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Oral probiotics influence oral and respiratory tract infections in pediatric population: a randomized double-blinded placebo-controlled pilot study

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Abstract. – OBJECTIVE: Acute oral and respiratory tract infections (RTIs) are highly present in the general population, and they represent one of the most impacting causes of morbidity and mortality every year. The aim of our study was to assess the clinical impact of oral probiotics on acute oral and respiratory tract infections affecting paediatric patients.

PATIENTS AND METHODS: This is a randomized, double-blinded placebo-controlled clinical study, where probiotics have been compared with placebo in a double-blinded investigation. 40 subjects with a recent clinical history reporting oral and respiratory tract infections were randomly selected and assigned to control (n=20) or the probiotics (n=20) group. During the 3 months before the starting, all subjects were assessed with several salivary examinations.

RESULTS: We found significant differences in the incidence of oral and respiratory tract infections between control and probiotic group, with a marked reduction of oral and respiratory tract infections episodes in the probiotic group. Salivary examinations gave similar results in both the groups, however, despite the salivary flow, viscosity and buffering were similar, on the contrary, pH values were found to be more alkaline in patients of probiotic group.

CONCLUSIONS: This study showed the main positive effects related to a supplementation with probiotics in order to prevent or reduce the incidence of infections onset in oral and respiratory tracts without any drugs-related adverse effects.

Key Words:
Oral infections, Oral diseases, Probiotics.

Introduction

The respiratory system is the district with the highest incidence and prevalence of infections due to its evident and easy access to foreign agents. Respiratory viruses are the second largest cause of mortality, after malaria, in infants outside the neonatal period and are the leading cause of hospitalization in infancy in developed countries[1].

The acute Respiratory Tract Infections (RTIs) are highly reported in worldwide paediatric population, and their effects may be related to the tract affected, considering that the lower respiratory tract is usually the most difficult to treat[2].

The upper respiratory tract infections are typically represented by sinusitis, chronic tonsillitis, and adenoiditis; they are triggered in most cases by viral infections and are often complicated by bacterial infections. Usually, we can observe a spontaneous resolution of the infections, even with no antibiotic administration, in order to avoid changes in bacterial biofilms following their exposure to antimicrobial agents[3].

Infections of the lower respiratory tract, such as tracheitis, bronchiolitis, bronchitis, and pneumonia may have, on the contrary, serious conse-

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quences if they are not properly and timely diagnosed and treated[4]. Although viruses are often responsible for RTIs, bacterial super-infections commonly occur[5,6]. The most prevalent bacterial respiratory pathogens are species such as *Streptococcus pneumoniae*, *Mycoplasma pneumoniae* and *Streptococcus pyogenes*[7]. There is also evidence of a synergistic effects between viruses and bacteria in the pathogenesis of oral and respiratory infections[8], in fact, recurrent infections may result in virus-induced immune dysfunction and can lead to a vicious cycle of recurrent RTIs with bacterial super-infections, exacerbation of cough, and increased risk of asthma development[9-11].

One serious problem of such infections is related to patient's age, in fact, most children experience RTIs when they are younger than 2 years, and 25% of them suffer from recurrent or prolonged infections in developed countries[12,13]. Because children affected by RTIs are severely weak, these pathological forms lead to limiting physical and scholastic activities, in fact, RTIs are a major cause for school absenteeism and hospitalizations[14,15].

Acute infections of the upper respiratory tract and oral tract are still the most common reason for reporting to the general practitioner or ENT specialist. Despite the fact that the most common causative agent of these diseases is a virus, antibiotics are still administered in about 60-80% of patients who report to the doctor, showing that many bacterial superinfections could be depending from a diffuse antibiotics abuse[16-18].

