing the incidence and duration of episodes of AGE and the incidence of URTIs to those of a control group of 109 healthy, untreated subjects. In a subgroup consisting of 34 healthy, treated children, we also evaluated salivary IgA levels.

RESULTS: The use of the probiotic formula significantly reduced the incidence and duration of episodes of AGE by 82% and 45%, respectively, and the incidence and duration of episodes of URTIs by 84% and 50%. Salivary IgA levels significantly increased three-fold after 90 days of probiotic treatment. The probiotic formula was well tolerated and no side effects occurred.

CONCLUSIONS: According to our results, use of the probiotic strains BB-12 and L3 statistically reduced the risk of AGE and URTIs in healthy children and increased levels of salivary IgA.

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KEY WORDS: Influenza, human; Common cold; Diarrhea; Bifidobacterium animalis; Enterococcus faecium.

Clinical health records, retrospectively reviewed with the aim of assessing access to a pediatric emergency department in a large Italian (Monza) hospital, have shown that between October and November 2017, out of 2364 cases, the most frequent diagnoses were upper respiratory tract infections (URTIs) (29.5% of cases), followed by gastroenteritis (7.0%) and abdominal

pain (7.0%).[1] Similar findings were observed in 2012, between January and June, in an observational study performed in the Lombardy region (Italy), where, out of 133,275 children (10.6% of the pediatric population), the most common reasons for hospital presentation, not including traumatic injuries (26.1%), were respiratory tract infections (19.9%) and gastrointestinal illnesses (8.5%).[2] These numbers should be taken into serious consideration as they demonstrate: 1) how seriously acute gastroenteritis (AGE) and URTIs are perceived by families, entities which, for the most part, should be managed by the family pediatrician; and 2) the substantial impact the occurrence of AGE and URTIs has on the prescription of antibiotics, prescribed in at least one out of five cases of the total accessing the pediatric emergency room and in at least one out of two cases of respiratory infection,[2, 3] this having a certain effect on the phenomenon of antibiotic resistance.[4] As recently reported on the basis of the analysis of a number of controlled trials carried out with high-quality methodology,[5] probiotics have been shown to have some positive effects, decreasing the incidence and the severity of symptoms, with respect to the occurrence of URTIs in the pediatric population. Comparable results have been obtained from a similar analysis performed evaluating the relationship between probiotics and pediatric AGE.[6] Although still a long way from becoming a globally accepted treatment modality, the small positive changes observed and the prophylactic role played by probiotics with respect to these pathologies are important, and should encourage researchers and clinicians to consider further testing of probiotic agents, particularly relying on well-defined (in terms of genomics, stability, vitality and colonizing features) and highly documented (regarding safety profile and clinical effects) strains.

Bifidobacterium is a bacterial genus that has a considerable presence and dominance in the gut microbiota of newborns and children.[7] *Bifidobacterium animalis* subspecies *lactis* BB-12 is one of the most scientifically documented probiotic strains,[8] being the subject of more than 300 scientific publications, out of which, more than 130 involve human clinical studies. The strain, the complete genome sequence of which has been determined and published, exhibits excellent gastric acid and bile tolerance, contains bile salt hydrolase and has strong mucus adherence properties. Colonization, pathogen inhibition and barrier function enhancement are mechanisms by which BB-12 exerts its beneficial health effects, supporting a healthy gastrointestinal microbiota. Furthermore, BB-12 has been shown to improve bowel function, to have a protective effect against diarrhea and to reduce side effects of antibiotic treatment, such as antibiotic-associated diarrhea. Finally, in terms of immune function, clinical studies have shown that BB-12 increases the body's resistance to common respiratory infections, as well as reducing the incidence of acute respiratory tract infections.

