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Probiotics for the management of upper respiratory diseases: new insights

CONTENTS

Probiotics for the management of upper respiratory diseases

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Probiotics represent an intriguing challenge in clinical practice. They are currently used worldwide in all fields of Medicine. The present Supplement reports some Italian experiences concerning a probiotic mixture (Abincol®) employed in patients with upper respiratory diseases. A group of Italian otolaryngologists conducted these experiences in a real-world setting. The results demonstrated that this compound might represent a useful therapeutic option in clinical practice. In particularly, this probiotic mixture was tested in patients with rhinosinusitis, pharyngotonsillitis, otitis media, and laryngotracheitis.

About 100 trillion colonies of microorganisms inhabit various parts of the body, including the respiratory tract, the digestive tube, the skin, and other mucosae (1). These microbes are commensal microbiota and exist in different proportions, with diverse functions: developing and maintaining a healthy immune system and resisting colonization against pathogens (2). During childhood, the composition of commensal microbiota modifies over time, and adequate microbial exposure allows the physiological maturation of airways, intestine, and brain (3). The alterations of the equilibrium of the composition of commensal microbiota are defined dysbiosis. Dysbiosis can cause impairment of the immune system and promote an inflammatory response.

In this regard, upper respiratory diseases are increasing worldwide (4). Acute and chronic respiratory disorders represent a clinical challenge (5). High-fat, low-fiber diet and great antibiotic use are the leading causes of the increase is the dysbiosis. Consequently, restoration of dysbiosis using probiotics could be a fruitful intervention for upper respiratory diseases (6).

The inhibition of the growth of pathogenic bacteria is the most relevant mechanism of probiotic action (7). Other mechanisms of action include the antagonistic effects of probiotics, such as adhesion and co-aggregation ability, competition for binding sites and nutritional sources, secretion of antimicrobial substances, enhancement of intestinal barrier function by regulation of tight junctions and mucins expression, along with immunomodulation by interaction to receptors of microorganism-associated molecular patterns (8, 9). Moreover, production of short-chain fatty acids, such as butyrate, propionate, and acetate, derive from microbiota fermentation exerts immunoregulatory functions modulating extracellular and intracellular signaling molecules. They bind to cell surface G protein-coupled receptors, modulate the immune function indirectly, inhibit histone deacetylases, the cell interior, and regulate gene transcription in the immune response. They also promote B-cell differentiation and antibody production (10).

Microbiota metabolites are also essential amino acids, including tryptophan, which attenuates

Key words: Probiotics, upper airways, infection, inflammation, otolaryngologist, clinical experience

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TNF-α-induced activation of NF-κB and reduce expression of the proinflammatory chemokine. The intestinal microbiota can also synthesize vitamins from complex B, and vitamins play a vital role in regulating the immune system (11). The anti-inflammatory effects of probiotics entail significant increase in anti-inflammatory IL-10 production and decrease of proinflammatory cytokines IL-1β and IL-6 (12) Moreover, probiotics can decrease Type 3 polarization, favoring the differentiation of anti-inflammatory Treg/Type 1 regulatory intestinal T cell (13). Lactobacilli activate human dendritic cells and lead toward a Type 1 T-helper polarization, characterized by increased IFN-γ and IL12 production, against infections (14).

On the other hand, the gut-lung axis, such as the cross-talk between the intestinal tract and the lungs, involves continuous bidirectional communication *via* the blood system and lymphatic drainage, modulates the local immunity of both the gut and the lungs, as well as their respective microbial patterns and composition (15). This relevant concept is the basis for using oral probiotics also in upper airway infections (16). Based on this background, this Supplement reports some clinical experiences concerning the therapeutic effectiveness and safety of a nutraceutical compound, Abincol® (tested in patients with different upper respiratory diseases), in clinical practice. These experiences have been conducted on a group of Italian otolaryngologists.

Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (formerly *Lactobacillus plantarum*) (1 billion of living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million of living cells), and *Lactobacillus delbrueckii* subspecies *delbruekii* LDD01 (200 millions of living cells). The rationale for using this probiotic mixture is that probiotics are living microorganisms and confer a health benefit to the host when administered in adequate amounts. These probiotics produce microbial transformation in the intestinal microbiota and exert several health-promoting properties, including maintenance of the gut barrier function and modulation of the host immune system (17). Moreover, the effects of probiotic mixtures may be complementary (also referred to as additive) or synergistic (18). These probiotic strains produce growth factors that strengthen the gut epithelium and antimicrobial-anti-inflammatory mediators (short-chain fatty acids, bacteriocins, hydroperoxides, bile acids, and lactic acids), killing harmful microorganisms (19).

Consequently, their cellular components are released in the gut environment, activating immune responses by modulating pro-inflammatory cytokine production and immunoglobulin synthesis, besides improving macrophage and lymphocyte activity (20). Also, non-immunological benefits associated with these probiotics include the digestion and absorption processes, competition with potential pathogens for nutrients and intestinal adhesion sites, pH alterations, agglutination of pathogenic microorganisms, and sequestration of metabolic toxins (21). Animal models and *in vitro* assays described that these probiotics also decrease the apoptosis, increase the mucus synthesis, tissue repair, redistribution, and production of tight junctions in gut epithelial cells, thus reducing the intestinal permeability and enhancing the barrier protection and function (22). However, it must underline that the underlying mechanisms of probiotics are dependent on the specific microbial strain, and the effectiveness is also disease specific. Thus, the probiotic choice should be carefully oriented to a specific strain in a particular disease.

Abincol® has been fruitfully used in patients with chronic intestinal diseases (23), in patients undergoing bowel preparation (24), and in post-surgical intestinal dysbiosis (25). Therefore, the current experiences considered the evaluation of doctors' point of view in managing patients with upper respiratory diseases, and the point of view of patients with rhinosinusitis, pharyngotonsillitis, and otitis media and laryngotracheitis. The present outcomes have clinical relevance as they were obtained in real-world settings. There are also some implications considering the close link between airways and gut. Therefore, these probiotics may represent a reliable therapeutic option in clinical practice.

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

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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.

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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

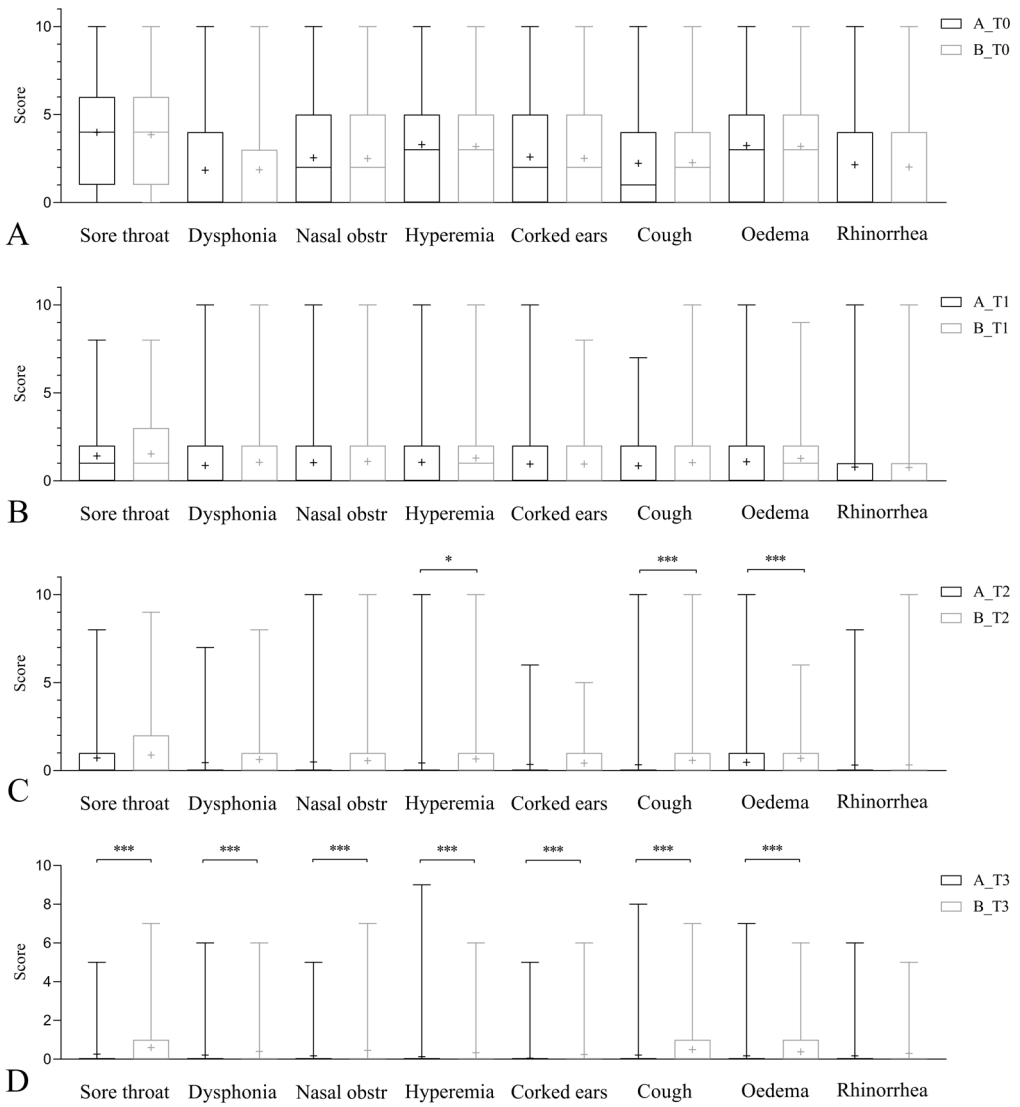

Fig. 1. *Comparison of symptoms between group A (active) and B (control) in the four times. The data are represented by boxplot, the horizontal line represents the median, while the mean is indicated with the sign "+". (* p-value: 0.05-0.01; ** p-value: <0.01; *** p value: <0.001).*

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.

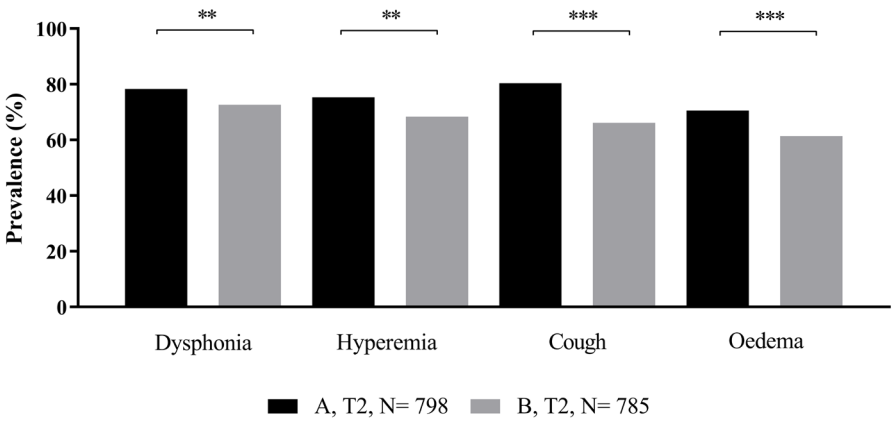

Fig. 2. *Comparison of patients' percentage without a symptom between group A (active) and B (control) at T2. (* p value: 0.05-0.01; ** p value: <0.01; *** p value: <0.001).*

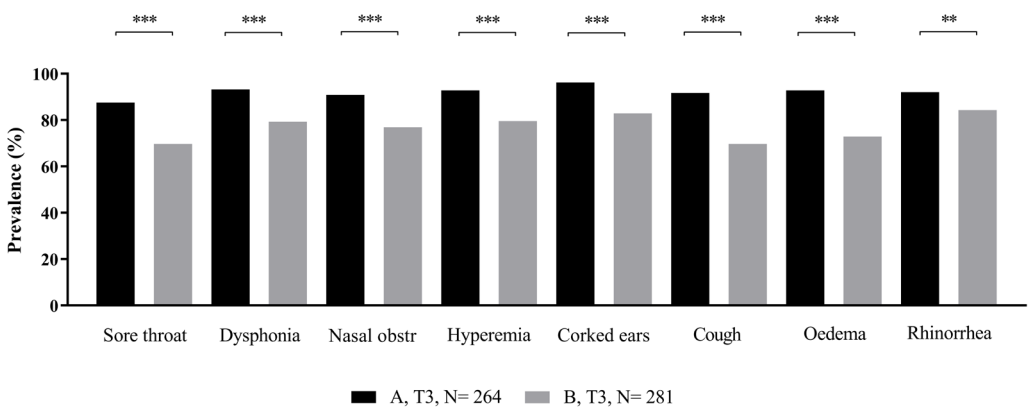

Fig. 3. *Comparison of patients' percentage without a symptom between group A (active) and B (control) at T3. (* p value: 0.05-0.01; ** p value: <0.01; *** p value: <0.001).*

