A probiotic mixture in patients with upper respiratory diseases: the point of view of the otorhinolaringologist.

M. Gelardi[1], I. La Mantia[2], L. Drago[3], G. Meroni[3], S.E. Aragona[4], G. Cupido[5], C. Vicini[6], C. Berardi[7], G. Ciprandi[8] and the Italian Study Group on Upper Respiratory Infections*

*[1]Department of Otolaryngology, University of Foggia, Foggia, Italy; [2]Unit of Otolaryngology, Department of General Surgery and Medical-Surgical Specialties, University of Catania, Catania, Italy; [3]Microbiology Department, University of Milan, Milan, Italy; [4]Center of Regenerative Medicine, Humanitas Mater Domini, Castellanza (VA), Italy; [5]ENT Department, University of Palermo, Palermo, Italy; [6]ENT Department, Forlì-Faenza Hospital, Italy, University of Ferrara e Bologna; [7]ENT Department, Istituto Clinico Città Studi di Milano, Milan, Italy; Allergy Clinic, Casa di Cura Villa Montallegro, Genoa, Italy; *Italian Study Group on Upper Respiratory Infections*

*the Italian Study Group on Upper Respiratory Infections: G. Albanese, A. Anni, G. Antoniacomi, S. Artoni, G. Asprella, R. Azzaro, A. Azzolin, A. Balduzzi, I. Barbarino, C. Berardi, B.I. Bertetto, a. Bianchi, N. Bianco, A. Bonanno, C. Bordonaro, A.M. Brindisi, S. Bucolo, A.G. Bulzomì, G. Caligo, P. Capaccio, M. Capelli, A. Capone, S. Carboni, G. Carluccio, M. Casaula, M. Cassano, M. Cavaliere, A. Ciabattoni, A. Conti, A. Cordier, F. Cortellessa, M. Costanzo, F. Cupido, L. D'Ascanio, C. Danza, C. D'Auria, M. De Ciccio, C. De Luca, M. D'Emila, R. Dessi, A. Di Lullo, M. D'Urso, S. Falcetti, R. Falciglia, G. Fera, G. Ferrari, S.M. Ferraro, O. Fini, M. Fiorella, A. Floriddia, F. Asprea, C. Fusco, R. Fuson , A. Gallo, T. Gambardella, G. Gambardella, F. Giangregorio, G. Gramellini, M. Ierace, F. Ingria. A. La Paglia, L. Lanza, R. Lauletta, P. Lavazza, M. Leone, P. Lovotti, P. Luperto, F. Maniscalco, I. Marincolo, R. Martone, A. Melis, A. Messina, V. Milone, N. Mirra, S.C. Montanaro, F. Muià, A. Nacci, E. Nardello, L. Paderno, D. Padovani, A. Palma, M. Paoletti, I. Pedrotti, F. Petrillo, M. Piccolo, P. Pinto, M. Policarpo, M. Raguso, A. Ranieri, G. Romano, M. Rondinelli, C. Russo, L. Scotto Di Santillo, G. Sequino, E.M. Serraino, I. Spahiu, G. Spanò, C. Stabile, G. Stagni, M. Stellin, S. Tassi, G. Tomacelli, A. Tombolini, V. Valenzise, A. Zirone

Upper respiratory infections are widespread in clinical practice. Antibiotics are frequently used in the management of patients with airways infection. However, antibiotics can induce intestinal and respiratory dysbiosis that, in turn, worsens respiratory symptoms. Moreover, respiratory infections *per se* can cause dysbiosis. Consequently, probiotics may counterbalance the disturbed microbiota. The current clinical experience evaluated the efficacy and safety of an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion of living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii subspecies delbrueckii* LDD01 (200 million living cells), in 2928 outpatients with an upper respiratory infection and treated with antibiotics. Patients took one stick/daily for four weeks. Simultaneously, 2877 patients with an upper respiratory infection and treated with antibiotics were recruited as control. This probiotic mixture significantly diminished the presence and the severity of respiratory symptoms at the end of the probiotic course and, more evidently, after a 3-month follow-up. In conclusion, the current clinical experience suggested that this probiotic mixture may be considered an effective and safe therapeutic option in managing patients with an upper respiratory infection and treated with antibiotics.

Key words: upper respiratory infection, mucosal microbiota, antibiotics, probiotics, randomized study.