Inappropriate and wide use of antibiotics may lead to the development of bacterial resistance and disturb the normal balance of human microbiota, facilitating the pathogen colonization and reducing the availability of vaccines for viruses[19,20]. A Cochrane review in 2011 found that, in randomized controlled trials (RCTs) of specific patient populations, probiotic prophylaxis significantly reduces both upper respiratory tract infections and antibiotic prescribing rates for these infections[21]. Bacteria play an essential role in defence of their territory against the entry of other bacteria that may be pathogenic to systemic health. Metchnikoff, about a century ago, invented probiotics, assuming that the use of certain bacteria could be beneficial to maintaining health. The alterations of oral bacterial flora are responsible for numerous diseases of the oral cavity, and the idea of the use of probiotics is leading the way to new therapeutic perspectives[22]. Considerable human illness can be linked to the development of oral microbiota disequilibria. The predominant oral cavity commensal, *Streptococcus salivarius* has emerged as an important source of safe and efficacious probiotics, capable of fostering more balanced, health-associated oral microbiota[23]. *Streptococcus salivarius K12* (SSK12) is a probiotic strain strongly antagonistic to the growth of Streptococcus pyogenes, the most important bacterial cause of pharyngeal infections in humans; recent studies reported that the daily administration of SSK12 to children was associated with a significant reduction in episodes of streptococcal pharyngitis and acute otitis media, thus creating a strong link among probiotics administration and oral and respiratory tracts infections[24].

In this context, the present randomized, double-blinded placebo-controlled clinical study aimed to evaluate the effect of oral probiotics in paediatric patients reporting oral and respiratory tract infections.

Patients and Methods

This is a randomized, double-blinded placebo-controlled clinical study. This project was approved by the local Ethical Committee (Study consent and registration code: TRI2018-03-01BA). Advice leaflets reporting understandable recommendations to take a probiotic supplement daily, for a specific period, with the aim of reducing antibiotic prescription in participants, were given to guardians after careful explanation. All guardians involved in this study were requested to read, clearly understand and sign an informed consent for a voluntary participation to the research. The study was carried out among February and May 2018, in compliance with guidelines approved by the local Ethics Committee and in accordance with Italian laws and regulations on clinical studies. The study followed the ethical principles for medical research involving human subjects of the Helsinki Declaration.

Sample Characteristics

All participants were recruited with a cluster-sampling in the south Italy healthcare centers. Inclusion criteria were age under 16 years old (y.o.), a current diagnosis of oral or upper respiratory tract infections. A total amount of 40 children (11 females and 29 males aged 12-15y.o.) affected by recurrent oral and respiratory infections (RTIs) were selected and recruited in the study with at least one recent episode of RTIs, specifically, we recruited patients diagnosed for

stomatitis, tonsillitis, pharyngitis, laryngitis, rhinosinusitis.

Exclusion criteria for this study were nasal polyps, immunodeficiency, gastroesophageal reflux, cystic fibrosis, bronchial asthma, pertussis, and allergic rhinosinusitis or conjunctivitis.

Study Protocol

None of subjects were administered with antibiotics for the last 3 months and/or during the study. Patients were randomly assigned into 2 groups: Test group (A:20 Subjects, 4 females and 16 males) treated with probiotics (PRO-Kids ENT Hyperbiotics, USA) administered three oral tablets per day, for the first month, and one tablet per day, for the following two months. Placebo group (B: 20 Subjects, 7 females and 13 males) was treated with the same posology of oral tablets looking identical to probiotics administered in group A. Patients were subjected to oral and ENT visits at 30 (first milestone – T1) and 90 (second milestone – T2) days after baseline (T0). All subjects who did not follow the indications reported in the study protocol were also reported and not considered in the final outcomes.

Biological Samples Collection and Management

Samples of whole saliva (10ml) were collected during the study from each patient at different time-points. Samples collection was carried out in the morning, at least 1h after eating. Patients were asked to do twice oral rinses with distilled water; then, they were instructed on how to spit out a small amount of saliva in a graduated tube. All the collected samples were analysed for salivary flow rate, pH, buffering capacity and viscosity. The salivary flow rate for each patient was obtained by analysing the volume of saliva collected in 3 different time-points, including baseline. Saliva can act as a buffer which neutralizes oral acids. This ability is mainly based on the phosphate system and the carbonic acid/bicarbonate system; however, in stimulated saliva, the carbonic acid/bicarbonate system is the most important buffer. Several methods can be used to determine the saliva buffer effect. The "Buffer Test" was used to estimate the buffering capacity of saliva. The relative viscosity of saliva with respect to water was measured using a commercial viscometer. Salivary pH values were measured by means of pH-strips (Advantec MFS, Tokyo, Japan) soaked with unstimulated saliva for 10 seconds; the results were obtained by comparing the strips with a reference chart. The saliva samples of all the patients were identified by a code-number and tested on the same day for salivary pH.

