BACKGROUND: Probiotics may prevent the allergic response’s development due to their anti-inflammatory and immunomodulatory effects.

The aim of this study is to determine if the prophylactic treatment with a mixture of *Bifidobacterium animalis* subsp. *Lactis* BB12 and *Enterococcus faecium* L3, would reduce symptoms and need for drug use in children with allergic rhinitis (AR).

METHODS: The study included 250 children aged from 6 to 17 years, affected by AR. Patients were randomly assigned to the intervention group (117) or to the placebo group (86). Patients of the intervention group, in addition to conventional therapy (local corticosteroids and/or antihistamines), were treated, in the 3 months preceding the development of AR symptoms, with a daily oral administration of a probiotic mixture containing the *Bifidobacterium animalis* subsp *Lactis* BB12 DSM 15954 and the
Enterococcus faecium L3 LMG P-27496 strain. Nasal Symptoms Score (NSS) was used to evaluate AR severity before and after the treatment with probiotics or placebo.

RESULTS: 96% of the patients in the intervention group showed a significant decrease in their NSS after the probiotic treatment as well as a decrease in the intake of pharmacological therapy. GPower software was used to calculate the test power. Given the probability of error α = 0.05, the total sample size n = 117 and the effect size ρ = 2.0651316, the power of the test is 1 - β = 1.

CONCLUSIONS: When administered as a prophylactic treatment the mixture of BB12 and L3 statistically decrease signs and symptoms of AR and reduces significantly the need of drugs.

Keywords: Allergic rhinitis, Probiotics, Bifidobacterium animalis subsp lactis BB12, Enterococcus faecium L3, children

INTRODUCTION

Allergic diseases are increasing considerably in both adults and children and represent a public health problem, given the risk of serious complications, poor quality of life and related cost. It is estimated that more than 20% of the population is affected by an allergic pathology such as allergic rhinitis (AR), asthma, food allergy, and/or atopic dermatitis [1]. AR affects 10 to 25% of the general population and the prevalence of this condition has increased during the past decades, making it a global health problem [2]. The presence of specific bacterial strains could influence the development of allergic diseases [3, 4]. Recently the intestinal microbiota hypothesis has been proposed to explain the rising incidence of allergic diseases [5].

AR is a non-infectious inflammatory disease of nasal mucosa, induced by an IgE mediated reaction, which occurs following the exposure to one or more allergens to which the patient is sensitized [6]. It is one of the most common chronic conditions in pediatric patients and it is clinically characterized by nasal symptoms such as congestion, sneezing, itching, rhinorrhea, often associated with ocular symptoms, ear infections and general symptoms such as asthenia and malaise [7]. Furthermore, AR is closely linked to other airway diseases like asthma, nasal polyps, sinusitis and otitis media. Therefore AR can significantly compromise the quality of life of children leading to poor sleep quality and lack of concentration affecting also their growth and...
Allergen immunotherapy (ITS) consists in the administration of increasing quantities of allergen in order to reduce allergic symptoms and it is the only treatment that allows to modify the natural course of the atopic march [9]. Traditional medical treatment involves the use of local corticosteroids and antihistamines, which, however, do not allow the complete resolution of allergic symptoms and are often associated with side effects such as fatigue and sleepiness that limit children to continue their daily activities [10]. Moreover, the management of allergic disease is expensive, both for the drugs’ costs and for the reduction in children school attendance with loss of several working days of their parents [11]. Recently, it has been suggested that probiotics could modulate the immunologic and inflammatory response and they could represent a possible preventive treatment for allergic disease including AR. Recent evidences support the role of Bifidobacteria strains, such as Bifidobacterium animalis and Bifidobacterium longum in this regard [12]. The human gastrointestinal tract harbors 500-1000 distinct bacterial species [13]. The intestinal microbiota phyla in adult belong to six prevalent groups: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Tenericutes and Fusobacteria [14]. Instead Actinobacteria and specifically the genus Bifidobacterium are the dominant bacteria in infants [15]. In breast-fed newborns, B. breve is the dominant species, followed by B. bifidum, B. longum and B. adolescentis [16, 17]. Bacterial colonization of the intestine begins even before birth, through the ingestion by the fetus of bacteria contained in the amniotic fluid. Several environmental interactions, such as the mode of delivery, gestational age, type of breastfeeding and nutrition, are fundamental in the development of the intestinal microbiome [18, 19].