Corresponding Author:
Dr Giorgio Ciprandi
Via P. Boselli 5,
16146 Genoa Italy
e-mail: gio.cip@libero.it

0393-974X (2020)

The human body contains many diverse microbes, which are also associated with the digestive and respiratory tracts. These microorganisms are mainly bacteria and constitute a unique and dense ecosystem named microbiota (1). Many studies investigated human microbiota, including the Human Microbiome Project in the United States, to define its physiological and pathological role (2). The microbiota is characterized by a beneficial diversity to guarantee adequate defence against pathogens. Unbalanced microbiota is called dysbiosis. A dysbiosis can promote respiratory infections as well as a respiratory infection may *per se* promote dysbiosis (3). Moreover, antibiotics may significantly affect the intestinal and respiratory microbiota, worsening the dysbiosis (4).

Upper respiratory infections are a common challenge in otorhinolaryngological (ORL) practice. They may be acute, chronic, or recurring. Antibiotic prescription is frequent in patients with respiratory infections, even though many patients could not require antibiotic therapy as affected by viral infections. The antibiotic abuse entails two main consequences: antibiotic resistance and mucosal dysbiosis (5). Considering these reasons, many doctors prescribe probiotics to restore the microbiota environment in patients with respiratory infections and treated with antibiotics (6).

Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (formerly *Lactobacillus pntarum*) (1 billion of living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* subspecies *delbruekii* LDD01 (200 million living cells). It has been recently placed on the market. Based on this background, a group of Italian ORL doctors performed a clinical experience concerning the use of probiotics in patients with upper airway infection. Therefore, this clinical experience aimed to evaluate the efficacy and safety of Abincol® in outpatients suffering a respiratory infection and treated with antibiotics.

MATERIALS AND METHODS

The current experience was conducted in Italian otorhinolaryngological (ORL) centres, distributed across Italy, therefore ensuring an extensive and complete national coverage, during the fall-winter 2019-2020. ORL specialists were asked to recruit all consecutive outpatients with an upper respiratory infection.

Patients were consecutively recruited during the specialist visit. The inclusion criteria were to have upper airways infection, both genders, adulthood, and ongoing antibiotic treatment. Exclusion criteria were to have comorbidities and concomitant medications able to interfere with the evaluation of the outcomes.

All patients signed informed consent. All the procedures were conducted in real-world setting.

The patients were randomly subdivided into two groups: Group A (active group) was treated with an oral antibiotic for 7-10 days associated with a 4-week course of Abincol®, and Group B (control group) was treated with antibiotics alone. The oral nutraceutical was taken following the specific indications, such as one stick/daily. Patients were visited at baseline (T0), after 7-10 days (T1), such as at the end of antibiotic therapy, after four weeks (T2), such as at the end of the probiotic course in Group A, and after a 3-month follow-up (T3).

Clinical examination was performed in all patients at each visit. Doctors used a visual analog scale (VAS) to assess the perception of symptom and sign severity. The VAS scale ranged from 0 (no symptom) to 10 (very intense symptom). The following symptoms and signs were investigated: sore throat, dysphonia, nasal obstruction, corked ears, cough, rhinorrhea, and mucosal hyperemia and edema. Safety was measured by reporting the occurrence of adverse events. All clinical data were inserted in an internet-platform that guaranteed the patients' anonymity and the findings' recording accuracy.

Statistical analysis of the data was conducted with GraphPad Prism software version 8.0.1 for Windows (GraphPad Software®, San Diego, USA, www.graphpad.com). The following tests were used to verify the normality of the data expressed with numerical variables (except mucus consistency and temperature): D'Agostino and Pearson omnibus normality test, Shapiro - Wilk normality test, and Kolmogorov - Smirnov normality test. One-way ANOVA test and multiple comparisons with Dunn's test were used to check for any statistical differences between groups. The Mann - Whitney test was used to compare data that were not normally distributed, and vice versa, the

Student's T-test. The data were subsequently transformed into categorical variables using a scale of scores from absent to severe (0=absent, 1-3=mild, 4-6=moderate, 7-10=severe). The prevalence thus calculated were compared with the Chi-square test. For all tests, the accepted minimum significance limit was 0.05.