The probiotics used in this study are commercially known as Hyperbiotic PRO-Kids ENT (Hyperbiotics, Henderson, NV, USA) and belong to a new generation of advanced probiotics highly effective on the homeostasis of the oral microbiome of children. Hyperbiotic PRO-Kids ENT contains several probiotic strains (*Streptococcus salivarius* K12, *Streptococcus salivarius* M18, *Lactobacillus reuteri*, *Lactobacillus sakei*, and *Lactobacillus paracasei*) and is characterized by the controlled-releasing of active principles usually residing on the tongue, tonsils, throat-ear-nose, and surrounding area.

Statistical Analysis

The mean values of pH, flow, buffering capacity and salivary viscosity for Groups A and B were analysed using the GraphPad Prism (version 6.01, La Jolla, CA, USA) and IBM Statistical Package for the Social Sciences (SPSS Inc. Version 16.0, Chicago, IL, USA) software.

Statistical analyses were performed using SPSS Statistics 21 software (USA). Data were analysed with ANOVA tests. A value of $p<0.05$ was considered statistically significant.

Results

The salivary flow rate (SFR) in healthy individuals may vary, according to different factors.

The monitoring of salivary flow rate and pH is of primary importance for preventing oral and respiratory pathologies; indeed, it has been shown that alteration of these values reduces oropharyngeal and dental health[25]. In our study, we obtained salivary flow values quite similar among the A and B groups reported in this study (Figure 1). The viscosity of saliva depends on its compounds and on the percentage of water present in its composition. The stimulated salivary flow is characterized by an increased presence of bicarbonate ions, which may increase the salivary buffering power. The presence of macromolecules and salts may influence salivary viscosity and can consequently reduce the overall amount of saliva in patients. In our study, we found similar levels of viscosity in both our test (A) group and control (B) group (Figure 2). The obtained data showed that despite the reported flow rate values, viscosity and total proteins amount were quite

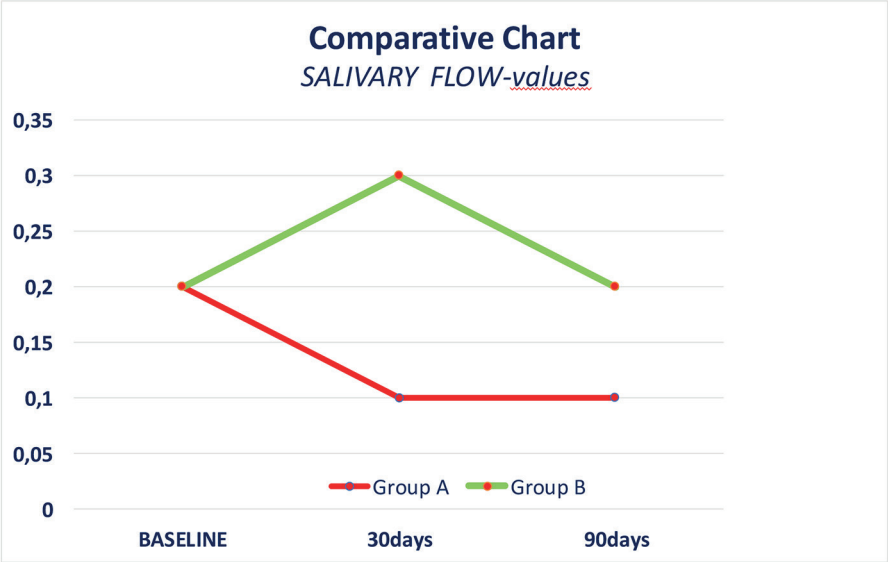

Figure 1. Salivary flow evaluation.

similar among the 2 groups, while the buffering capacity of saliva was slightly improved in group A (Figure 3). Finally, there was a significant difference in the mean values of salivary pH, if we compare the 2 study groups ($p < 0.001$). Group A had a significantly higher mean value of salivary pH, in comparison with Group B. The results obtained are in agreement with the aim of our study; in fact, we assumed that probiotics were able to basically re-equilibrate oral bacteria and their action on PH values (Figure 4). A salivary pH of 7.0 usually indicates a healthy dental and clinical periodontal condition, characterized by a low incidence of dental caries and oral and respiratory infections. Therefore, these environments should be monitored and maintained with a stable microbiome[26]. Salivary pH with values less than 7.0 usually indicates an increased susceptibility to oral and respiratory tract pathologies and inflammations[27,28].