Results of the Fujimura study[20] and of the Bjorksten study [21] showed that even from the first weeks of life there are differences between the intestinal microflora of an allergic child compared to a healthy one (before the development of any clinical manifestation of atopy), and that these differences still remain at two years of age, namely, a low level of Bifidobacteria and Bacterioides and, on the contrary, an increased level of Enterobacteria (E.Coli), Staphylococcus aureus and, only in the first three weeks, Clostridium difficile. Microbiome is a key element for the development of the immune response, particularly during early childhood, establishing an effective "cross-talking" with the host. Within the normal microbiome there is a balance between gram- positive and negative bacteria. Disruption of this balance and the increase in gram- negative bacteria (Bacteroidetes,
Proteobacteria) with a consequent increase in microbial products such as lipopolysaccharide (LPS), causes alteration in the immune system leading to macrophage activation with the production of tumor necrosis factor α (TNF-α) which promotes the transition of naïve T cells to Th2 cells with a negative effect on tight junctions. Excess of LPS also leads to a reduction of regulatory T cells (TREGS) and therefore to an amplification of the effects of TNF-α and Th2 differentiation. The components of the normal microflora are able to induce a state of "physiological" inflammatory response in the intestine, maintained by balanced and controlled responses [22]. Probiotics help to preserve intestinal homeostasis by modulating the immune response and inducing the development of Tregs [23].

The World Health Organization (WHO) defines “probiotics as live microorganisms that when administered in adequate amounts, are able to confer a health benefit on the host”[24]. They must already be normally present in the gastrointestinal tract, are able to colonize and adhere to the epithelium and, protect the host by modulating the intestinal microbiota. This may be possible by improving the natural functions of the gastrointestinal barrier (tight junctions, mucous barrier); modulating the immune response (increase in secretory IgA, anti-inflammatory cytokines, TregS and NK) and antagonizing pathogens (bacteriocin production and short-chain fatty acids) [25].

Bifidobacteria have been shown to interact with human immune cells by modulating specific pathways, involving both innate and adaptive immunity [26]. In particular, the increase of Bifidobacteria leads to a decrease in gram- negative bacteria and consequently LPS; they also strengthen the tight junctions by decreasing the permeation of LPS. BB12 in particular promotes the Th1 response and increases the production of secretory IgA [27]. Enterococcus faecium L3 is a natural enhancer of BB12: it creates the space that is occupied by the Bifidobacteria, increasing their growth. It also produces bacteriocins with a microbicidal action that antagonize pro-inflammatory gram-negative bacteria (Proteobacteria) with a reduction of LPS-mediated subclinical inflammation, promoting an increase in IL-10 [28]. Some preliminary studies have shown that the prophylactic administration of a probiotic mixture containing Bifidobacterium animalis subsp. Lactis BB12 and Enterococcus faecium L3 in children affected by seasonal allergic diseases, reduces the rhinitis’symptoms by about 50%; it also reduces the need of oral and local cortisones and
antihistamines, limiting their use and any side effects [29]. We performed a randomized controlled trial to evaluate whether children affected by AR treated with conventional therapies (local corticosteroids and antihistamines) and a prophylactic treatment of a probiotic containing *Bifidobacterium animalis* subsp. *Lactis BB12* and *Enterococcus faecium L3*, before the periods of pollens’ allergic exposure, could reduce allergic signs and symptoms and the need of conventional therapies (local corticosteroids and oral antihistamines).