RESULTS

Globally, 5805 outpatients (mean age 56 years) were enrolled: 2928 (50.4%) in Group A and 2877 (49.6%) in Group B. The global analysis of the data (expressed as score 0-10) highlighted some differences between the two groups at T2 [for mucosal hyperemia (p=0.0479)], cough (p <0.0001) and mucosal edema (p=0.0006) and at T3 (for all symptoms except rhinorrhea), as reported in Fig. 1.

By transforming the scoring assigned by the patients into a categorical variable (0=absent, 1-3=mild, 4-6=moderate, 7-10=severe), it was possible to calculate the relative frequencies of each symptom to better identify any statistical

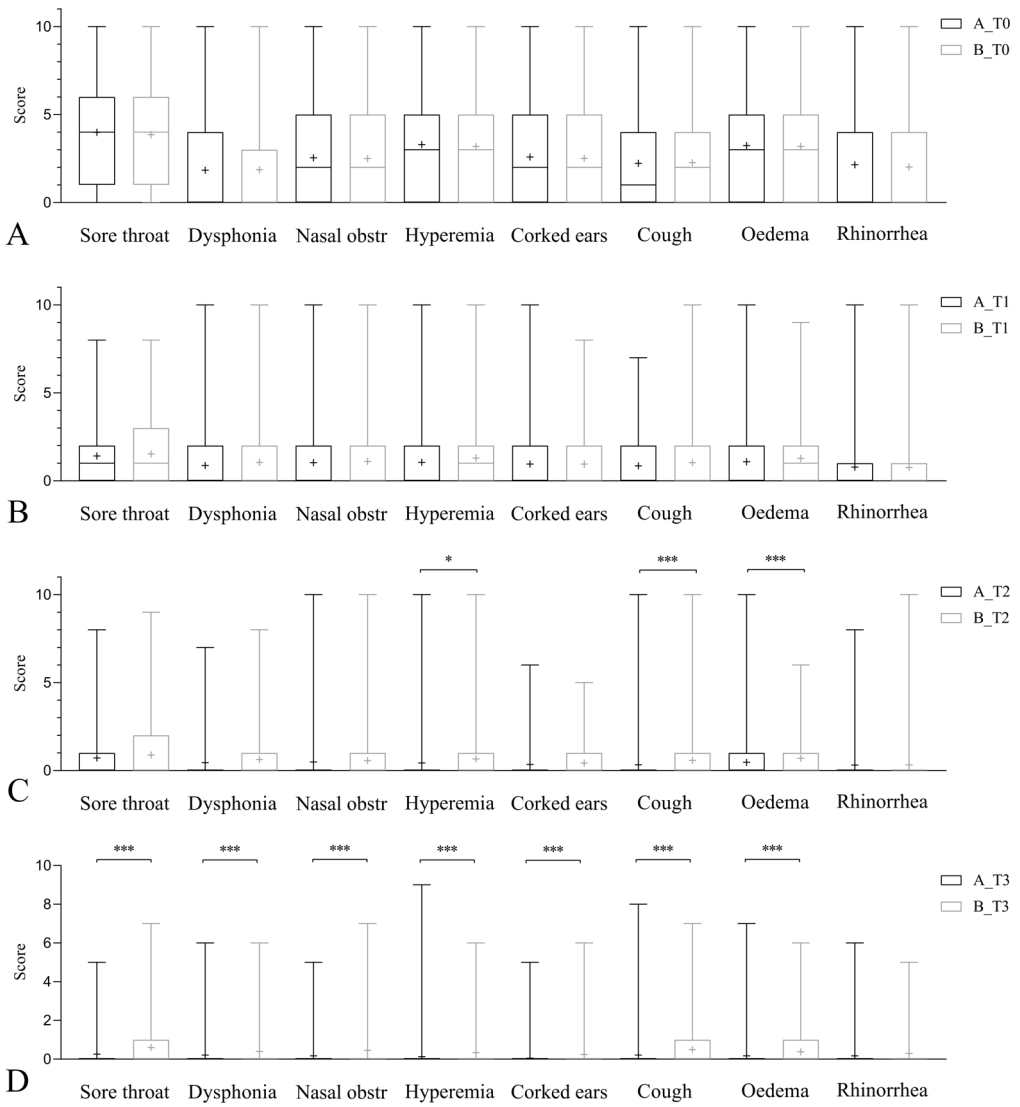

Fig. 1. *Comparison of symptoms between group A (active) and B (control) in the four times. The data are represented by boxplot, the horizontal line represents the median, while the mean is indicated with the sign "+". (* p-value: 0.05-0.01; ** p-value: <0.01; *** p value: <0.001).*

significance. Considering the quota of patients with absence of symptoms, there were significant intergroup differences for dysphonia, mucosal hyperemia, cough, and mucosal edema at T2, namely the percentage of asymptomatic patients was higher in Group A (Fig. 2).