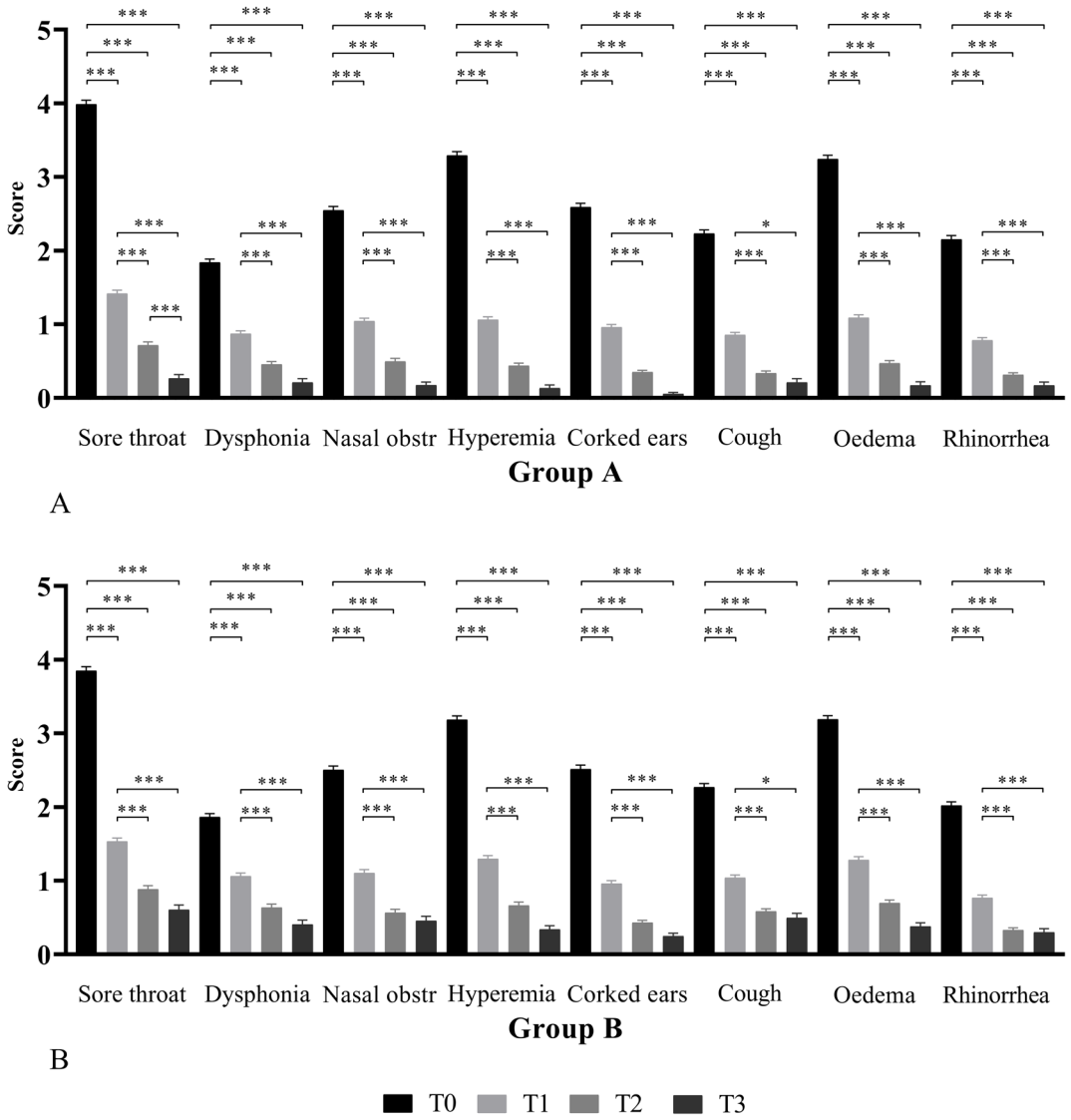

Fig. 4. *Intragroup analysis of all symptoms in the four times in group A and B. (* p value: 0.05-0.01; ** p value: <0.01; *** p value: <0.001).*

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.

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Probiotics in the add-on treatment of pharyngotonsillitis: a clinical experience

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Pharyngotonsillitis is a common disease, mainly characterized by a sore throat. It may be classified as acute or chronic, based on duration. The diagnosis is usually performed on the clinical ground, and antibiotic therapy is frequently used in clinical practice. However, antibiotics frequently induce intestinal dysbiosis associated with some clinical problems. Therefore, probiotics are commonly prescribed in patients treated with antibiotics. The current clinical experience was conducted in patients with pharyngotonsillitis and treated with antibiotics. A one-month course of a probiotic mixture (Abincol® containing *Lactobacillus plantarum* LP01 (1 billion of living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million of living cells), and *Lactobacillus delbrueckii subspecies delbrueckii* LDD01 (200 million of living cells), was prescribed in the Group A, and was compared with no add-on treatment, such as the Group B. Patients were evaluated at baseline (T0), at the end of antibiotic treatment (T1), at the end of probiotic course (T2), and at the end of 3-month follow-up (T3). Globally, 1118 outpatients were enrolled. Acute pharyngotonsillitis affected 795 subjects: 396 in Group A and 399 in Group B. Chronic pharyngotonsillitis affected 323 outpatients: 158 in Group A and 165 in Group B. All patients were usually treated with a 7-10-day course of antibiotic therapy. In patients with acute pharyngotonsillitis, the probiotic mixture significantly reduced the duration of all the symptoms ($p<0.001$ for all), except

Key words: pharyngotonsillitis, acute, chronic, antibiotic therapy, dysbiosis, probiotics

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for the urinary tract infection, associated with antibiotic therapy which was already at the end of the antibiotic cycle (T1). The intergroup analysis showed that patients with chronic pharyngotonsillitis in Group A had significantly less tiredness, pain, and malaise ($p<0.001$ for all) than patients in Group B at T1. The probiotic course reduced the possible clinical relapse, and the use of additional medications at T2 and T3 in patients with both acute and chronic pharyngotonsillitis. In conclusion, the present clinical experience demonstrated that a probiotic mixture containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii*, was able to quickly reduce symptoms, possible relapse, and use of additional medications, associated with antibiotic therapy, in patients with both acute and chronic pharyngotonsillitis.

Pharyngitis is clinically described as a sore throat and represents the inflammation of the posterior oropharynx. Bacteria are responsible for a substantial portion of pharyngeal infections, and these infections present differently, have different complications, and require different treatment (1). Namely, identifying bacterial pharyngitis is clinically relevant to treat the infection properly (2). On the other hand, the palatine tonsils are an anatomic structure positioned in the pharynx and are composed of lymphatic tissue as components of the Waldeyer's ring along with the adenoids (nasopharyngeal tonsil), tubal, and lingual tonsil. These structures serve as an essential defense against inhaled or ingested pathogens by providing the initial immunological response. If defensive mechanisms defect, tonsillitis occurs. Tonsillitis is predominantly the result of a viral or bacterial infection and, when uncomplicated, presents a sore throat (3). Acute tonsillitis is a clinical diagnosis. Differentiation between bacterial and viral causes can be difficult. Consequently, antibiotics are overused in clinical practice. As tonsillitis and pharyngitis are very frequently associated, the term pharyngotonsillitis is used in everyday practice.

Pharyngotonsillitis may be acute and chronic, and the distinction is based on the disease duration: if it lasts < one month, it is acute (4, 5). The etiology may be viral or bacterial. Acute viral pharyngotonsillitis is shared, the acute bacterial form is widespread, and group A beta-hemolytic *Streptococcus* (GABHS) is commonly referred to as strep throat (6). Patients experiencing sore throat are frequent in medical offices, emergency departments, and urgent care centers (7). As complications are feared, antibiotics are widely prescribed in patients with pharyngotonsillitis (8). However, antibiotic treatment is frequently associated with microbiota perturbation, such as dysbiosis. To restore the physiological balance of microbiota, probiotics are usually prescribed along with antibiotics in clinical practice.

Probiotics could improve intestinal microbiota equilibrium, reduce respiratory symptoms, and aid general wellbeing (9). Moreover, thanks to the gut-lung axis, oral probiotics could affect also respiratory microbiota (9). In this regard, 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.

This product has been previously tested in patients with gastroenterological disorders (10-12). Therefore, the present clinical experience evaluated the potential role of add-on treatment to antibiotic therapy in patients with pharyngotonsillitis recruited by a group of Italian otorhinolaryngologists (ORL) in clinical practice.

MATERIALS AND METHODS

The current experience was conducted in Italian otorhinolaryngology centres, distributed across Italy, ensuring an extensive and complete national coverage, during the fall-winter 2019-2020. ORL specialists were asked to recruit all consecutive outpatients with pharyngotonsillitis. Patients were consecutively recruited during the specialist visit. The inclusion criteria were to

have pharyngotonsillitis, 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 a real-world setting. Pharyngotonsillitis was diagnosed on a clinical ground according to validated criteria (13). The patients were randomly subdivided into 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. Patients were further subdivided in two subgroups considering the acute and chronic pharyngotonsillitis diagnosis.

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 gave the patients a clinical diary to record clinical information.

In the first period (7-10 days), patients had to report the duration (expressed in days) of symptoms, including fever, tiredness, headache, pain, malaise, diarrhea, urinary tract problems, and nausea. In the second (first month) and third (3-month follow-up) periods, patients had to report clinical relapse, use of concomitant medications, and adverse events. 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). For all tests, the accepted minimum significance limit was 0.05.

RESULTS

Globally, 1118 outpatients were enrolled. Acute pharyngotonsillitis affected 795 subjects: 396 in Group A and 399 in Group B. Chronic pharyngotonsillitis affected 323 outpatients: 158 in Group A and 165 in Group B.

Acute Pharyngotonsillitis

The intergroup analysis showed that all reported symptoms mainly diminished in Group A at T1, as reported in Fig. 1. In particular, patients in the Group A had 29% fewer days with fever, 33% fewer days with tiredness, 26% fewer days with headache, 28% fewer days with pain, 30% fewer days with malaise, 20% fewer days with diarrhea, 13% fewer days with

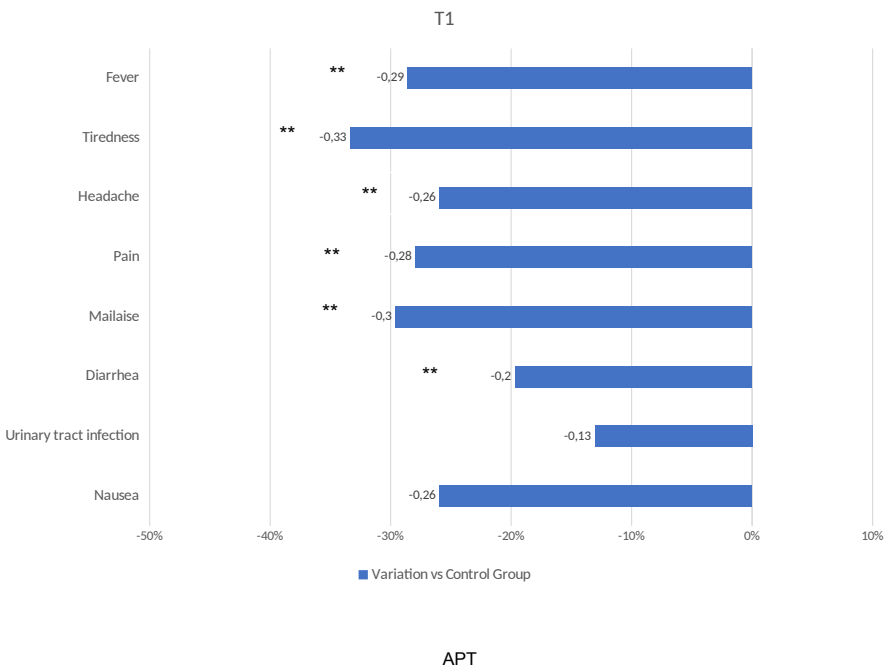

Fig. 1 *Percentage of difference between Group A and Group B concerning the evaluated symptoms in patients with acute pharyngotonsillitis at T1.*

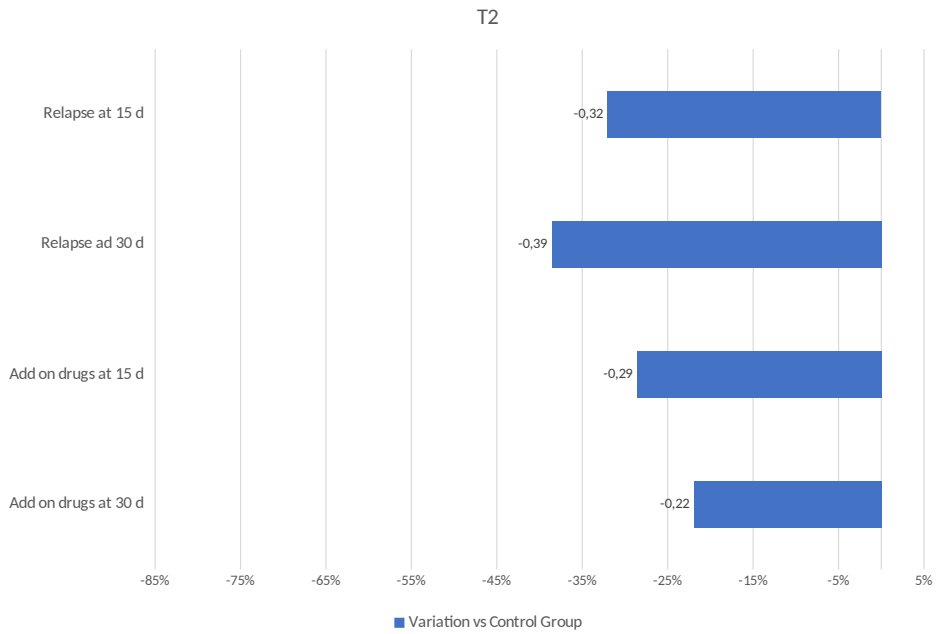

Fig. 2 *Percentage of difference between Group A and Group B concerning the clinical relapse and the use of additional medications in patients with acute pharyngotonsillitis at T2.*