**METHODS**

**Participants and design**

The present study was conducted as a prospective and double-blind, randomized, placebo-controlled trial. From July 2019 to November 2020, children and adolescents affected by AR between 6 and 17 years of age, were consecutively enrolled at the Department of Pediatrics, Division of Allergy and Immunology, “Sapienza” University of Rome. The diagnosis of AR was confirmed by pediatricians trained in allergic diseases on the basis of clinical history, skin prick tests (SPTs), serum allergen-specific IgE towards aeroallergens and according to ARIA guidelines [28].

All the patients had a prescription with a conventional pharmacological treatment for AR such as local corticosteroids and/or oral antihistamines.

The selected patients were randomly assigned to group A (probiotic treatment group) or to group B (placebo group) according to computer-generated permuted-block randomization. In addition to conventional therapy, a daily oral administration of probiotics in the 3 months preceding the onset of symptoms, were prescribed to group A children; on the other hand, placebo was prescribed to group B patients. Both subjects and investigators were blind to the treatment groups. Study duration was 16 months.

- **Inclusion criteria** were the following: children and adolescents aged between 6 and 17 years who attend our clinics with a diagnosis of AR based on clinical examination, SPTs and serum allergen-specific IgE. Patients had to be already being treated...
with conventional AR therapies. The patients enrolled were characterized by sensitization versus inhaled allergens occurring in the 3 months followed probiotic treatment.

- Exclusion criteria include the following: patients with primary or secondary immunodeficiency, intrinsic asthma or wheezing secondary to infectious etiology, current systemic infections, use of probiotics, prebiotics, antibiotics, current or previous treatment with desensitizing therapy.

Nasal Symptom Score (NSS), a validated pediatric questionnaire, was used to assess the severity of rhinitis before and after treatment with the probiotic [30, 31].

The NSS is a written, four item questionnaire and its score is the sum of the values reported by the patient (or their parents) for each of the individual items that compose it, evaluating both the severity (S) and duration (D). The rating scale is divided into 4 grades (0-3), where 0 indicates no symptoms, 1 mild symptoms that are easily tolerated, 2 symptoms that are bothersome but tolerable and 3 for severe symptoms difficult to be tolerated and that interfere with daily activities. The following symptoms were taken into consideration:

- nasal obstruction (stuffy nose)
- rhinorrhea (runny nose)
- sneezing and
- nasal itching

The total score can vary from a minimum of 0 to a maximum of 24 points

no symptoms = 0; mild rhinitis: 1 to 8; moderate rhinitis: 9 to 16; severe rhinitis: 17 to 24.

Probiotic supplementation consisted of a mixture containing $2 \times 10^9$ colony-forming units (CFUs) of *Bifidobacterium animalis* subsp. Lactis BB12 and $2 \times 10^9$ of *Enterococcus faecium* L3. This probiotic also contains maltodextrin, oligofructose mono- and di-glycerides of fatty acids. It is a pack containing 14 sticks of 2.5 g. The product must be stored at a temperature between $+2^\circ$ C.
and + 8 °C and transported at temperatures below 25 °C for periods not exceeding 48 hours. It must also be kept away from light and heat sources. The placebo consist of maltodextrin that looked and tasted the same as the probiotics.

All patients were included in the safety analysis and adverse events were registered.

This study was approved by the medical ethics review board of Sapienza University of Rome, Policlinico Umberto I. Patients’ parents or guardians signed a written consent form.

Outcomes

The primary outcome measure of this study was:

to evaluate whether children affected by AR, already treated with conventional therapies, if previously undergone to a probiotic containing *Bifidobacterium animalis* subsp. Lactis BB12 and *Enterococcus faecium* L3 in the periods preceding allergen exposure, could have a reduction in their allergic signs and symptoms and in the need of conventional therapies (local corticosteroids and oral antihistamines).

Statistical analysis

The NSS before and after the treatment was tested with the Shapiro-Wilk test to verify their Gaussianity. Since the NSS after the treatment resulted “non Gaussian”, we applied Wilcoxon signed rank test obtaining a significant p-value $<10^{-4}$ with a confidence $\alpha=0.05$. GPower software was used to calculate the test power. Given the probability of error $\alpha = 0.05$, the total sample size $n = 117$ and the effect size $\rho = 2.0651316$, the power of the test is $1 - \beta = 1$.