Within the gut microbiota, lactic acid bacteria belonging to the genus *Enterococcus* play a very substantial role and probiotic enterococci are widely used by pediatricians as a means of counteracting dysbiosis and functional gut disease such as irritable bowel syndrome, and in the treatment and/or prevention of different organic chronic intestinal diseases.[9] Among probiotic enterococci, *Enterococcus faecium* L3 has been extensively investigated in pediatric populations, preterm infants included, where it has been shown to preserve and increase the growth of endogenous bifidobacterial and lactobacillus species, in addition to reducing the number of opportunistic microorganisms[10] and the incidence of URTIs.[11] We have therefore prospectively evaluated, in a randomized and controlled study, whether the administration of a mixture of the two well-documented probiotic strains *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Enterococcus faecium* L3 was able to reduce the incidence of URTIs and AGE in healthy children. Moreover, as immunoglobulin A (IgA)-deficient patients predominantly suffer from respiratory and gastrointestinal infections,[12] and since secretory IgA has an important function in protecting mucosal surfaces, and a significant increase in the salivary IgA secretion rate is associated with a decrease in URTI incidence and symptoms, and gut infections,[13, 14] we also carried out an analysis of salivary IgA in a subgroup of treated children.

Materials and methods

Product

The probiotic mixture of *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) and *Enterococcus faecium* L3 (L3) was manufactured by Farmaceutici Procemsa (Turin, Italy) as single-dose sachets. The product was notified to the Italian Ministry of Health on June 16, 2016 as iNatal Ped® by Pharmextracta SpA (Piacenza, Italy) according to the provisions of Law No. 169 of 2004 (notification number: 87470). The preparation used in the study contained no fewer than 2 billion CFU/sachet of each strain (total dose: no fewer than 4 billion CFU/sachet) within the expiry date.

Clinical trial

Our prospective, randomized clinical study was conducted in 2019 between January and May, on 203 (105 male and 98 female) children (age: 6-36 months) living in the Milan, Cosenza, Crotone, Catanzaro or Messina areas of Italy. Some of the children (group A; N.=94) were treated with the probiotic mixture, while the remaining children (group B; N.=109) acted as untreated controls. Among the 94 treated children of group A, 34 were also assessed, at baseline and after 90 days of treatment, with respect to salivary IgA levels. Children were randomized to the treated or control group depending on whether a flipped coin landed heads (treated group) or tails (control group) up. Children who also underwent salivary IgA analysis were those from group A whose parents approved saliva sampling and testing. IgA analysis was performed according to the method described by Pacifici *et al.*[15] The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee in Milan (Italy). The parents of all the study participants were informed of the study methods and signed the appropriate consent and privacy policy documents.

Inclusion and exclusion criteria

We included healthy children living in the nearby areas of Milan, Crotone, Cosenza, Catanzaro and Messina, who were 6-36 months old and belonging to one of our pediatric outpatient clinics. We excluded children with immunosuppression, cardiomyopathy, lung, liver or kidney diseases, asthma, who had undergone recent surgery and those with celiac disease. We also excluded children not attending nursery or kindergarten and/or vaccinated for rotavirus and/or taking proton pump inhibitors and/or taking antibiotics for prophylaxis of urinary tract infections. Finally, we excluded children with a gluten-free and/or low-FODMAP diet.

Treatments

Parents were asked not to give their children any other probiotics, and/or dead bacterial cells (tyndallized bacteria, bacterial lysate, postbiotics) for the duration of the study. Even prebiotics were excluded. With respect to antibiotic use, parents were requested to stop probiotic administration and to resume this after the last antibiotic dose had been given. Acetaminophen or ibuprofen could be used to treat pain, and probiotic use could be continued. Parents were asked not to administer the probiotics when the child was hungry or had consumed a large meal so that the probiotics would have better survival in order to colonize the gut. Probiotic treatment lasted 90 days starting from the day of enrollment. Any conditions or diseases compatible with the aim of the study were managed in accordance with Italian pediatric guidelines. There were no cases of diseases incompatible with our study and all enrolled children completed the study.