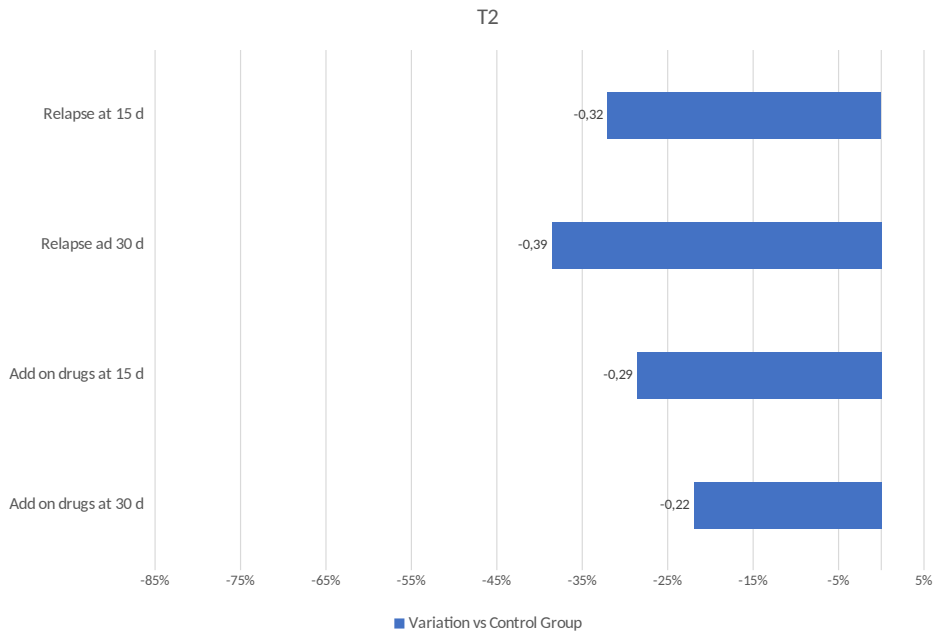

Fig. 3 *Percentage of difference between Group A and Group B concerning the clinical relapse and the use of additional medications in patients with acute pharyngotonsillitis at T3.*

urinary tract problems, and 26% fewer days with nausea than Group B patients (Fig 1).

At T2, patients in Group A had 32% fewer episodes of clinical relapse at 15 days, 39% fewer episodes of clinical relapse between 16 and 30 days, 29% less use of additional drugs at 15 days, and 22% less use of additional drugs between 16 and 30 days than patients belonging to Group B (Fig. 2).

At T3, patients in Group A had 29% fewer episodes of clinical relapse between 30 and 75 days, 52% fewer episodes of clinical relapse between 75 and 120 days, 27% less use of additional drugs between 30 and 75 days, and 45% less use of additional drugs between 75 and 120 days than patients belonging to Group B (Fig. 3). The intergroup analysis showed that patients in Group A had significantly less fever, tiredness, headache, pain, malaise, diarrhea, and nausea ($p<0.001$ for all) than patients in Group B at T1.

Chronic Pharyngotonsillitis

The intergroup analysis showed that all reported symptoms mainly diminished in Group A at T1, as reported in Fig. 4. In particular, patients in the Group A had 4% fewer days with fever, 33% fewer days with tiredness, 14% fewer days with headache, 16% fewer days with pain, 19% fewer days with malaise, 11% fewer days with diarrhea, and 8% fewer days with nausea than Group B patients (Fig. 4).

At T2, patients in Group A had 31% fewer episodes of clinical relapse at 15 days, 41% fewer episodes of clinical relapse between 16 and 30 days, 22% less use of additional drugs at 15 days, and 32% less use of additional drugs between 16 and 30 days than patients belonging to Group B (Fig. 5). At T3, patients in Group A had 40% fewer episodes of clinical relapse between 30 and 75 days, 61% fewer episodes of clinical relapse between 75 and 120 days, 37% less use of additional drugs between 30 and 75 days, and 58% less use of additional drugs between 75 and 120 days than patients belonging to Group B (Fig. 6). The intergroup analysis showed that patients in Group A had significantly less tiredness, pain, and malaise ($p<0.001$ for all) than patients in Group B at T1. The nutraceutical compound was well-tolerated in all subjects.

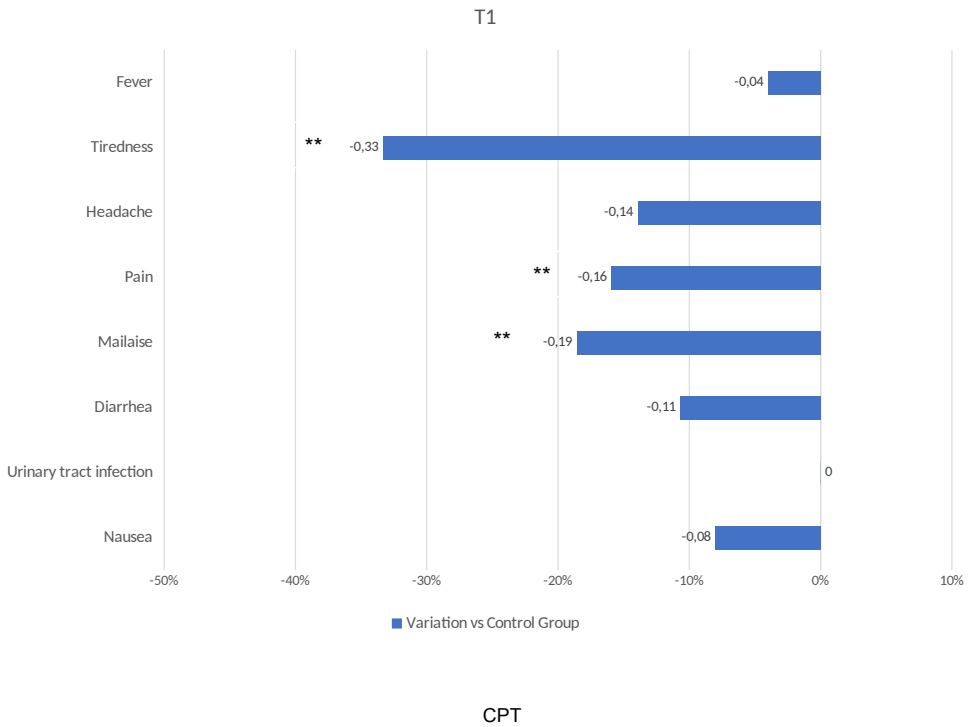

Fig. 4 *Percentage of difference between Group A and Group B concerning the evaluated symptoms in patients with chronic pharyngotonsillitis at T1.*

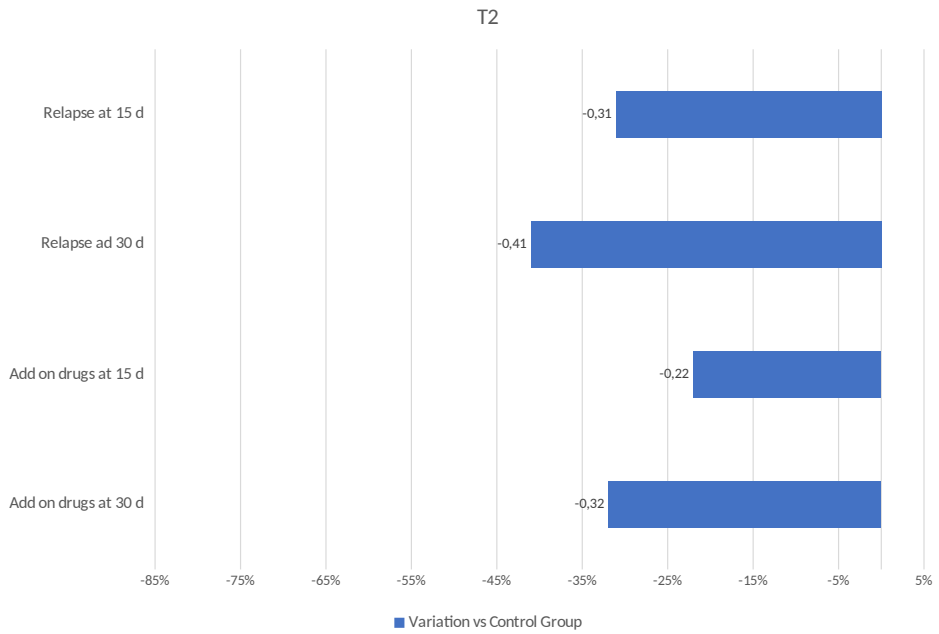

Fig. 5 *Percentage of difference between Group A and Group B concerning the clinical relapse and the use of additional medications in patients with chronic pharyngotonsillitis at T2.*

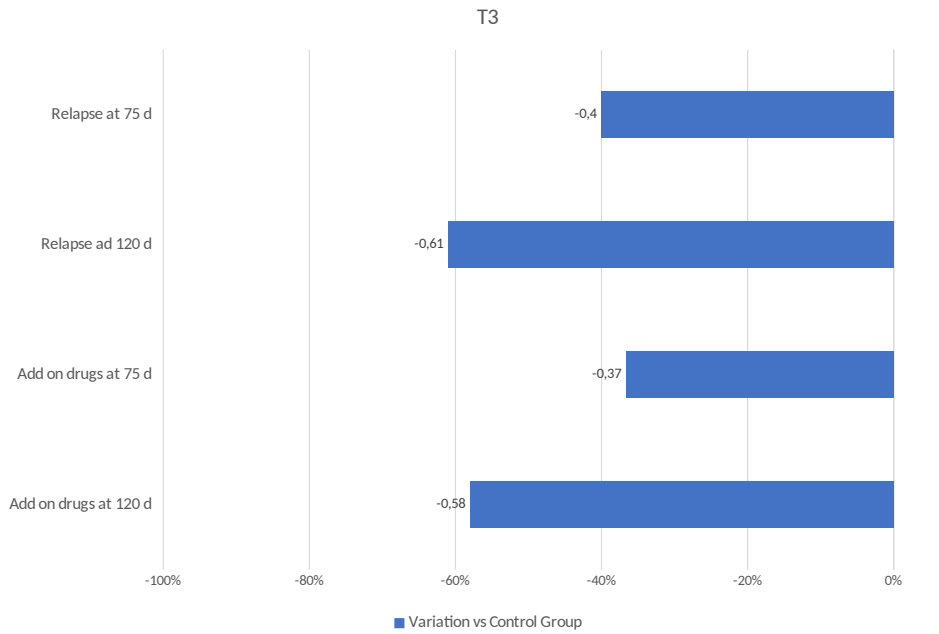

Fig. 6 *Percentage of difference between Group A and Group B concerning the clinical relapse and the use of additional medications in patients with chronic pharyngotonsillitis at T3.*

DISCUSSION

Patients with pharyngotonsillitis usually present sore throat. Even though a myriad of diagnosis is possible, the infectious form is the most common, mainly in the acute presentation. The diagnosis is based on the clinical ground, and antibiotic therapy is frequently prescribed. However, antibiotic treatment may frequently cause dysbiosis, both at the intestinal and respiratory levels. Consequently, the use of probiotics, to restore a physiological microbiota, has become a widespread practice.

On the other hand, a considerable number of probiotics are available on the market. Unfortunately, most of them lack adequate scientific documentation, or only in vitro studies are available. In this regard, Abincol® is an oral nutraceutical containing a probiotic mixture with *Lactobacillus plantarum* LP01 (1 billion of living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million of living cells), and *Lactobacillus delbrueckii subspecies delbrueckii* LDD01 (200 million of living cells). Some clinical studies showed that Abincol® improved symptom severity in patients with intestinal problems (10-12).

The current experience was conducted on a large group of patients with acute pharyngotonsillitis and a small number of patients with chronic pharyngotonsillitis. The outcomes showed that the probiotic mixture reduced the intensity of symptoms during the antibiotic treatment (7-10 days), the probiotic course (one month), and the 90-day follow-up. Patients with both acute and chronic pharyngotonsillitis had a clinically relevant improvement. Moreover, the probiotic mixture reduced the frequency of clinical relapse and the use of additional medications for both acute and chronic pharyngotonsillitis.

The current experience has some limitations: the most relevant was the open design. However, the strength of the current experience was the many randomly recruited outpatients, mainly concerning the acute pharyngotonsillitis. Further rigorous studies, such as placebo-controlled, should be conducted to confirm these preliminary findings.