RESULTS

Among 250 enrolled patients, a total of 203 children of which 117 in the probiotic group (males 73; mean age 10.5±3.1SD) and 86 in the control group (males 49; mean age 8.8±3.5 SD), completed the study (Fig 1, Fig 2) . Baseline data and characteristics of participants randomized to receive or not probiotic supplementation are showed in Table 1.

**TABLE 1 Baseline characteristics of the study population**
|                        | Probiotic (n. 117) | Placebo (n. 86) |
|------------------------|--------------------|----------------|
| Age (y)                | 10.5 ± 3.1         | 8.8 ± 3.5      |
| Sex (M/F)              | 73/44              | 49/36          |
| Rhinitis               | 55 (47.0%)         | 37 (43.02%)    |
| Asthma + Rhinitis      | 50 (42.7%)         | 39 (45.34%)    |
| Asthma + dermatitis    | 1 (0.9%)           | 1 (1.17%)      |
| Rhinitis + dermatitis  | 6 (5.1%)           | 6 (6.97%)      |
| Rhinitis+asthma+dermatitis | 5 (4.3%)    | 3 (3.49%)      |
| SPT_DPT                | 88 (75.2%)         | 48 (62.3%)     |
| SPT_DPF                | 86 (73.5%)         | 47 (61.0%)     |
| SPT_Parietaria         | 29 (24.8%)         | 10 (13%)       |
| SPT_Olea               | 29 (24.8%)         | 26 (33.8%)     |
| SPT_Cynodon            | 15 (12.8%)         | 14 (18.2%)     |
| SPT_Lolium             | 26 (22.2%)         | 18 (23.4%)     |
| SPT_Grass pollen       | 76 (65.0%)         | 38 (49.4%)     |
| SPT_Alternaria         | 12 (10.0%)         | 8 (10.4%)      |

SPT= Skin prick test; DPT=Dermatophagoides pteronissimum; DPF= Dermatophagoides farinae;

In Table 2, are reported the percentages of sensitization among the enrolled patients.

### TABLE 2 Prevalent Allergens in study population

| Allergen | % |
|----------|---|
| PARIETARIA | 0.85 |
| DPT, DPF, GRASS POLLEN | 13.68 |
| OLEA, GRASS POLLEN | 0.85 |
| DPT, DPF, PARIETARIA, GRASS POLLEN | 7.70 |
| DPT, DPF, LOLIUM, GRASS POLLEN | 2.60 |
| DPT, DPF | 17.95 |
| DPT, DPF, PARIETARIA, OLEA, CYNODON, LOLIUM, GRASS POLLEN | 2.60 |
| DPT, DPF, PARIETARIA, OLEA, GRASS POLLEN | 4.27 |
| GRASS POLLEN | 5.13 |
| LOLIUM | 1.71 |
| DPT, LOLIUM, GRASS POLLEN | 3.42 |
| DPT, DPF, ALTERNARIA | 1.71 |
| DPT, DPF, PARIETARIA, LOLIUM, GRASS POLLEN | 0.85 |
| DPT, DPF, PARIETARIA, CYNODON | 1.71 |
The probiotic treatment was well tolerated and there were no clinically relevant side effects.

We obtained significant statistical difference between the NSS before and after treatment in the intervention group (group A).

(Table 3.)