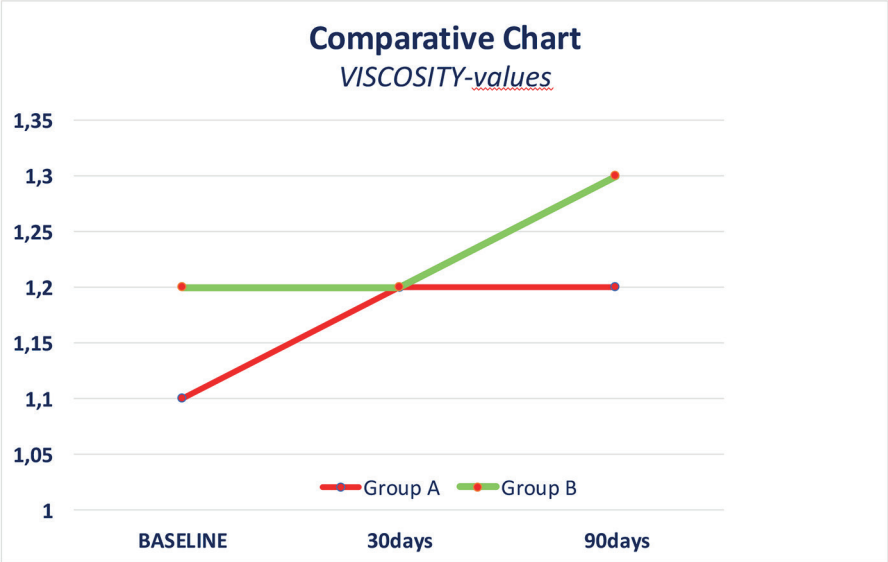

Figure 2. Salivary viscosity evaluation.

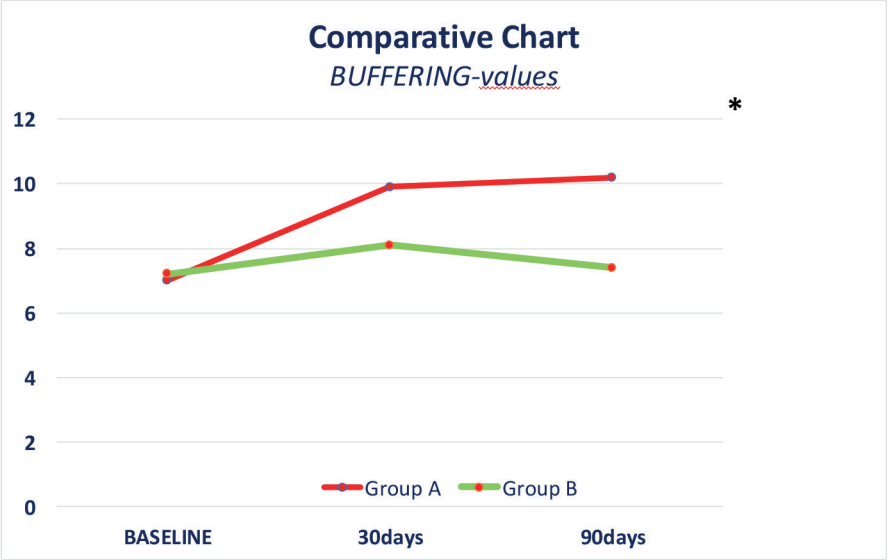

Figure 3. Salivary buffering ability evaluation.

Along with salivary analysis, the occurrence of RTIs episodes was also recorded among both the groups during the study period, as described in Table I: the reported data highlighted that the groups were markedly different in the incidence of relapses of RTIs, during the study monitoring. In fact, group A, treated with probiotics, showed a marked decreasing of respiratory infections in almost all the reported cases. On the other hands, untreated group B reported a similar trend of RTIs, compared to baseline; moreover, in some cases, patients within the B group showed a worsening of their general and respiratory conditions.

Table I. Analysis of the relapses of oral and respiratory tract infections in the 2 groups at 2 different time-points.