In conclusion, the present clinical experience demonstrated that a probiotic mixture containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii*, was able to significantly reduce symptoms associated with antibiotic therapy in patients with both acute and chronic pharyngotonsillitis already during the antibiotic treatment.

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Probiotics in the add-on treatment of otitis media in clinical practice

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Otitis media (OM) affects the middle ear and is typically characterized by earache. OM may be classified as acute (AOM) or chronic (COM), based on symptom duration. OM may be clinically suspected, but the diagnosis is usually confirmed by the otoscopy. Antibiotic therapy is frequently used in clinical practice. However, antibiotics often induce intestinal and respiratory dysbiosis associated with some clinical problems. A one-month course of a probiotic mixture (Abincol® containing *Lactobacillus plantarum* LP01 (1 billion of living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* LDD01 (200 million living cells), was prescribed in the Group A, and was compared with no add-on treatment, such as the Group B. Patients were evaluated at baseline (T0), at the end of antibiotic treatment (T1), at the end of probiotic course (T2), and at the end of 3-month follow-up (T3).

In total, 1164 outpatients with OM were enrolled. Acute OM affected 1058 subjects: 563 in Group A and 495 in Group B. Chronic OM affected 106 outpatients: 49 in Group A and 57 in Group B. All of them were treated with a 7-10-day course of antibiotic therapy. The probiotic mixture reduced the duration of symptoms associated with antibiotic therapy already at the end of the antibiotic cycle. Group A patients with acute otitis media experienced significantly less fever, tiredness, headache, pain,

Key words: otitis media, acute, chronic, antibiotic therapy, dysbiosis, probiotics.

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malaise, nausea ($p<0.001$ for all), and diarrhea ($p=0.023$) than patients in Group B at T1. Group A patients with chronic otitis media experienced less tiredness ($p<0.01$) and malaise ($p<0.001$) than patients in Group B at T1.

The probiotic course reduced the possible clinical relapse, and the use of additional medications in both diseases at T2 and T3. In conclusion, the present clinical experience demonstrated that a probiotic mixture containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii*, was able to reduce symptoms associated with antibiotic therapy in patients with both acute and chronic OM. Otitis media (OM) is an inflammation of the middle ear (1).

However, the term OM is an umbrella term that comprises three main, distinct conditions: acute OM (AOM), OM with effusion (OME), and chronic OM (COM). These conditions, even though different, are, however, closely related and can also overlap (2-4). OM is one of the most common diseases in young children (5, 6). OM is also a leading cause of medical consultation, antibiotic prescription, and surgery, mainly in young people (7-10).

On the other hand, OM may also affect adult subjects, frequently after an upper airway infection. It entails a relevant burden for healthcare resources and a negative impact on both patients and their families' quality of life. From a practical point of view, there is a need to use clear and unambiguous terms. Literature abounds with misleading terms that significantly affect the methodologic rigor and consequently, the outcomes. Moreover, it is essential to differentiate patients according to the OM type (i.e. AOM, OME, and COM).

Bacteria may infect the middle ear primitively or successively to a viral infection. OM can be a severe condition that should be promptly treated. Therefore, antibiotics are commonly used in daily practice to treat AOM or COM when a bacterial cause is suspected (11). On the other hand, antibiotic treatment is frequently associated with microbiota perturbation, such as dysbiosis. To restore the physiological balance of microbiota, probiotics are usually prescribed along with antibiotics in clinical practice.

Probiotics could improve intestinal and respiratory microbiota equilibrium, reduce respiratory symptoms, and promote general wellbeing (12). In this regard, 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. This product has been previously tested in patients with gastroenterological disorders (13-15). Therefore, the present clinical experience evaluated the potential role of probiotic add-on treatment to antibiotic therapy in patients with otitis media, recruited by a group of Italian otorhinolaryngologists (ORL) in clinical practice.

MATERIALS AND METHODS

The current experience was conducted in Italian otorhinolaryngology centres, distributed across Italy, so assuring an extensive and complete national coverage, during the fall-winter 2019-2020. ORL specialists were asked to recruit all consecutive outpatients with otitis media. Patients were consecutively recruited during the specialist visit. The inclusion criteria were to have otitis media diagnosis, 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 a real-world setting.

OM was diagnosed on a clinical ground according to validated criteria (16). The patients were subdivided into 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. Patients were also subdivided into two subgroups considering the acute or chronic OM diagnosis. 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 gave the patients a clinical diary to record clinical information. In the first period (7-10 days), patients had to report the duration (expressed in days) of symptoms, including fever, tiredness, headache, pain, malaise, diarrhea, urinary tract problems, and nausea. In the second (first month) and third (3-month follow-up) periods, patients had to report clinical relapse, use of concomitant medications, and adverse events. 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). For all tests, the accepted minimum significance limit was 0.05.

RESULTS

Globally, 1164 outpatients were enrolled. Acute OM affected 1058 subjects: 563 in Group A and 495 in Group B. Chronic OM affected 106 outpatients: 49 in Group A and 57 in Group B.

Acute OM

The intergroup analysis showed that all reported symptoms mainly diminished in Group A at T1, as reported in Fig. 1. In particular, patients in the Group A had 35% fewer days with fever, 33% fewer days with tiredness, 26% fewer days with headache, 26% fewer days with pain, 29% fewer days with malaise, 11% fewer days with diarrhea, and 13% fewer days with urinary tract infection, and 41% fewer days with nausea than Group B patients (Fig. 1).

At T2, patients in Group A had 28% fewer episodes of clinical relapse at 15 days, 31% fewer episodes of clinical relapse between 16 and 30 days, 29% less use of additional drugs at 15 days, and 23% less use of additional medications between 16 and 30 days than patients belonging to Group B (Fig. 2).

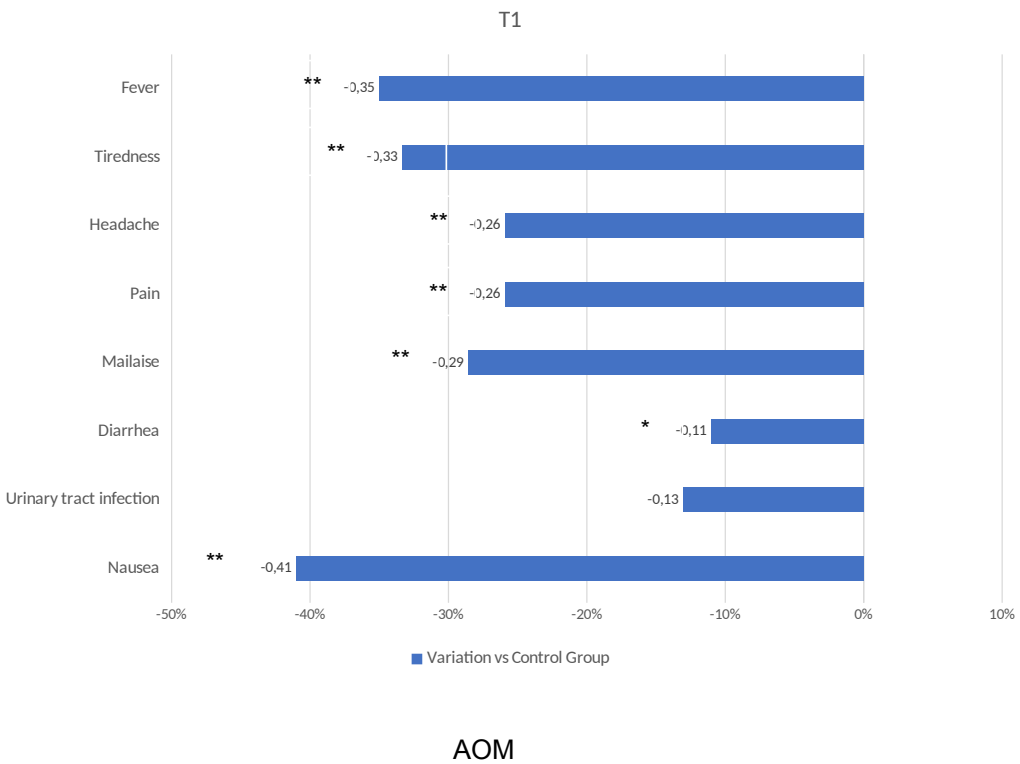

Fig. 1. *Percentage of difference between Group A and Group B concerning the evaluated symptoms in patients with acute otitis media at T1.*

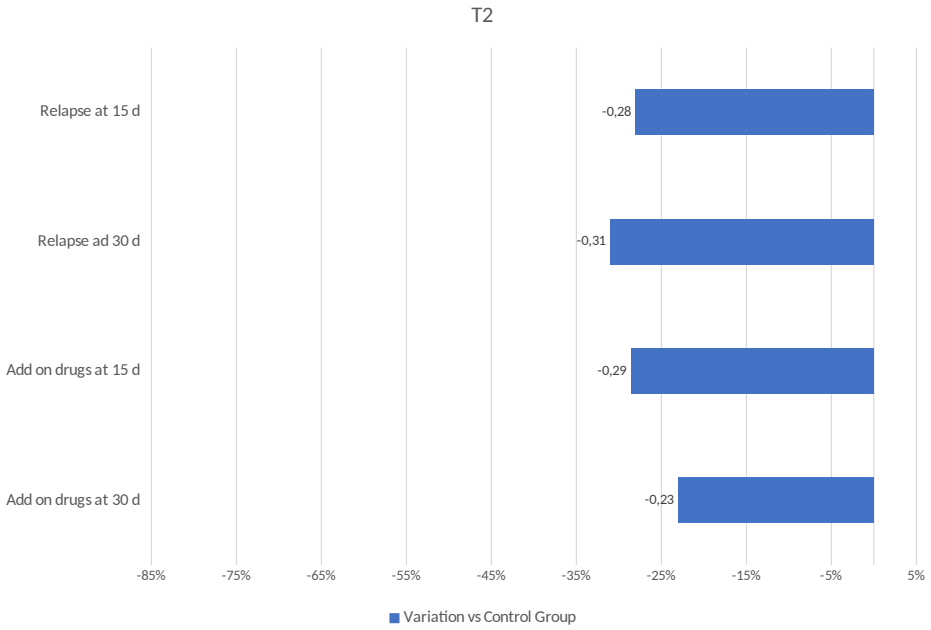

Fig. 2. *Percentage of difference between Group A and Group B concerning the clinical relapse and additional medications in patients with acute otitis media at T2.*

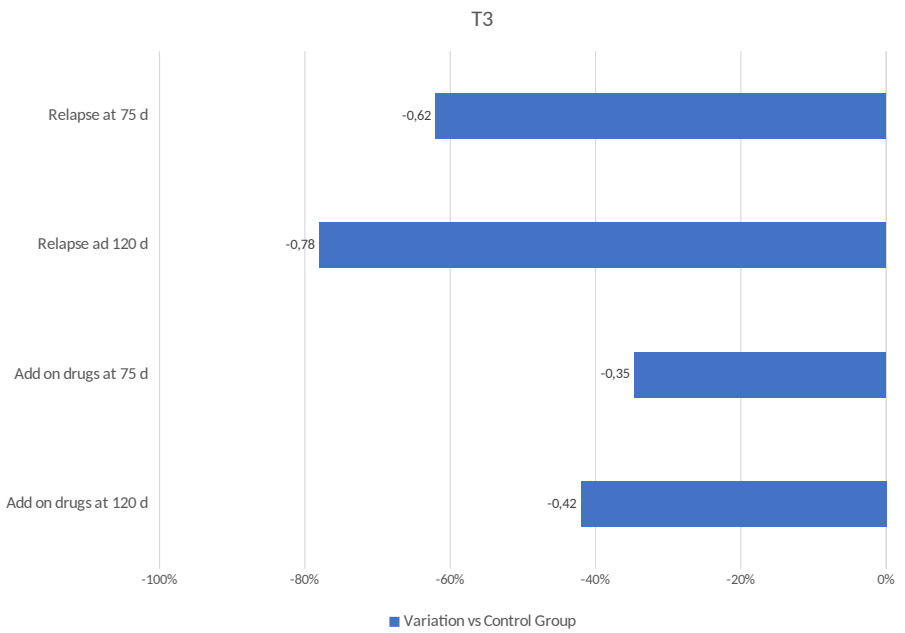

Fig. 3. *Percentage of difference between Group A and Group B concerning the clinical relapse and additional medications in patients with acute otitis media at T3.*

At T3, patients in Group A had 62% fewer episodes of clinical relapse between 30 and 75 days, 78% fewer episodes of clinical relapse between 75 and 120 days, 35% less use of additional drugs between 30 and 75 days, and 42% less use of additional medications between 75 and 120 days than patients belonging to Group B (Fig. 3).

The intergroup analysis showed that the Group A had a significantly greater reduction of fever ($p<0.001$), tiredness ($p<0.001$), headache ($p<0.001$), pain ($p<0.001$), malaise ($p<0.001$), diarrhea ($p=0.023$), and nausea ($p<0.001$) than Group B at T1.