TABLE 3. Nasal Symptoms Score (NSS)

|                          | Intervention group | Placebo group | P value |
|--------------------------|--------------------|---------------|---------|
| LOLIUM, GRASS POLLEN    | 4.27               |               |         |
| OLÉA                    | 0.85               |               |         |
| ALTERNARIA              | 0.85               |               |         |
| CYNODON, LOLIUM, GRASS POLLEN | 1.71         |               |         |
| DPT, DPF, OLÉA          | 3.42               |               |         |
| OLÉA, GRASS POLLEN      | 1.71               |               |         |
| PARIETARIA, GRASS POLLEN | 0.85           |               |         |
| OLÉA, LOLIUM, GRASS POLLEN | 0.85           |               |         |
| DPT                      | 0.85               |               |         |
| CYNODON, LOLIUM         | 1.71               |               |         |
| ALTERNARIA, GRASS POLLEN | 0.85           |               |         |
| PARIETARIA, LOLIUM      | 0.85               |               |         |
| DPT, DPF, LOLIUM        | 0.85               |               |         |
| DPF, DPT, OLÉA, GRASS POLLEN | 3.42         |               |         |
| DPF, DPT, ALTERNARIA, GRASS POLLEN | 2.60     |               |         |
| DPT, DPF, PARIETARIA    | 1.71               |               |         |
| DPT, DPF, PARIETARIA, ALTERNARIA | 0.85   |               |         |
| DPT, DPF, OLÉA, CYNODON, LOLIUM | 0.85 |               |         |
| PARIETARIA, OLÉA, GRASS POLLEN | 0.85 |               |         |
| PARIETARIA, OLÉA, LOLIUM, GRAMINACEE | 0.85 |               |         |
| OLÉA, CYNODON, GRASS POLLEN | 0.85     |               |         |
| LOLIUM, ALTERNARIA, GRASS POLLEN | 0.85 |               |         |
| DPF, DPT, OLÉA, ALTERNARIA, GRASS POLLEN | 2.60 |               |         |
| DPT, DPF, CYNODON, LOLIUM, GRASS POLLEN | 0.85 |               |         |
| DPT, DPF, CYNODON, GRASS POLLEN | 0.85 |               |         |
The treatment showed a decrease of the severity score in the 96% of children in the intervention group (group A) and also a decrease in the intake of local corticosteroids and oral antihistamines. In fact, 56.41% patients in group A had no need to take conventional therapy in addition to the probiotic and only 43.59% of them had to continue therapy with the combination of local corticosteroid or oral antihistamine.

Change in the NNS score from baseline over the three months in the intervention (group A) and placebo (group B) groups are described in the boxplots below (Fig.3, Fig 4).

|                        | (group A) | (group B) |
|------------------------|-----------|-----------|
| Before treatment       | 14.07     | 14.51     | 0.5       |
| After treatment        | 5.43      | 13.60     | <2.2e-16  |

Fig 3. Boxplot of NSS before and after treatment in the intervention group (group A)
DISCUSSION

In this study, we obtained statistically significant results of the efficacy of the preventive probiotic treatment in AR in children.

When administered as a prophylactic treatment the mixture of *Bifidobacterium animalis* subsp. Lactis BB12 and *Enterococcus faecium* L3 statistically decreases the signs and symptoms of AR and reduces significantly the use of drugs, including oral anti-histamines and local corticosteroids. The results of our study are well in line with data of previous trial reporting that *Bifidobacterium animalis* subsp. Lactis BB12 and *Enterococcus faecium* L3 strains when administered 3 months before the development of AR statistically decreases allergic sign and symptoms. So this study represents a further contribution to the...
literature concerning probiotics in the prevention of allergic disease [13]. Microbial exposure may direct the immune system away from allergic-type responses, but until now probiotic interventions have had limited success in the prevention and treatment of allergic diseases and currently available evidence does not indicate that probiotic supplementation reduces the risk of developing allergic disease in children [5]. To the best of our knowledge, there have been few published double-blind, randomized, placebo-controlled trials examining the effect of probiotics in preventing AR in pediatric population. Lin et al. in a randomized double-blind controlled trial demonstrated that *Lactobacillus salivarius* treatment reduces clinical symptoms and drug use among children with AR [33]. A systematic review conducted by Peng et al. examined the role of probiotics in the prevention and treatment of allergic diseases. In this review five studies analyzed the preventive role of probiotics in AR in children and they found no effect in preventing this condition [34]. Cuello-Garcia et al. carried out a systematic review of randomized trials assessing the effects of any probiotic administered to pregnant women, breast-feeding mothers, and/or infants and they found that supplementation with probiotics decreases the risk of atopic eczema in infants but does reduce the risk of other allergies including AR [35]. Miraglia Del Giudice et al. [36] performed a randomized, double-blind, placebo-controlled study to investigate whether *Bifidobacterium* mixture (*B. longum* BB536, *B. infantis* M-63, *B. Breve* M-16V) is effective in children with seasonal allergic rhinitis and intermittent asthma. They reported that *Bifidobacterium* mixture significantly improved symptoms of AR and quality of life (QoL). *Bifidobacteria* are frequently depleted in atopic children [20] and adults [26], and L3 promotes the preservation of endogenous gut *Bifidobacteria* in children [27]. On the basis of this background we conducted the present study and we found the beneficial effects of a probiotic treatment (*Bifidobacterium animalis* subsp. Lactis BB12 and *Enterococcus faecium* L3) in preventing symptoms of AR and in reducing the use of conventional therapies in AR children.