At T3, there was a significant difference in all symptoms between the two groups, namely the percentage of asymptomatic patients was higher in Group A (Fig. 3). Slightly higher differences were identified at T3; 260/264 (98.5%) patients of group A did not report the presence of exudate, while 263/281 (93.6%) subjects of group B indicated the absence of this symptom (p = 0.0037).

The intragroup analysis showed that all symptoms diminished over time, as reported in Fig. 4. The nutraceutical was well-tolerated in all subjects.

DISCUSSION

Popular medicine uses natural non-pharmacological remedies since ancient times. There is growing scientific interest in the use of medical devices and food supplements to improve human diseases, so sparing the use of conventional medications. Despite significant scientific and technological progress in combinatorial chemistry, products derived from natural products, including microbial derivates, still make enormous contributions to medication discovery today.

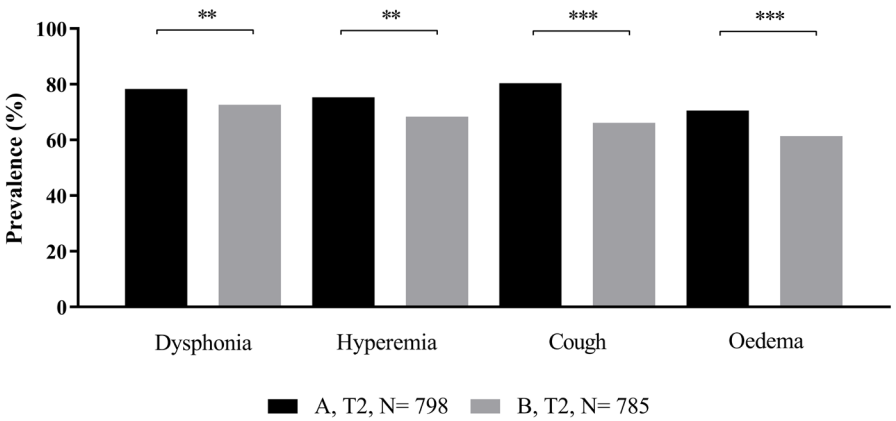

Fig. 2. *Comparison of patients' percentage without a symptom between group A (active) and B (control) at T2. (* p value: 0.05-0.01; ** p value: <0.01; *** p value: <0.001).*

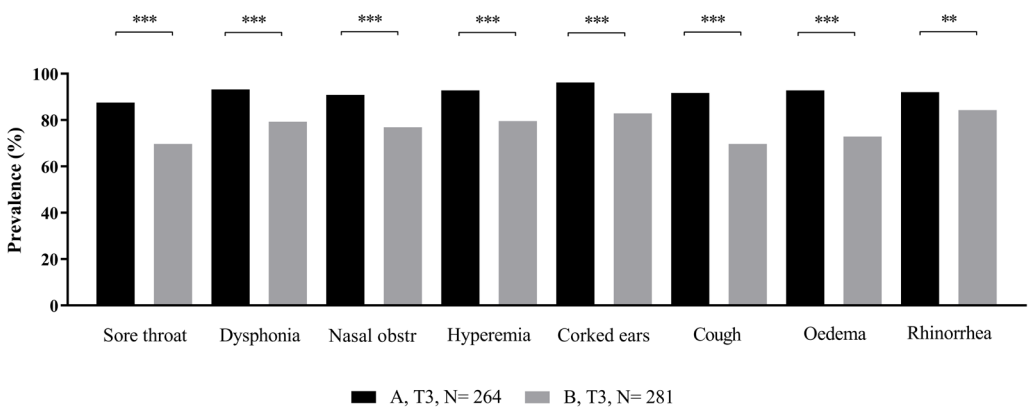

Fig. 3. *Comparison of patients' percentage without a symptom between group A (active) and B (control) at T3. (* p value: 0.05-0.01; ** p value: <0.01; *** p value: <0.001).*