Chronic OM

The intergroup analysis showed that all reported symptoms mainly diminished in Group A at T1, as reported in Fig. 4. In particular, patients in the Group A had 9% fewer days with fever, 16% fewer days with tiredness, 10% fewer days with headache, 13% fewer days with pain, 39% fewer days with malaise, 9% fewer days with diarrhea, 2% fewer days with urinary tract infection, and 22% fewer days with nausea than Group B patients (Fig. 4).

At T2, patients in Group A had 31% fewer episodes of clinical relapse at 15 days, 45% fewer episodes of clinical relapse between 16 and 30 days, 13% less use of additional drugs at 15 days, and 9% less use of additional medications between 16 and 30 days than patients belonging to Group B (Fig. 5).

At T3, patients in Group A had 61% fewer episodes of clinical relapse between 30 and 75 days, 73% fewer episodes of clinical relapse between 75 and 120 days, 49% less use of additional drugs between 30 and 75 days, and 60% less use of additional medications between 75 and 120 days than patients belonging to Group B (Fig 6). The intergroup analysis showed that the Group A had a significantly greater reduction of tiredness ($p<0.01$), and malaise ($p<0.001$) than Group B at T1. The nutraceutical compound was well-tolerated in all subjects.

DISCUSSION

Even though otitis media frequently affects

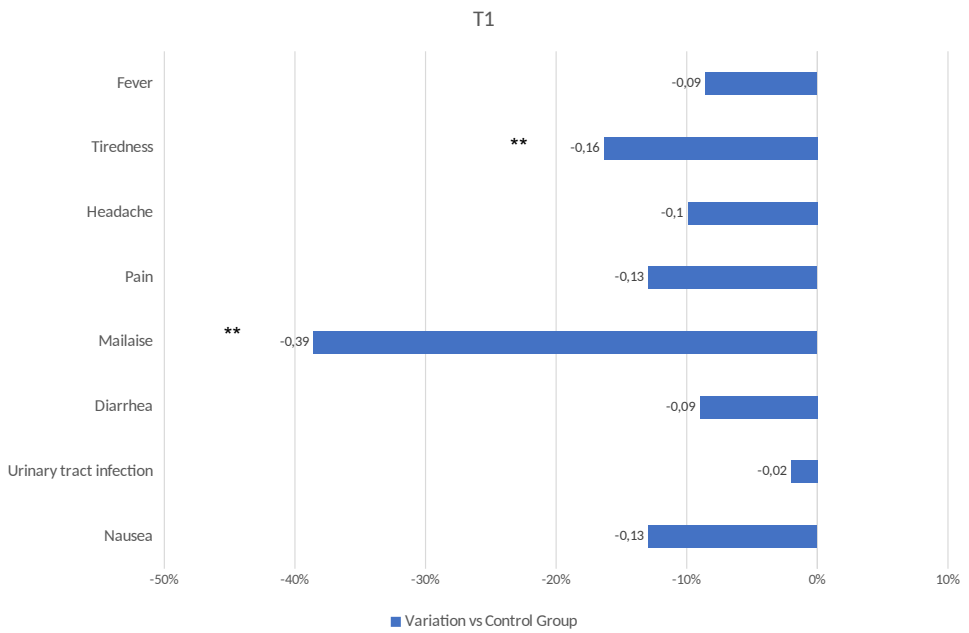

Fig. 4. *Percentage of difference between Group A and Group B concerning the evaluated symptoms in patients with chronic otitis media at T1.*

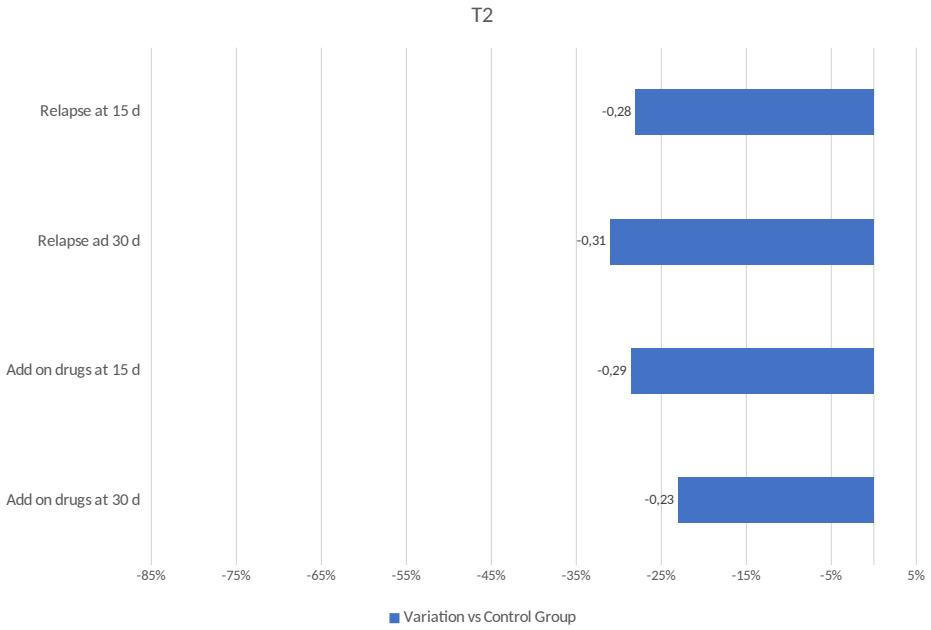

Fig. 5. *Percentage of difference between Group A and Group B concerning the clinical relapse and additional medications in patients with chronic otitis media at T2.*

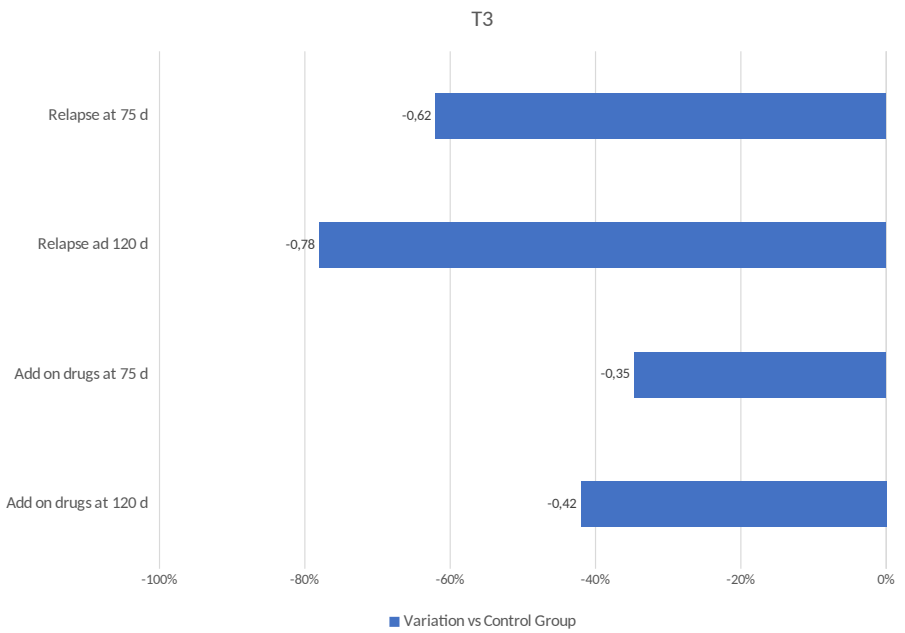

Fig. 6. *Percentage of difference between Group A and Group B concerning the clinical relapse and additional medications in patients with chronic otitis media at T3.*

young people, it is quite common also in adults. AOM represents a common problem facing general practitioners, paediatricians, and otolaryngologists. Acute OM usually follows an acute respiratory infection, mainly caused by viral or bacterial pathogens (17). AOM places a large burden on health care systems worldwide. Although symptomatic relief could be enough for some patients, other cases require antibiotics, especially if complications are suspected.

COM is also a common problem facing general practitioners and otolaryngologists (18). COM most commonly presents with painless otorrhoea and hearing loss. Treatment options vary according to the activity and type of disease encountered. COM carries significant patient morbidity and is characterized by an inflammatory process potentially associated with an infectious episode (18).

In both acute and chronic OM, the diagnosis is based on clinical features and otoscopic outcomes. When an infection is suspected, clinicians prescribe antibiotic treatment. However, antibiotics may frequently cause dysbiosis, both at the intestinal and respiratory levels. Consequently, the use of probiotics has become a common practice to contrast dysbiosis.

In the vast panorama of probiotic products, existing on the market, Abincol® is an oral nutraceutical containing a 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). Some clinical studies showed that Abincol® improved symptom severity in patients with different intestinal problems (13-15). The current experience was conducted on a large group of patients with acute or chronic OM. The outcomes showed that the probiotic mixture reduced the intensity of symptoms during the antibiotic treatment (7-10 days), the probiotic course (one month), and the 90-day follow-up. Patients with both acute and chronic OM had an improvement. Moreover, the probiotic mixture reduced the frequency of clinical relapse and additional medications for both acute and chronic OM.

The current experience has some limitations, and the most relevant was the open design. However, the strength of the present experience was many randomly recruited outpatients, mainly concerning the acute pharyngotonsillitis. Further rigorous studies, such as placebo-controlled, should be conducted to confirm these preliminary findings. In conclusion, the present clinical experience demonstrated that a probiotic mixture containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii*, was able to reduce symptoms associated with antibiotic therapy in patients with both acute and chronic OM.

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Probiotics in the add-on treatment of rhinosinusitis: a clinical experience

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Rhinosinusitis (RS) affects the nose and the paranasal sinus and is characterized by nasal and systemic symptoms. It may be classified as acute or chronic, based on duration. Rhinosinusitis may be clinically suspected, but the diagnosis is usually based on the endoscopy. Antibiotic therapy is frequently used for RS patients in clinical practice. However, antibiotics often induce intestinal dysbiosis associated with some clinical problems and respiratory microbiota impairment. The current clinical experience was conducted in patients with pharyngotonsillitis and treated with antibiotics. A one-month course of a probiotic mixture (Abincol® containing *Lactobacillus plantarum* LP01 (1 billion of living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* LDD01 (200 million living cells), was prescribed in the Group A, and was compared with no add-on treatment, such as the Group B. Patients were evaluated at baseline (T0), at the end of antibiotic treatment (T1), at the end of probiotic course (T2), and at the end of 3-month follow-up (T3).

Globally, 789 outpatients were enrolled. Acute rhinosinusitis (ARS) affected 726 subjects: 371 in Group A and 355 in Group B. Chronic rhinosinusitis (CRS) affected 63 outpatients: 32 in Group A and 31 in Group B. All of them were treated with a 7-10-day course of antibiotic therapy. The probiotic mixture

Key words: rhinosinusitis, acute, chronic, antibiotic therapy, dysbiosis, probiotics

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reduced the duration of symptoms associated with antibiotic therapy already at the end of the antibiotic cycle. ARS patients treated with probiotics experienced less fever, tiredness, headache, pain, malaise, and diarrhea ($p<0.001$ for all) than control patients at T1. CRS patients treated with probiotics experienced less tiredness, headache, pain, and malaise ($p<0.001$ for all) than control patients at T1.

The probiotic course reduced the possible clinical relapse, and the use of additional medications in both diseases at T2 and T3. In conclusion, the present clinical experience demonstrated that a probiotic mixture containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii*, was able to quickly reduce symptoms associated with antibiotic therapy in patients with both acute and chronic rhinosinusitis.

The term rhinosinusitis (RS) is a compound word that includes two diseases, such as rhinitis and sinusitis. Rhinitis is the inflammation of the nasal mucosa; sinusitis is the inflammatory process involving the paranasal sinus. As the sinusitis is very frequently associated with rhinitis, it was considered appropriate to coin the new term rhinosinusitis instead of sinusitis (1).

Rhinosinusitis may be acute and chronic, and the distinction is based on the disease duration: if RS lasts < one month, RS is acute (2). Acute rhinosinusitis (ARS) accounts for approximately 30 million primary care visits and $11 billion in US healthcare expenditure annually (3). It is also a common reason for antibiotic prescriptions worldwide. Due to recent guidelines and concerns for antibiotic resistance and the judicious use of antibiotics, it is essential to perform a correct diagnosis (4, 5).

Chronic rhinosinusitis (CRS) affects about 10-12% of the European population (1). Chronic rhinosinusitis (CRS) consists of two main phenotypes based on endoscopy and computed tomography (CT) findings: CRS with nasal polyposis (CRSwNP) and CRS without nasal polyposis (CRSsNP) (6). CRSwNP is defined by the presence of nasal polyps and signs and symptoms lasting longer than 8-12 weeks (7). From a pathophysiological point of view, nasal polyps are benign edematous masses in the nasal and paranasal cavities. Their occurrence depends on an exaggerated inflammatory reaction. As nasal polyps occupy space into nasal cavities, they can cause nasal obstruction, rhinorrhea, postnasal drip, and hypo- or anosmia (8).

The CRSwNP overall prevalence is approximately estimated to be 2% to 4% of the general population. In adolescence, the CRSwNP is undoubtedly lower than in older subjects, even though exact data are not available. On the other hand, the adolescent is an "at-risk" patient as emotional problems frequently affect this age, and the severity of the disease may also depend on low adherence to treatment. Treatment options for CRSwNP consist of local or systemic corticosteroids as the first-line choice. If ineffective, there is a need for functional endoscopic sinus surgery. However, CRS patients frequently experience symptoms attributable to an infectious form.