**Conclusions**

Several clinical trials have reported the effectiveness of probiotics in controlling symptoms and improving quality of life in patients with AR. However, most of the conducted studies have shown a significant heterogeneity regarding the type of strains,
dosage, timing, outcomes, so currently available evidence does not recommend the use of probiotics for the primary prevention of allergic diseases. The World Allergy Organization (WAO) suggests anyway probiotic supplementation in pregnant/lactating women and in infants with a family history of allergic disease. The current study demonstrates a reduced incidence of AR symptoms and a reduction of medical conventional therapies in children and adolescents previously treated with probiotic containing specific strains (Bifidobacterium animalis subsp. Lactis BB12 and Enterococcus faecium L3). Probiotic intervention may have a promising role in the prevention of AR but there is still a need of a well-designed randomized trials to define the role of probiotics in preventing allergic diseases including AR.

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REFERENCES

1) Ebert, C.S. Jr.; Pillsbury, H.C. 3rd. Epidemiology of allergy. Otolaryngol Clin North Am. 2011;44:537-548

2) Dykewicz, M.S.; Hamilos, D.L. Rhinitis and sinusitis. J Allergy Clin Immunol 2010;125: S103-115.

3) De Martinis, M.; Sifuro, M.M.; Suppa, M.; Ginaldi, L. New prospective in food allergy. Int J Mol Sci. 2020;21:1474.

4) Mastrorilli, C.; Caffarelli, C.; Hoffmann-Sommergruber, K. Food allergy and atopic dermatitis: Prediction, progression, and prevention. Pediatr Allergy Immunol. 2017;28:831-840.
5) Fiocchi, A.; Pawankar, R.; Cuello-Garcia, C., Ahn, K, Al-Hammadi S, Agarwal A, Beyer K, Burks W, Canonica GW, Ebisawa M, et al. World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics. World Allergy Organ J. 2015;8:4.

6) Bousquet, J.; Khaltaev, N.; Cruz, A.A.; Denburg, J.; Fokkens, W.J.; Togias, A.; Zuberbier, T., Baena-Cagnani, C.E.; Canonica, G.W.; van Weel, C.; Agache, I.; et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA (2) LEN and AllerGen). Allergy 2008; 63 Suppl 86:8-160.

7) Zicari, A.M.; De Castro, G.; Leonardi, L.; Duse, M. Update on rhinitis and rhinosinusitis. Pediatr Allergy Immunol 2020;31(Suppl 24):32-33.

8) Cao, X.; Zhong, P.; Li, G.; Zhu, J.; Zheng, Y. Application of probiotics in adjuvant treatment of infant allergic rhinitis. A randomized controlled study. Medicine 2020;99:e20095.

9) Calderón, M.A.; Penagos, M.; Sheikh, A.; Canonica, G.W.; Durham, S.R. Sublingual immunotherapy for treating allergic conjunctivitis: Cochrane Database Systematic review and meta-analysis. Clin Exp Allergy. 2011;41:1263-72.