Study aims

The primary endpoint of the study was the development of signs and symptoms of URTI (pharyngitis, tonsillitis, sore throat, otitis, otorrhea, otalgia, rhinitis, sinusitis, acute upper respiratory infections of multiple or unspecified sites, cough) and/or gastroenteritis. Another primary endpoint was the evaluation of salivary IgA levels at baseline and after 90 days of probiotic treatment. The secondary endpoints were tolerability, side effects and compliance with the probiotic treatment. Both primary and secondary endpoints were evaluated throughout the 90 days, starting from the day of enrollment.

Statistical analysis

The comparison of IgA evolution between the same subjects was performed using the Wilcoxon signed-rank Test. The equivalence between groups in terms of sex, ethnicity, delivery mode, weight at childbirth, antibiotic treatment in the first month of life, nursery and/or kindergarten attendance, the type of feeding and weaning, number of siblings, the aggregated indicator for gastroenteritis and the aggregated indicator for respiratory tract infections was determined using Fisher's Exact two-tailed test. Differences between groups in terms of URTI and/or AGE episodes, the disease period length (expressed in days) and age (expressed in years) were determined using the two-tailed Wilcoxon-Mann-Whitney Test. All analysis was performed using JMP v12, by SAS.

Results

The characteristics of the enrolled children (N.=203) are detailed for the probiotic (A) and the control (B) groups in Table I. The data demonstrate that the two groups did not significantly differ in terms of number of subjects, sex, age, ethnicity, delivery mode, birth weight, number of siblings, feeding modality and attendance at nursery. Significant differences were observed for weaning and the use of antibiotics during the first month of life, with the children of the probiotic group (group A) having been weaned earlier and having been treated with more antibiotics in their first month of life. As shown in Table II, children within group A (probiotic; N.=94) had 11 episodes of AGE with an incidence of 0.12 and with an average duration for any single episode of 3.5 days. In contrast, children within group B (control; N.=109) had 71 episodes of AGE with an incidence of 0.65 and with an average episode duration of 6.3 days. Statistically significant differences were noted between the two groups of 82% and 45%, for incidence and duration of episodes respectively, in favor of the probiotic group. Regarding URTIs, there were 20 and 139 episodes respectively for group A and group B, with an incidence of 0.21 (A) and 1.28 (B), representing a significant difference of 84% in favor of group A (probiotic). Duration of URTIs (in days) was 12.94 (group B) and 6.49 (group A) with a significant different by 50% in favor of the probiotic group. As shown in Table III, salivary IgA levels, evaluated at baseline and after 90 days in a subgroup (N.=34) of children treated with the probiotic mixture, underwent a highly significant increase corresponding to 197% (value calculated between T=90 and baseline). A more detailed statistical analysis for any single parameter was also performed by

TABLE I.—*Characteristics of the children (N.=203) enrolled in the study.*

	Group A	Group B	P
Treatment	Probiotics	Control	
N.	94	109	NS
Sex M/F	42/52	52/57	NS
Age (years±SD)	2.2±0.7	2.2±0.7	NS
Ethnicity: C/As/Afr	92/1/1	109/0/0	NS
Delivery mode: E/D/CS	58/1/35	70/2/37	NS
Birth weight (kg): <3/>3	29/65	26/83	NS
Feeding: B/F	79/15	80/29	NS
Weaning (months): <6/>6	53/41	36/73	<0.05
Antibiotics 1st month: Y/N	55/39	11/98	<0.01
Siblings: 0/1/+1	42/40/12	54/44/11	NS
Nursery (year): 1°/2°	51/43	43/66	NS

M/F: male/female; SD: standard deviation; C/As/Afr: Caucasian/Asian/African; E/D/CS: eutocic/dystocic/cesarean section; B/F: breastfed/formula or mixed; Y/N: yes/no; NS: not significant.

TABLE II.—*Acute gastroenteritis (AGE) and upper respiratory tract infections (URTIs) in children enrolled in the study.*

	Group A	Group B	Δ%	P
Treatment	Probiotics	Control		
N.	94	109		
Episodes of AGE	11	71		
Incidence of AGE	0.12	0.65	-82	<0.01
Duration of AGE	3.5±1.8	6.3±2.3	-45	<0.01
Episodes of URTIs	20	139		
Incidence of URTIs	0.21	1.28	-84	<0.01
Duration of URTI	6.49±2.1	12.94±4.7	-50	<0.01

The duration of AGE and URTI is given as the mean±standard deviation (days).