Thus, antibiotics are commonly prescribed by doctors for patients with ARS or CRS with suspected infectious relapse. However, antibiotic treatment is commonly associated with microbiota perturbation, such as dysbiosis. To restore the physiological balance of microbiota, probiotics are usually prescribed in clinical practice.

Probiotics could improve intestinal and respiratory microbiota equilibrium, reduce respiratory symptoms, and induce a general wellbeing (9). In this regard, 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. This product has been previously tested in patients with gastroenterological disorders (10-12). Therefore, the present clinical experience evaluated the potential role of add-on treatment to antibiotic therapy in patients with rhinosinusitis recruited by a group of Italian otorhinolaryngologists (ORL) in clinical practice.

MATERIALS AND METHODS

The recent experience was conducted in Italian otorhinolaryngology centres, distributed across Italy, so

assuring an extensive and complete national coverage, during the fall-winter 2019-2020. ORL specialists were asked to recruit all consecutive outpatients with rhinosinusitis. Patients were consecutively recruited during the specialist visit. The inclusion criteria were to have rhinosinusitis diagnosis, 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 a real-world setting. Rhinosinusitis was diagnosed on a clinical ground according to validated criteria (13). The patients were randomly subdivided into 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. Patients were also subdivided into two subgroups, considering the acute or chronic rhinosinusitis diagnosis.

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 gave the patients a clinical diary to record clinical information.

In the first period (7-10 days), patients had to report the duration (expressed in days) of symptoms, including fever, tiredness, headache, pain, malaise, diarrhea, urinary tract problems, and nausea. In the second (first month) and third (3-month follow-up) periods, patients had to report clinical relapse, use of concomitant medications, and adverse events. 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). For all tests, the accepted minimum significance limit was 0.05.

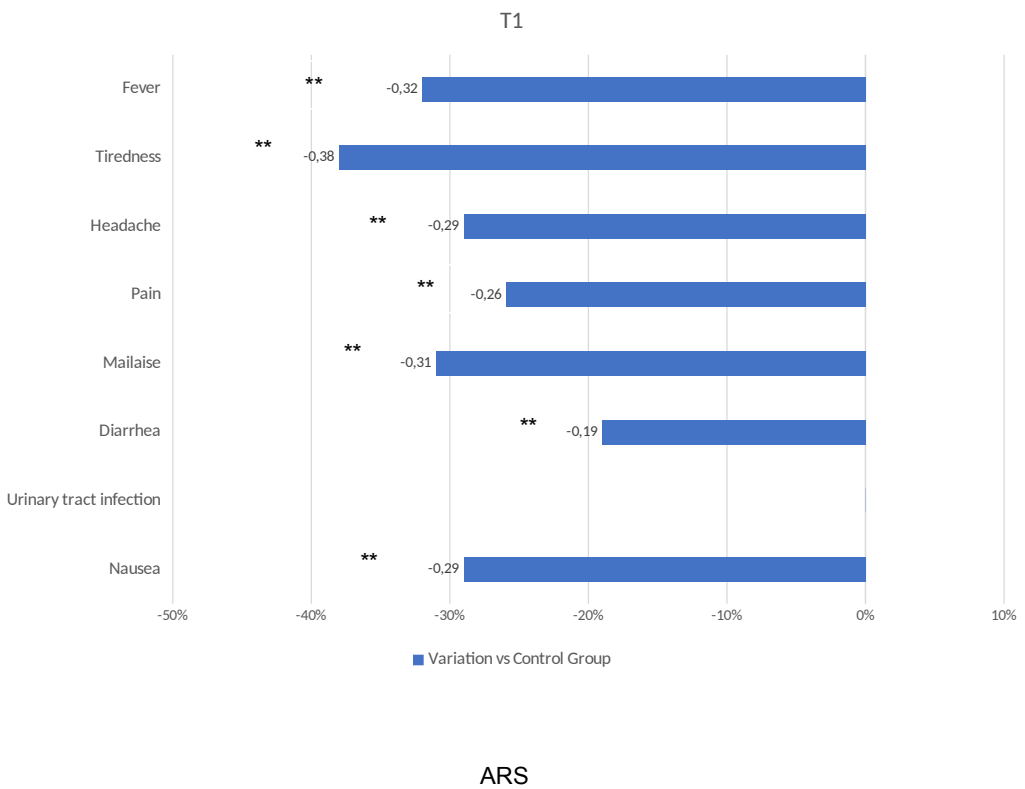

Fig. 1. *Percentage of difference between Group A and Group B concerning the evaluated symptoms in patients with acute rhinosinusitis at T1.*

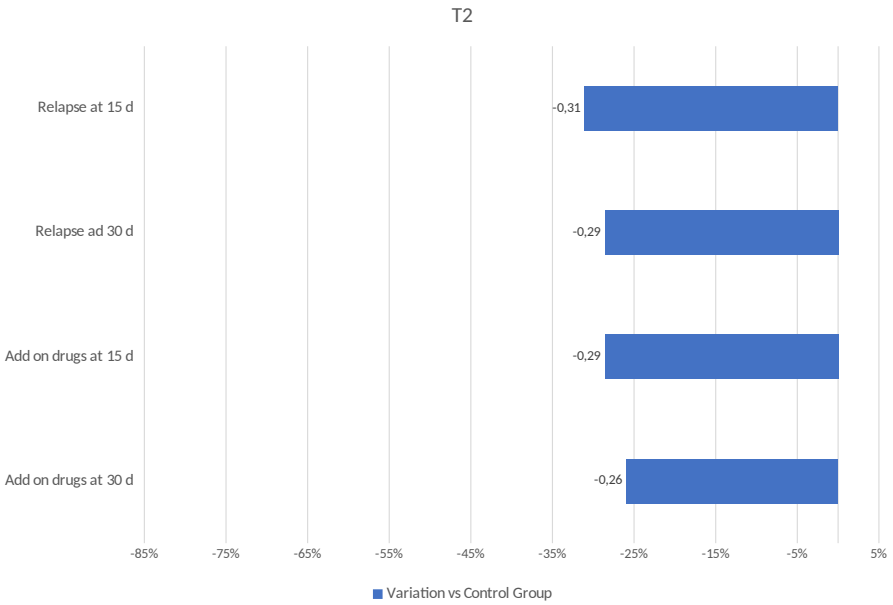

Fig. 2. *Percentage of difference between Group A and Group B concerning the clinical relapse and additional medications in patients with acute rhinosinusitis at T2.*

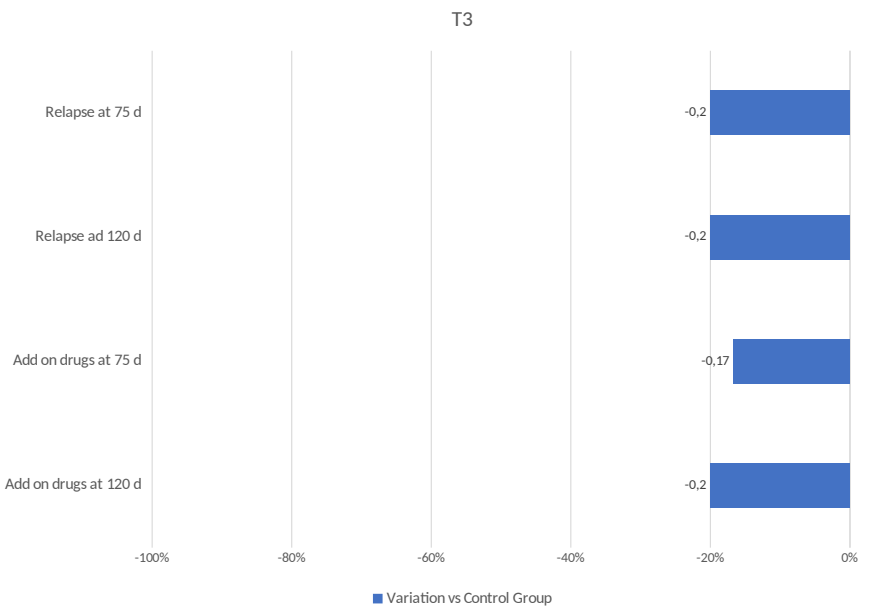

Fig. 3. *Percentage of difference between Group A and Group B concerning the clinical relapse and the use of additional medications in patients with acute rhinosinusitis at T3.*

RESULTS

Globally, 789 outpatients were enrolled. Acute rhinosinusitis affected 726 subjects: 371 in Group A and 355 in Group B. Chronic rhinosinusitis affected 63 outpatients: 32 in Group A and 31 in Group B.

Acute rhinosinusitis

The intergroup analysis showed that all reported symptoms mainly diminished in Group A at T1, as reported in Fig. 1. In particular, patients in the Group A had 32% fewer days with fever, 38% fewer days with tiredness, 29% fewer days with headache, 26% fewer days with pain, 31% fewer days with malaise, 19% fewer days with diarrhea, and 29% fewer days with nausea than Group B patients (Fig. 1).

At T2, patients in Group A had 31% fewer episodes of clinical relapse at 15 days, 29% fewer episodes of clinical relapse between 16 and 30 days, 29% less use of additional drugs at 15 days, and 26% less use of additional medications between 16 and 30 days than patients belonging to Group B (Fig. 2).

At T3, patients in Group A had 20% fewer episodes of clinical relapse between 30 and 75 days, 20% fewer episodes of clinical relapse between 75 and 120 days, 17% less use of additional drugs between 30 and 75 days, and 20% less use of additional medications between 75 and 120 days than patients belonging to Group B (Fig. 3). The intergroup comparison showed that patients treated with probiotics experienced less fever, tiredness, headache, pain, malaise, and diarrhea ($p<0.001$ for all) than control patients at T1.

Chronic rhinosinusitis

The intergroup analysis showed that all reported symptoms mainly diminished in Group A at T1, as reported in Fig. 4. In particular, patients in the Group A had 7% fewer days with fever, 33% fewer days with tiredness, 21% fewer days with headache, 19% fewer days with pain, 29% fewer days with malaise, 11% fewer days with diarrhea, and 22% fewer days

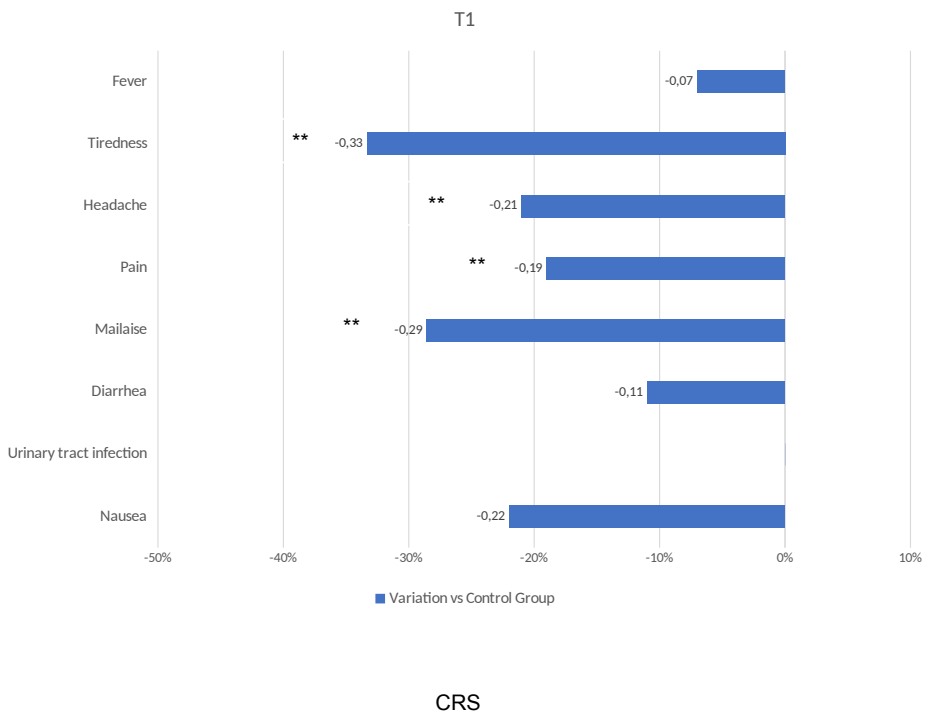

Fig. 4. *Percentage of difference between Group A and Group B concerning the evaluated symptoms in patients with chronic rhinosinusitis at T1.*