Subjects	Group A (Baseline)	Group A (T2)	Group B (Baseline)	Group B (T2)
1	3 (4.85)	1 (1.15)	4 (4.25)	4 (4.35)
2	2	1	5	1
3	4	2	4	2
4	4	1	2	7
5	5	2	9	7
6	5	3	8	13
7	3	0	9	11
8	2	0	8	7
9	5	1	9	7
10	5	2	4	5
11	8	0	2	1
12	3	0	1	1
13	5	0	1	2
14	6	2	1	2
15	9	3	5	7
16	1	0	2	1
17	6	0	3	1
18	4	0	2	1
19	9	2	1	1
20	8	3	5	6

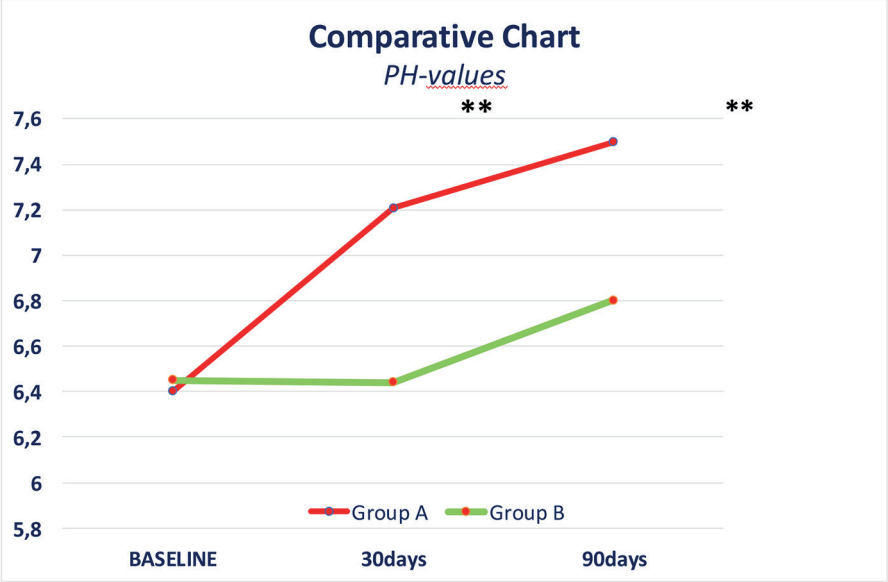

Figure 4. Salivary pH values evaluation.

Discussion

Probiotics have been showed to have local effects on the gut microbiome, inhibiting the potential pathogens, modulating the gut functionality and permeability, and acting as immunomodulatory players in the host subjects. Probiotics can also influence both innate and adaptive immune responses by producing exopolysaccharides[29]. A study showed that intestinal probiotics could be effective in relieving clinical symptoms of severe hand-foot-and-mouth disease, maintaining intestinal immunity and anti-inflammatory responses[30] Furthermore, probiotics can influence the salivary immunoglobulin A levels and induce a better antimicrobial activity on oral pathogens[31,32]. Different probiotic bacteria have been associated with several severe syndromes on intestinal mucosae: recently, the beneficial effect of probiotics has been reported also on chronic constipation, via a significant decrease of methane production[33]. In this context, our results on Hyperbiotic administration in paediatric patients suffering from RTIs, are in strong agreement with the recent scientific literature. In our work subjects administered with probiotics reported the 76% reduction of RTIs incidence, compared to control group. Moreover, no adverse effects were reported in both the groups. Differences in the onset of illness between the probiotic group and the placebo group became apparent after approximately two weeks after the study start. After a careful revision of the current literature on this topic, our study adds important new information regarding the effects of probiotics on oral and respiratory inflammatory diseases.

Literature has widely reported how probiotics may increase the clinical course of several pathologies; one of the most interesting results was related to the effects of probiotics and gut microbiota on prevention and treatment of rotavirus-related gastro-intestinal severe pathologies. Few researches reported effects of probiotics on oral and respiratory tracts: Santagati et al[34] identified 13 α-haemolytic *Streptococci* bactericidin-producers capable of inhibiting different Gram-positive pathogens and found that *Streptococcus salivarius* 24SMB does not possess any virulence factor and is a strong producer of bactericidins against *Streptococcus pneumoniae*, the most common respiratory bacterial pathogen. *Lactobacillus* residing on of the respiratory mucosa resulted effective in suppression of multiple cytokines, associated with the inflammatory ENT pathologies[35-44]. A better understanding of the specific effects by different probiotic strains and a deeper insight into their mechanisms of action are needed for the validation of specific strains carrying a po-

tential to modify the frequency and severity of RTIs in infants and children.

Conclusions

We reported and discussed in this study assessed that specific probiotic strains act as a useful and safe tool for the decreasing of RTIs episodes in the paediatric population. This approach may provide a valuable addition to the current therapeutic strategies and could improve the antibiotics effect on chronic oral and respiratory inflammatory conditions.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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