TABLE III.—*Salivary IgA levels (mean±standard deviation) in a subgroup of children treated with the probiotic mixture, evaluated at baseline and after 90 days of treatment.*

	T=0	T=90	Δ%	P
N.	34	34		
IgA	19.8±18.3	58.9±40.0	+197%	<0.01

IgA values expressed as ng/mL.

comparing group A, group B and the subgroup of the 34 children who were subject to salivary IgA analysis. The results observed through this comparison were identical to the ones obtained, as described earlier, which considered group A *versus* group B (data not shown). Adherence was greater than 95%; compliance and tolerability were very good. There was one case of taste aversion, while there were no side effects attributable to the treatment, with no significant differences in episodes of nausea, vomiting, insomnia, fussiness, crying, meteorism, flatulence or skin rash between the two groups (data not shown). Parental opinion of the product tested, collected by clinicians using the question "Will you use such a therapy again on your child?," manifested as a positive response in more than 95% of cases (data not shown).

Discussion

AGE is defined as a diarrheal disease of rapid onset, with or without nausea, vomiting, fever or abdominal pain.[16] AGE is a highly preventable and clinically significant health issue in pediatric medicine. It is the third most common cause of death in children less than five years old in the developing world.[17] In the United States, AGE accounts for 1.5 million general practitioner visits, 200,000 hospitalizations and 300 deaths in children each year.[16] Other western countries show proportionally (to the number of inhabitants) similar figures. Evaluation of a child with AGE should include a recent history of fluid intake and output. Significant dehydration is unlikely if parents report no decrease in oral intake or urine output and no vomiting. Physical examination is the best way to evaluate hydration status. The four-item Clinical Dehydration Scale can be used to determine the severity of dehydration based on physical examination findings.[16] In children with mild illness, stool microbiological tests are not routinely needed when viral gastroenteritis is the likely diagnosis. Moreover, mild gastroenteritis in children can be managed at home. Oral rehydration therapy, such as provision of the child's preferred liquids, is in fact the mainstay of treatment for mild dehydration and is as effective as intravenous rehydration in preventing hospitalization and a return to the emergency department. Oral rehydration solutions are therefore always recommended for moderate dehydration. Drugs such as ondansetron may be prescribed if needed to prevent vomiting and improve tolerance of oral rehydration solutions. Hospitalization and intravenous fluids are then only recommended for children who do not respond to oral rehydration therapy. Handwashing, breastfeeding and rotavirus vaccination are reported to reduce the incidence of AGE in young children.[16] Regarding URTIs, they also remain one of the leading causes of global morbidity and mortality among children at different ages. Most children have experienced several URTIs during the first year of life, and one-quarter suffer from recurrent or prolonged infections in developed countries.[18, 19] URTIs are a major cause of parental concern and medical visits for preschool and elementary school children, leading to school absenteeism and hospitalizations.[20, 21] They also result in inappropriate prescription of antibiotics in pediatric practice.[22, 23] Inappropriate and broad use of antibiotics may lead to the development of bacterial resistance and disturb the normal balance of the human microbiota, facilitating pathogen colonization and disease.[24, 25] The economic impact of URTIs is also significant among countries, making URTIs in children an important global challenge for public health.[26, 27] In any case, despite guidelines well describing which approaches should be kept under strong consideration to prevent AGE and URTIs, these pathologies continue to show very high incidence and recurrence. According to recent clinical findings, meta-analysis and clinical guidelines, the use of very well-defined and documented bacterial strains has been recommended, for the prevention of both URTIs and AGE (in addition to the prevention of nosocomial diarrhea, the prevention of antibiotic-associated diarrhea and the treatment of infantile colic in breastfed babies).[28] On this basis, we decided to clinically test the prophylactic role of a recently developed mixture of two well-documented probiotics, *Bifidobacterium animalis* subspecies *lactis* BB-12 and *Enterococcus faecium* L3,[8-11] *versus* AGE and URTI occurrence in healthy children. We randomized 203 enrolled children