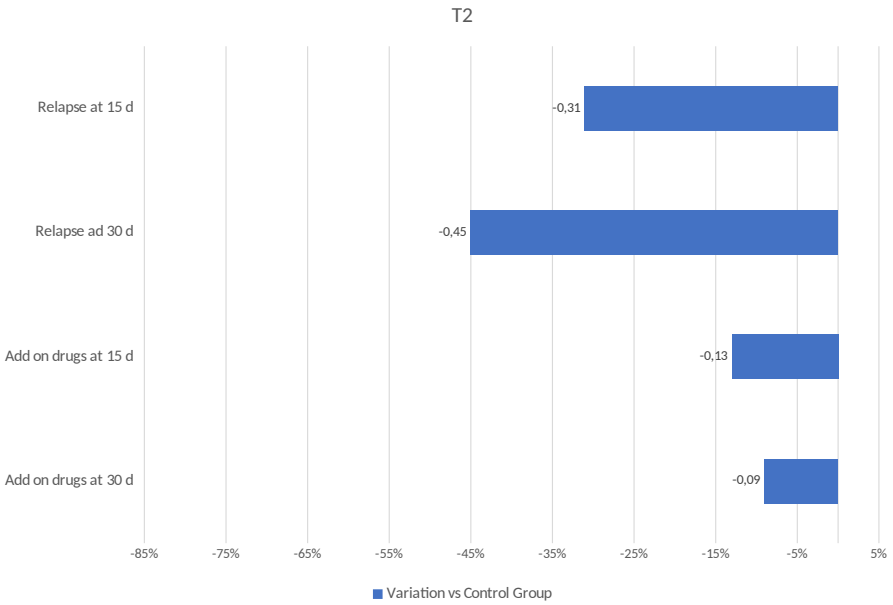

Fig. 5. *Percentage of difference between Group A and Group B concerning the clinical relapse and the use of additional medications in patients with chronic rhinosinusitis at T2.*

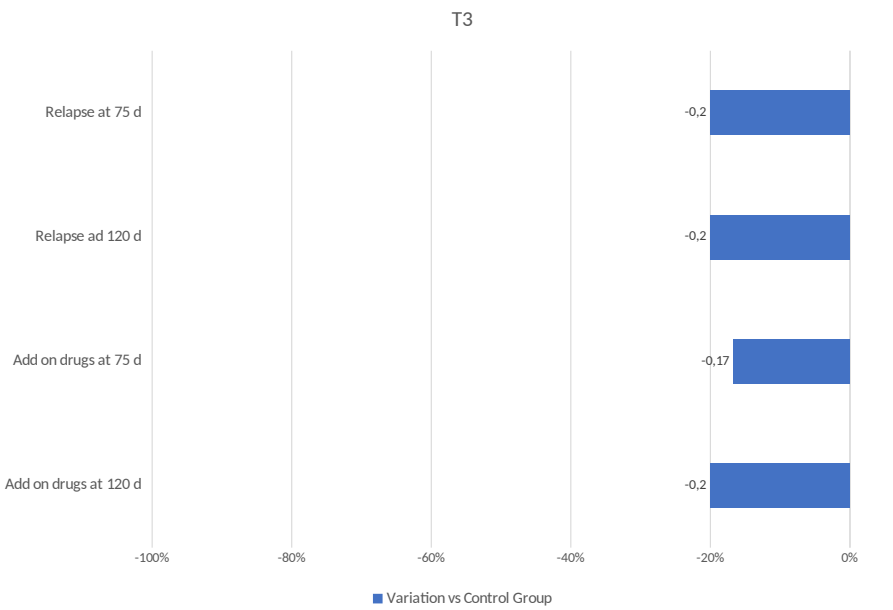

Fig. 6. *Percentage of difference between Group A and Group B concerning the clinical relapse and the use of additional medications in patients with chronic rhinosinusitis at T3.*

with nausea than Group B patients (Fig. 4).

At T2, patients in Group A had 31% fewer episodes of clinical relapse at 15 days, 45% fewer episodes of clinical relapse between 16 and 30 days, 13% less use of additional drugs at 15 days, and 9% less use of additional medications between 16 and 30 days than patients belonging to Group B (Fig. 5).
At T3, patients in Group A had 20% fewer episodes of clinical relapse between 30 and 75 days, 20% fewer episodes of clinical relapse between 75 and 120 days, 17% less use of additional drugs between 30 and 75 days, and 20% less use of additional medications between 75 and 120 days than patients belonging to Group B (Fig. 6). The intergroup comparison showed that patients treated with probiotics experienced less tiredness, headache, pain, and malaise ($p<0.001$ for all) than control patients at T1. The nutraceutical compound was well-tolerated in all subjects.

DISCUSSION

Rhinosinusitis is a widespread disease in clinical practice. Acute rhinosinusitis is usually caused by a respiratory infection, mainly concerning viral or bacterial pathogens (14). Chronic rhinosinusitis is characterized by an inflammatory process that may be potentially associated with an infectious episode (15). In both acute and chronic rhinosinusitis, the diagnosis is based on clinical features and endoscopic outcomes. When an infection is suspected, clinicians prescribe antibiotic treatment. However, antibiotics may frequently cause dysbiosis, both at the intestinal and respiratory levels. Consequently, the use of probiotics has become a common practice for antibiotic-treated patients.

In the vast panorama of probiotic products existing on the market, Abincol® is an oral nutraceutical containing a 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). Some clinical studies showed that Abincol® improved symptom severity in patients with intestinal problems (10-12). The recent experience was conducted on a large group of patients with acute rhinosinusitis or chronic rhinosinusitis.

The outcomes showed that the probiotic mixture reduced the intensity of symptoms during the antibiotic treatment (7-10 days), the probiotic course (one month), and the 90-day follow-up. Patients with both acute and chronic rhinosinusitis had an improvement. Moreover, the probiotic mixture reduced the frequency of clinical relapse and additional medications for acute and chronic rhinosinusitis.

The recent experience has some limitations, and the most relevant was the open design. However, the strength of the current experience was many randomly recruited outpatients, mainly concerning the acute pharyngotonsillitis. Further rigorous studies, such as placebo-controlled, should be conducted to confirm these preliminary findings. In conclusion, the present clinical experience demonstrated that a probiotic mixture containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii*, was able to reduce symptoms associated with antibiotic therapy in patients with both acute and chronic rhinosinusitis just during antibiotic therapy.

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Probiotics in the add-on treatment of laryngotracheitis: a clinical experience

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Laryngotracheitis is a common disease, mainly characterized by dysphonia, cough, and sore throat. The diagnosis is usually based on the clinical ground, and antibiotic therapy is frequently used in clinical practice. However, antibiotics frequently induce intestinal dysbiosis associated with some clinical problems. The current clinical experience was conducted in patients with pharyngotonsillitis and treated with antibiotics. A one-month course of a probiotic mixture (Abincol® containing *Lactobacillus plantarum* LP01 (1 billion of living cells), *Lactobacillus lactis subspecies cremoris* LLC02 (800 million living cells), and *Lactobacillus delbrueckii* LDD01 (200 million living cells), was prescribed in the Group A, and was compared with no add-on treatment, such as the Group B. Patients were evaluated at baseline (T0), at the end of antibiotic treatment (T1), at the end of probiotic course (T2), and at the end of 3-month follow-up (T3). Globally, 833 outpatients with laryngotracheitis were enrolled: 425 in Group A and 408 in Group B. All of them were treated with a 7-10-day course of antibiotic therapy. The probiotic mixture reduced the duration of symptoms associated with antibiotic therapy already at the end of the antibiotic cycle. The intergroup comparison showed that probiotic group patients experienced less fever, tiredness, headache, pain, malaise, diarrhea, and nausea ($p<0.001$ for all) than control patients at T1. The probiotic course reduced the possible clinical relapse, and the use of additional medications at T2 and T3. In conclusion, the present clinical experience demonstrated that a probiotic mixture containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris*

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LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii*, was able to rapidly reduce symptoms associated with antibiotic therapy in patients with laryngotracheitis.

Key words: pharyngotonsillitis, acute, chronic, antibiotic therapy, dysbiosis, probiotics.

Laryngitis is the larynx inflammation and can present in both acute and chronic forms (1). Patients with acute laryngitis usually experience mild and self-limiting symptoms lasting 3 to 7 days (2). Chronic laryngitis lasts for more than four weeks (3). The most common cause of laryngitis is an acute upper respiratory infection. The diagnosis is based on a thorough history. The most common symptoms include voice changes (hoarseness or "raspy" voice), early vocal fatigue (mainly in professional voice users), or a dry and irritating cough. Breathing difficulties, including dyspnea, shortness of breath, or audible stridor, suggest that a more severe disease process may be present. Suspicion should be considered in smokers and immunocompromised patients. Infectious laryngitis may be due to viral or bacterial pathogens. Viral agents such as rhinovirus, parainfluenza virus, respiratory syncytial virus, coronavirus, adenovirus, and influenza are all potential etiologic agents (listed in roughly descending order of frequency). Bacterial superinfection can occur in the setting of viral laryngitis; this classically occurs approximately seven days after symptoms begin. The most common bacterial pathogens are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. Laryngitis caused by fungal infection is very rare in immunocompetent individuals, and more often presents as chronic laryngitis in the immunocompromised or patients using inhaled steroid medications for a long time.

Bacterial tracheitis was first described in the 1920s despite the name not being coined until much later (4). The disease was initially referred to as acute laryngotracheobronchitis. Bacterial tracheitis, also known as bacterial croup, acute laryngotracheobronchitis, or membranous croup, is a potentially lethal infection of the subglottic trachea (5). It is often a secondary bacterial infection preceded by a viral infection, mainly affecting children. Concern for airway protection is the mainstay of treatment as thick, mucopurulent secretions can cause airway narrowing (6). Treatment is aimed at protecting the airway, assessing the need for endoscopy for therapeutic and/or diagnostic reasons, and antimicrobial therapy. On presentation, this must be distinguished from the more immediately lethal epiglottitis (7).

As laryngitis and tracheitis may be associated, the term laryngotracheitis is used in everyday practice. Laryngotracheitis is an infection-induced inflammation (8). As complications are feared, antibiotics are widely prescribed in patients with laryngotracheitis (9). However, antibiotic treatment is frequently associated with microbiota perturbation, such as dysbiosis. To restore the physiological balance of microbiota, probiotics may be, therefore, prescribed in clinical practice.

Probiotics could improve intestinal microbiota equilibrium, reduce respiratory symptoms, and general wellbeing (10). In this regard, 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. This product has been previously tested in patients with gastroenterological disorders (11-13). Therefore, the present clinical experience evaluated the potential role of add-on treatment to antibiotic therapy in patients with laryngotracheitis recruited by a group of Italian otorhinolaryngologists (ORL) in clinical practice.

MATERIALS AND METHODS

The current experience was conducted in Italian otorhinolaryngology centres, distributed across Italy, so

assuring an extensive and complete national coverage, during the fall-winter 2019-2020. ORL specialists were asked to recruit all consecutive outpatients with laryngotracheitis. Patients were consecutively recruited during the specialist visit. The inclusion criteria were to have laryngotracheitis, 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 a real-world setting. Laryngotracheitis was diagnosed on a clinical ground according to validated criteria (14). 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. Patients were further subdivided into two subgroups, considering the laryngotracheitis diagnosis.

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 gave the patients a clinical diary to record clinical information.

In the first period (7-10 days), patients had to report the duration (expressed in days) of symptoms, including fever, tiredness, headache, pain, malaise, diarrhea, urinary tract problems, and nausea. In the second (first month) and third (3-month follow-up) periods, patients had to report clinical relapse, use of concomitant medications, and adverse events. 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). For all tests, the accepted minimum significance limit was 0.05.