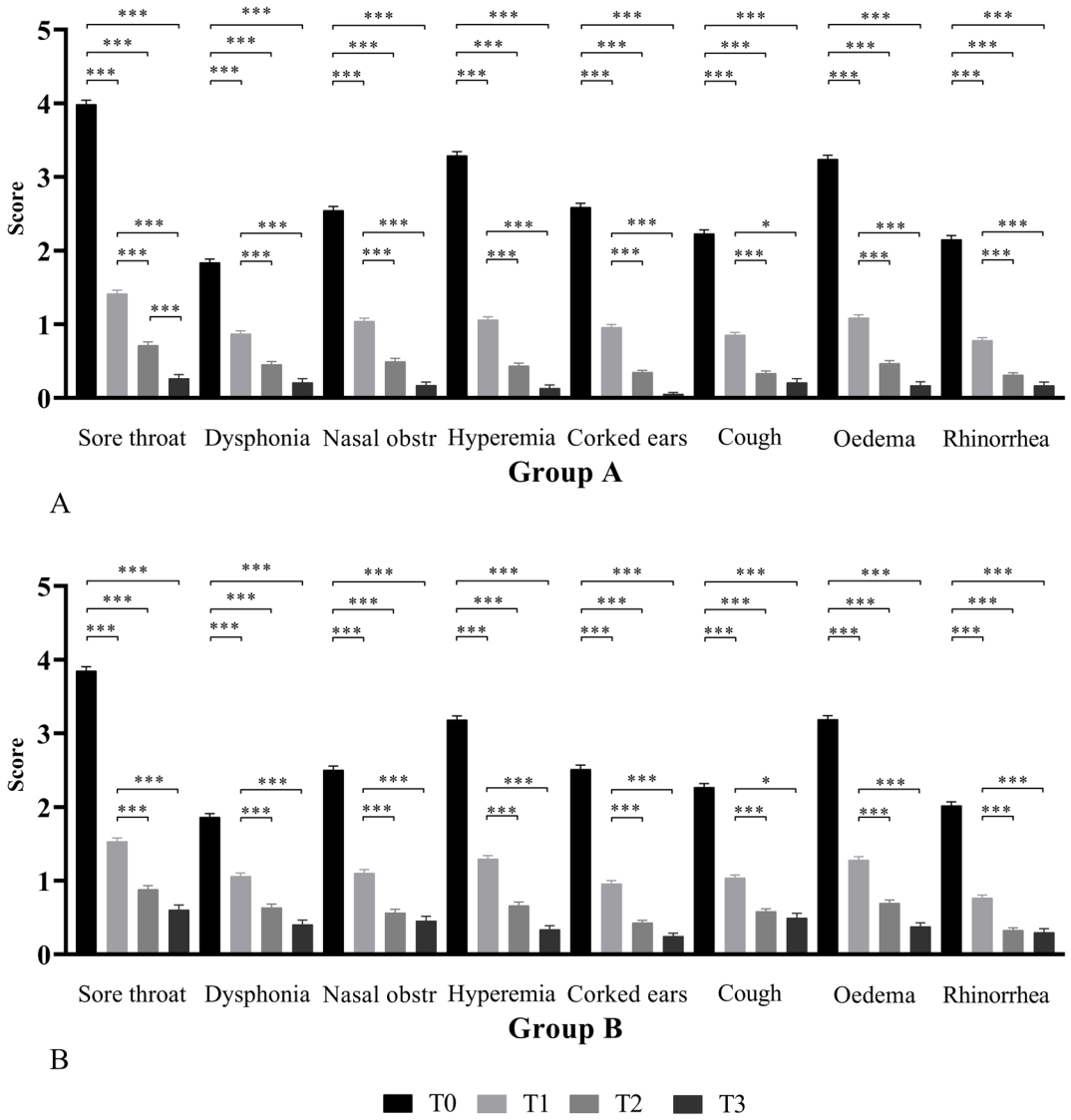

Fig. 4. *Intragroup analysis of all symptoms in the four times in group A and B. (* p value: 0.05-0.01; ** p value: <0.01; *** p value: <0.001).*

Respiratory infections are widespread at any age and represent a significant burden for both the single individual and society.