10) Yanai, K.; Rogala, B.; Chugh, K.; Paraskakis, E.; Pampura, A.N.; Boev, R. safety considerations in the management of allergic diseases: focus on antihistamines. Current Med Res Opin 2012,28:623-42.

11) Pawankar R, Canonica G, Holgate S, Lockey R, Blaiss M (Eds) World Allergy Organization (WAO) White Book on Allergy:Update2013; World Allergy Organization: Milwaukee, WI, USA, 2013.

12) Cukrowska, B.; Bierla, J.B.; Zakrzewska, M.; Klukowski, M.; Maciorkowska E. The relationship between the infant gut microbiota and allergy. The role of *Bifidobacterium breve* and prebiotic oligosaccharides in the activation of anti-allergic mechanisms in early life. Nutrients 2020;12:946.

13) Di Pierro, ; Basile, I.; Danza, M.L.; Venturelli, L.; Contini, R.; Risso, P.; Colombo, M. Use of probiotic mixture containing *Bifidobacterium animalis* subsp. *Lactis BB12* and *Enterococcus faecium L3* in atopic children. Minerva Pediatr 2018,70:418-424.
14) Turroni, F.; Milani, C.; Duranti, S.; Lugli, G.A.; Bernasconi, S.; Margolles, A.; Di Pierro, F.; van Sinderen, D.; Ventura, M. The infant gut microbiome as a microbial organ influencing host well-being. Ital J Pediatr 2020;46:16.

15) Turroni, F.; Peano, C.; Pass, D.A.; Foroni, E.; Severgnini, M.; Claesson, M.J.; Kerr, C.; Hourihane, J.; Murray, D.; Fuligni, F.; et al. Diversity of Bifidobacteria within the infant gut microbiota. PloS One 2012;7:e36957.

16) Milani, C.; Hevia, A.; Foroni, E.; Duranti, S.; Turroni, F.; Lugli, G.A.; Sanchez, B.; Martín, R.; Gueimonde, M.; van Sinderen, D.; et al. Assessing the fecal microbiota: an optimized ion torrent 16S rRNA gene-based analysis protocol. PloS One. 2013;8:e68739.

17) Robertson, R.C.; Manges, A.R.; Finlay, B.B.; Prendergast, A. The human microbiome and child growth- First 1000 days and beyond. Trend Microbiol. 2019;27:131-147.

18) Putignani, L.; Del Chierico, F.; Petrucca, A.; Vernocchi, P.; Dallapiccola, B. The human gut microbiota: a dynamic interplay with the host from birth to senescence settled during childhood. Pediatr Res 2014;76:2-10.

19) Sarkar, A.; Yoo, J.Y.; Valeria, Ozorio, Dutra, S.; Morgan, K.H.; Groer, M. The Association between Early-Life Gut Microbiota and Long-Term Health and Diseases. J Clin Med. 2021;10:459.

20) Fujimura, K.E.; Sitarik, A.R.; Havstad, S.; Lin, D.L.; Levan, S.; Fadrosch, D.; Panzer, A.R.; LaMere, B.; Rackaityte, E.; Lukacs, N.W.; et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. Nat Med 2016;22:1187-1191.

21) Björkstén, B.; Sepp, E.; Julge, K.; Voor, T.; Mikelsaar, M. Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol 2001;108:516-520.

22) Yamanishi, S.; Pawankar, R. Current advanced on the microbiome and role of probiotics in upper airways disease. Curr Opin Allergy Clin Immunol 2020;20:30-35.

23) Plaza-Diaz, J.; Ruiz-Ojeda, F.J.; Gi-Campos, M.; Gill, A. Mechanisms of action of probiotics. Adv Nutr 2019;10:549-566.
24) Fioramonti, J.; Theodorou, V.; Bueno, L. Probiotics: what are they? What are their effects on gut physiology? Best Pract Res Clin Gastroenterol 2003;17:711-724.