within two different groups (a probiotic-treated group and an untreated control group). Despite randomization procedures, the two groups differed in terms of weaning time and use of antibiotics in the first month of life, with both parameters "negative" for group A (the probiotic-treated group). However, since our aim was to evaluate the role played by probiotics in reducing AGE and URTIs, we considered our possible findings acceptable. As described in the Results section, we have shown that the incidence and duration of episodes of AGE and the incidence and duration of episodes of URTIs were significantly reduced by the prophylactic use of the probiotic mixture, by between 45% to 84%, demonstrating that these pathologies can be prevented by the appropriate use of bacterial strains that have been properly characterized and formulated in terms of stability, vitality, dosage and strain selection. Salivary IgA release serves as a good predictor of URTI incidence.[29] Salivary IgA is an important component of protection against infections at mucosal surfaces, along with the integrity of the epithelial barrier and regulatory T-lymphoid cells. Low levels of salivary IgA therefore pose a risk of URTI.[30] Microorganisms present in the oral cavity, mixed with salivary proteins and digestive enzymes, are swallowed and enter the lower gastrointestinal tract where, if they are resistant to the effects of gastric juice and bile secretion, they can colonize, possibly resulting in infection with consequences such as AGE. Salivary IgA therefore constitutes one of the possible defense mechanisms capable of reducing such risk. The barrier role played by salivary IgA to help prevent the occurrence of AGE is also applicable to URTIs. The mouth and nose provide a continuous flow of microorganisms to the upper respiratory tract, raising the risk of infection, and mucosal IgA presence can reduce such a risk.[31-33] We therefore decided to evaluate whether the protection against AGE and URTIs afforded by the probiotic formula correlated with salivary IgA release. Indeed, our results seem to confirm this. Salivary IgA levels were in fact increased approximately three-fold during the 90 days of treatment with the probiotic formula, consistent with reports that IgA controls host-microbial homeostasis.[34]

Limitations of the study

We fully acknowledge that our study presents some potential bias and limitations, such as the absence of a control formula or a real placebo; the study was not performed under blind conditions and, notwithstanding the difficulty associated with enrolling more than 200 healthy children here, we would need to confirm our results in greater cohorts of children. Furthermore, our results may seem to be incomplete since we were unable, for the URTI data, to differentiate the individual incidence rates for pharyngitis, tonsillitis, sore throat, otitis, otorrhea, otalgia, rhinitis, sinusitis, cough and others. This can be explained by the fact that our study was contributed to by several different pediatricians, some of whom did not accurately specify the different types of URTIs. Another potential bias to take into account when analyzing the results is that the evaluation of salivary IgA levels, limited to only 34 children, was performed without a real control and just within the same group by comparing the value reached at T=90 days *versus* baseline. We cannot therefore exclude the possibility that the observed increase could be due to phenomena other than the use of the probiotic mixture. However, we cannot dismiss the soundness of this result, as the analysis of the incidence of AGE episodes and URTIs was conducted *versus* a control group. In addition, the analysis of data from the 34 children enrolled in the subgroup undergoing IgA measurement completely overlaps with the analysis of the groups as a whole, suggesting that the observed event (the increase in salivary IgA levels) is due to and correlates well with the use of the probiotic mixture.

Conclusions

In conclusion, taking into proper consideration all of the above-listed bias and limitations, but bearing in mind the good statistical correlation within the various results obtained, also in relation to the IgA values, we propose that the correct use of stable, viable, colonizing and documented strains of probiotics, such as for our study the strains BB-12 and L3, confers a certain protection against AGE and URTIs in those children who, due to the patronage of communi-

ties like nurseries, kindergartens or schools, are surely exposed to a higher risk of oral, nose, ear, pharyngeal, laryngeal, tonsillar and bowel infections.

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