Antibiotics are frequently used in patients with an upper respiratory infection, but not always antibiotics are correctly prescribed. Overuse/abuse of antibiotics entails intestinal and respiratory dysbiosis. Moreover, infections *per se* promote dysbiosis. To interrupt this vicious circle, probiotics may represent a fruitful choice (7, 8).

The current clinical experience, conducted in patients with an upper respiratory infection and treated with antibiotics, demonstrated that a 4-week course of *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02 and *Lactobacillus delbrueckii* LDD01 significantly improved respiratory symptoms already after one month, but even more in 4 months. Consistently, the quote of patients without symptoms was significantly higher in the active group than in the control group

at the end of the probiotic course for dysphonia, mucosal hyperemia, cough, and mucosal edema. The quote of asymptomatic patients was significantly higher in the probiotic group for all symptoms at the end of the follow-up.

These outcomes are consistent with the literature, which underlines the decisive role of probiotics as add-on treatment in patients with respiratory infections (9). Moreover, there may be a promising role for probiotics in the pandemic COVID-19 era as recently advanced (10). As there is no specific cure or vaccine for SARS-Cov-2 infection, immune-modulating remedies could be reasonably employed. Probiotics are also frequently used to prevent respiratory infections, mainly in children with recurrent infections (11-13).

Therefore, the current survey demonstrated that an oral probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* subspecies *delbrueckii* LDD01 (200 million living cells), administered in patients with an upper respiratory infection for four weeks, was able to significantly reduce respiratory symptoms in comparison with the antibiotic therapy alone. It is conceivable that the present survey cannot be considered a formal investigative study. Consequently, further studies should be conducted by a rigorous methodology, such as designed according to placebo-controlled criteria. On the other hand, the strength of this survey is the considerable number of randomly enrolled patients and the real-world setting. The outcomes could, therefore, mirror the facts observable in clinical practice.

In conclusion, the current survey suggests that *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii* LDD01 may be considered an effective and safe therapeutic option in the management of patients with a respiratory infection and treated with antibiotics.

REFERENCES

1. Eckburg PB, Bik EM, Bernstein CN. Diversity of the human intestinal microbial flora. Science 2005; 308:1635-8.
2. Turnbaugh PJ, Ley RE, Hamady M. The human microbiome project. Nature 2007; 449:804-10.
3. Miraglia del Giudice M, Parisi GF, Indolfi C, Manti S, Leonardi S, Decimo F, et al. Nasal Microbiome in Chronic Rhinosinusitis. Minerva Ped 2020 (in press).
4. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci USA 2011; 108(S1):4554-61.
5. Ciprandi G, Aragona SE, Drago L, La Mantia I. The nutraceuticals: a new therapeutic strategy in the management of digestive and respiratory disorders. Acta Biomedica 2019; (7-S):5-7.
6. Varricchio A, La Mantia I, Brunese FP, Ciprandi G. Inflammation, infection, and allergy of upper airways: new insights from national and real-world studies. Ital J Pediatr 2020; 46:18.
7. He Y, Xu R, Wang W, Zhang J, Hu X. Probiotics, prebiotics, antibiotics, Chinese herbal medicine, and fecal microbiota transplantation in irritable bowel syndrome: Protocol for a systematic review and network meta-analysis. Medicine (Baltimore) 2020; 99(32):e21502.
8. Shahrokhi M, Nagalli S. Probiotics. StatPearls (Internet)2020.
9. Gaag EJV, Hummel TZ. Food or medication? The therapeutic effects of food on the duration and incidence of upper respiratory tract infections: A Review of the literature. Crit Rev Food Sci Nutr 2020; 1-14.
10. Sundararaman A, Ray M, Ravindra PV, Halami PM. Role of probiotics to combat viral infections with emphasis on COVID-19. Appl Microbiol Biotechnol 2020 (in press).
11. Clark SE. Clark SE. Commensal bacteria in the upper respiratory tract regulate susceptibility to infection. Curr Opin Immunol 2020; 66:42-9.
12. Santesso N. A Summary of a Cochrane Review: Probiotics to Prevent Acute Upper Respiratory Tract Infections. Glob Adv Health Med 2015; 4:18-9.
13. Ozen M, Kocabas Sandal G, Dinleyci EC. Probiotics for the prevention of pediatric upper respiratory tract infections: a systematic review. Expert Opin Biol Ther Expert Opin Biol Ther 2015; 15:9-20.