25) Ruiz, L.; Delgado, S.; Ruas-Madiedo, P.; Sanchez, B.; Margolles, A. Bifidobacteria and their molecular communication with the immune system. Front Microbiol 2017;8:2345.

26) Hevia, A.; Milani, C.; Lopez, P.; Donado, C.D.; Cuervo, A.; Gonzalez, S.; Suárez, A.; Turroni, F.; Gueimonde, M.; Ventura, M.; et al. Allergic patients with long-term asthma display low levels of Bifidobacterium adolescentis. PloS One 2016;11:e0147809.

27) Lo Skiavo, L.A.; Gonchar, N.V.; Fedorova, M.S.; Suvorov, A.N. Dynamics of contamination and persistence of Clostridium difficile in intestinal microbiota in newborn infants during therapy and use of probiotic strain Enterococcus faecium L3. Antibiot Khimioter 2013;58:13-18.

28) Jungersen, M.; Wind, A.; Johansen, E.; Christensen, J.E.; Stuer-Lauridsen, B.; Eskesen, D. The science behind the probiotic strains Bifidobacterium animalis ssp. Lactis BB-12®. Microorganisms 2014;2:92-110.

29) Mozzanica, F.; Urbani, E.; Atac, M.; Scottà, G.; Luciano, K.; Bulgheroni, C.; De Cristofaro, V.; Gera, R.; Schindler, A.; Ottaviani F. Reliability and validity of the Italian nose obstruction symptom evaluation (I-NOSE) scale. Eur Arch Otorhinolaryngol 2013;270:3087-3094.

30) Brozek, J.L.; Bousquet, J.; Agache, I.; Agarwal, A.; Bachert, C.; Bosnic-Anticevich, S.; Brignardello-Petersen, R.; Canonica, G.W.; Casale, T.; Chavannes, N.H. Allergic rhinitis and its impact on asthma (ARIA) guidelines-2016 revision. J Allergy Clin Immunol 2017;140:950-958.

31) Stewart, M.G.; Witsell, D.L.; Smith, T.L.; Weaver, E.M.; Yueh, B.; Hannley, M.T. Development and validation of the Nasal Obstruction Symptom Evaluation (NOSE) scale. Otolaryngol Head Neck Surg 2004;130:157-163.
32) Ellis, A.K.; Soliman, M.; Steacy, L.; Boulay, M.È.; Boulet, L.P.; Keith, P.K.; Vliagoftis, H.; Waserman, S.; Neighbour, H. The Allergic Rhinitis-Clinical Investigator Collaborative (AR-CIC): nasal allergen challenge protocol optimization for studying AR pathophysiology and evaluating novel therapies. Allergy Asthma Clin Immunol 2015;11:16.

33) Lin, T-Y.; Chen, C-J.; Chen, L-K.; Wen, S-H.; Jan R-H. Effect of probiotics on allergic rhinitis in Df, Dp or dust-sensitive children: a randomized double blind controlled trial. Indian Pediatrics 2013;50:209-213.

34) Peng, Y.; Li, A.; Yu, L.; Qin, G. The role of probiotics in prevention and treatment for patients with allergic rhinitis: a systematic review. Am J Rhinol Allergy 2015;29:292-298.

35) Cuello-Garcia, C.A.; Brožek, J.L.; Fiocchi, A.; Pawankar, R.; Yepes-Nuñez, J.J.; Terracciano, L.; Gandhi, S.; Agarwal, A.; Zhang, Y.; Schünemann, H.J. Probiotics for the prevention of allergy. A systematic review and meta-analysis of randomized controlled trials. J Allergy Clin Immunol 2015;136:952-961.

36) Del Giudice, M.; Indolfi, C.; Capasso, M.; Maiello, N.; Decimo, F.; Ciprandi, G. Bifidobacterium mixture (B longum BB536, B infantis M-63, breve M-16V) treatment in children with seasonal allergic rhinitis and intermittent asthma. Ital J Pediatr 2017;43:25.