RESULTS

Globally, 833 outpatients were enrolled: 425 in Group A and 408 in Group B. The intergroup analysis showed that all reported symptoms mainly

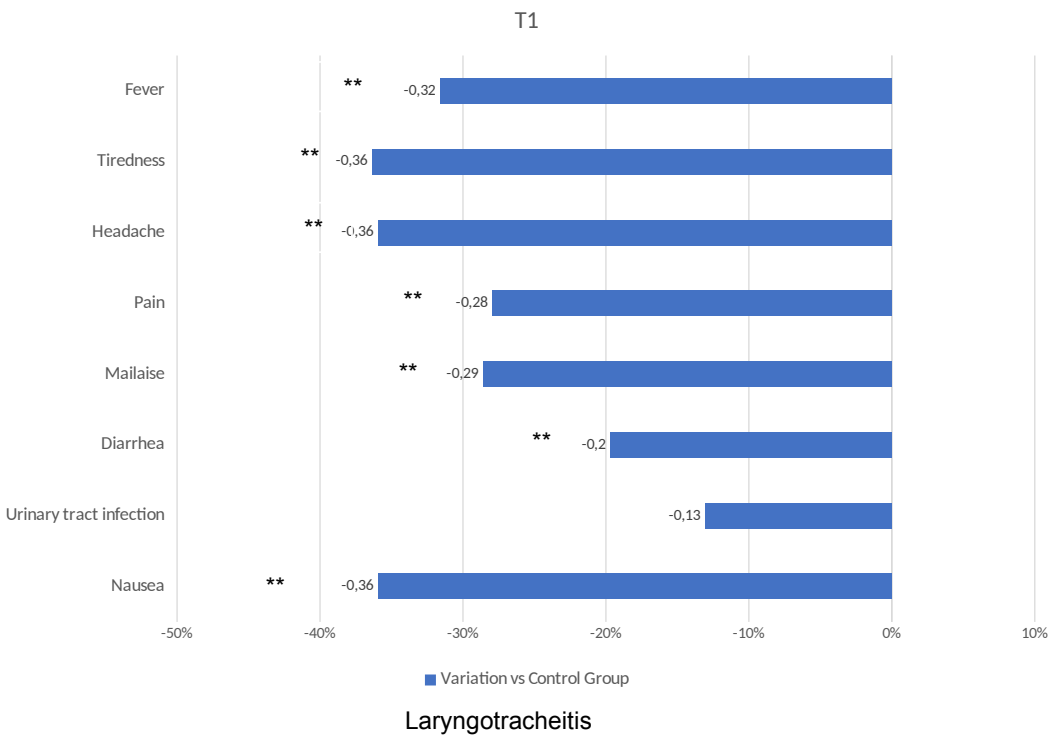

Fig. 1. *Percentage of difference between Group A and Group B concerning the evaluated symptoms in patients with laryngotracheitis at T1.*

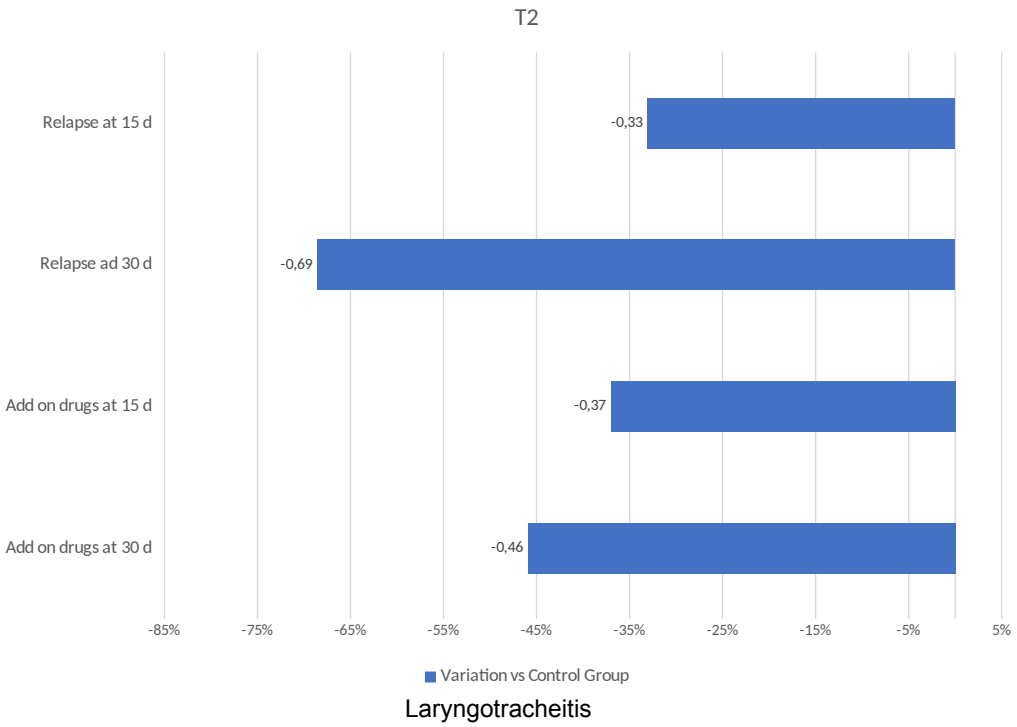

Fig. 2. *Percentage of difference between Group A and Group B concerning the clinical relapse and additional medications in patients with laryngotracheitis at T2.*

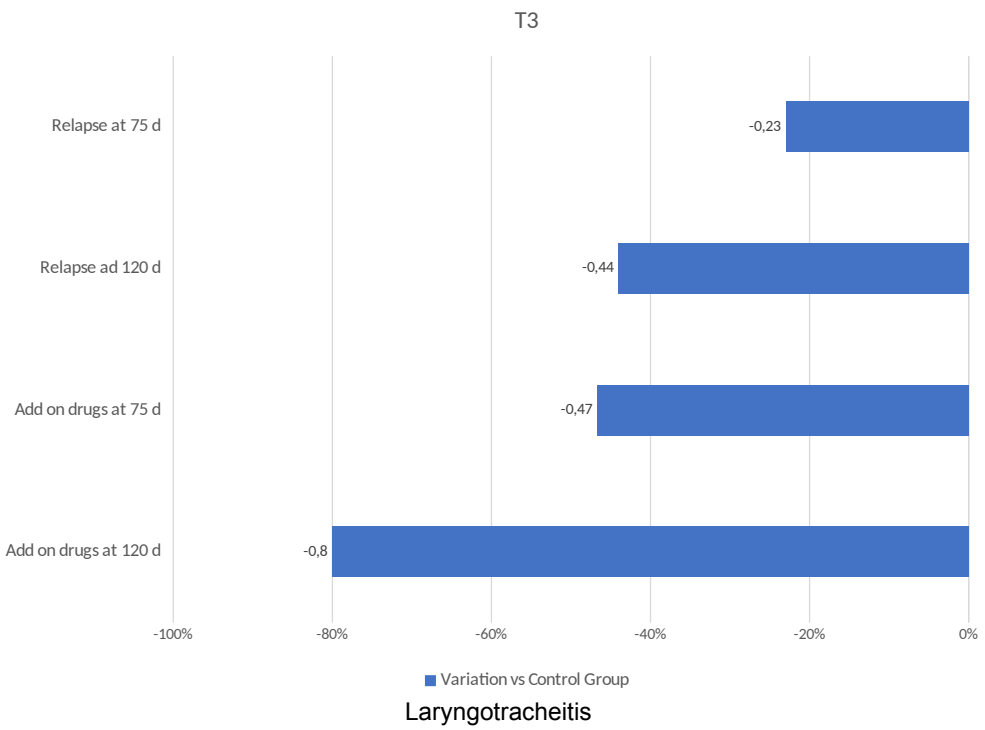

Fig. 3. *Percentage of difference between Group A and Group B concerning the clinical relapse and the use of additional medications in patients with laryngotracheitis at T3.*

diminished in Group A at T1, as reported in Fig. 1. In particular, patients in the Group A had 32% fewer days with fever, 36% fewer days with tiredness, 36% fewer days with headache, 28% fewer days with pain, 29% fewer days with malaise, 20% fewer days with diarrhea, 13% fewer days with urinary tract problems, and 36% fewer days with nausea than Group B patients (Fig. 1).

At T2, patients in Group A had 33% fewer episodes of clinical relapse at 15 days, 69% fewer episodes of clinical relapse between 16 and 30 days, 37% less use of additional drugs at 15 days, and 46% less use of additional drugs between 16 and 30 days than patients belonging to Group B (Fig. 2).

At T3, patients in Group A had 23% fewer episodes of clinical relapse between 30 and 75 days, 44% fewer episodes of clinical relapse between 75 and 120 days, 47% less use of additional drugs between 30 and 75 days, and 80% less use of additional drugs between 75 and 120 days than patients belonging to Group B (Fig. 3). The intergroup comparison showed that probiotic group patients experienced less fever, tiredness, headache, pain, malaise, diarrhea, and nausea ($p<0.001$ for all) than control patients at T1. The nutraceutical compound was well-tolerated in all subjects.

DISCUSSION

Patients with laryngotracheitis usually present relevant respiratory symptoms, and antibiotics may be prescribed in daily practice. However, antibiotic treatment may frequently cause dysbiosis, both at the intestinal and respiratory levels. Consequently, the use of probiotics has become a widespread practice to restore a physiologic balance of microbiota. On the other hand, many probiotics are available on the market. Unfortunately, most of them lack adequate scientific documentation, or only in vitro studies are available. In this regard, Abincol® is 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). Some clinical studies showed that Abincol® improved symptom severity in patients with intestinal problems (11-13).

The current experience was conducted on a large group of patients with laryngotracheitis and treated with antibiotics. The outcomes showed that the probiotic mixture reduced the intensity of symptoms during the antibiotic treatment (7-10 days), the probiotic course (one month), and the 90-day follow-up. Patients with laryngotracheitis had an improvement. Moreover, the probiotic mixture reduced the frequency of clinical relapse and the use of additional medications.

The current experience has some limitations, and the most relevant was the open design. However, the strength of the current experience was many randomly recruited outpatients, mainly concerning the acute pharyngotonsillitis. Further rigorous studies, such as placebo-controlled, should be conducted to confirm these preliminary findings. In conclusion, the present clinical experience demonstrated that a probiotic mixture containing *Lactobacillus plantarum* LP01, *Lactobacillus lactis subspecies cremoris* LLC02, and *Lactobacillus delbrueckii subspecies delbrueckii*, was able to rapidly, such as during the antibiotic therapy, reduce symptoms associated with antibiotic therapy in patients with laryngotracheitis.

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Non-pharmacological remedies for upper respiratory diseases in the pandemic COVID-19 era

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In the pandemic coronavirus disease 2019 (COVID-19) era, the need to use preventive-curative treatments is compelling. A series of non-pharmacological compounds, including supplements (oligo-elements and vitamins), probiotics, and nutraceuticals, might affect the risk of COVID-19 or reducing clinical severity. Non-pharmacological remedies are easily available and usually have no relevant side effects. There is evidence that bacterial and molecular substances may potentiate the immune system against respiratory viruses. Moreover, these compounds might exert essential anti-inflammatory and antioxidant activity in COVID-19. Furthermore, nasal lavage may be an additional resource for reducing the viral load and restore the integrity of respiratory patency. Therefore, preventive courses using non-pharmacological remedies could be prescribed to reinforce the immune response and adequate treatment of upper respiratory infection with natural compounds could be considered a reasonable way to manage people in the pandemic COVID-19 era.

Background

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has been initially reported in Wuhan (China) at the end of 2019. It has rapidly spread worldwide so that the World Health Organization (WHO) declared the pandemic on March 11, 2020 (1). At present (early September), more than 25 million cases have been officially reported, and about one million people deceased. This dramatic situation is continuously in progress, and the second wave of infections is probable that may occur soon. In this scenario, there is no specific treatment nor a vaccine.

However, COVID-19 has a broad clinical severity spectrum ranging from an asymptomatic state to very severe symptoms resulting in acute respiratory distress syndrome (2). A multiorgan failure may occur as well as septic shock, metabolic acidosis, and disseminated intravascular coagulation. These clinical features confer to COVID-19 the characteristics of a multifaceted, multiorgan, multi-system disease. Notably, all ages could be affected by COVID-19, even though elderly subjects are more prone to develop more severe disease than younger people.

From a pathophysiological point of view, two main aspects should be considered in the pathogenesis of severe COVID-19: a hyper-inflammation and hyperstimulation of the immune system by releasing an enormous quantity of cytokines, the so-called cytokine storm (3). Different pharmacological treatments have been used, including antiviral drugs, anti-inflammatory drugs (including corticosteroids and heparin), anti-cytokine biologics, and hyperimmune plasma.

On the other hand, there is a growing interest in using natural substances able to modulate the immune system and resolve inflammation, with the result of increasing the defenses against infection. In this regard, attention has been focused on

Keywords: COVID-19, upper respiratory infections, microbiota, probiotics, oligo-elements, nutraceuticals.

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non-pharmacological remedies characterized by consolidated efficacy and optimal tolerability and safety. These traditional remedies include probiotics, nutraceuticals, and diet supplementation. These natural compounds could be used both to potentially prevent COVID-19 and to adequately cure upper respiratory infection with the aim of preventing increased susceptibility to COVID-19.

Probiotics

As the gastrointestinal tract may be frequently affected during COVID-19, this concept has suggested of using probiotics to prevent or mitigate COVID-19 (4). It is known that SARS-CoV-2 infects human cells through the binding of its spike proteins (S) to Angiotensin-Converting-Enzyme-2 (ACE2) (5). ACE2 is highly expressed in respiratory epithelial cells, but also in esophagus epithelial cells and enterocytes in the ileum and colon (6). Consequently, SARS-CoV-2 RNA has been detected in the stool of COVID-19 patients (7).

The SARS-CoV-2 gastrointestinal colonization follows to a respiratory one, and the shedding, consistently, persists longer. The digestive symptoms during COVID-19 include diarrhea, nausea, vomiting, and intestinal pain. This clinical feature depends on a direct viral presence in the digestive tube. Namely, the viral replication in the gut entails a local increase of the SARS-CoV-2 load. This event promotes the impairment of mucosal integrity that, in turn, causes dysbiosis, such as unbalance microbiota, with significant consequences on the immune system, mainly concerning the massive release of pro-inflammatory cytokines. These mechanisms are based on the concept of the microbiota's relevance, such as the involved community of microorganisms, including bacteria, viruses, fungi, and other microbes, inhabiting the mucosal surfaces (8).

A good microbiota is fundamental to guarantee healthy metabolic and immune activities, mainly concerning the defense against pathogens (9). From a finalist point of view, a functional equilibrium between microbiota and host is called eubiosis. Physiological microbiota regulates the immune response during inflammatory and infectious disorders (10). The intestinal microbiota induces immunocompetent cells' maturation, including type 1, type 2, type 3, and regulatory cells (11). Similarly, to intestinal microbiota, respiratory microbiota performs a series of defensive functions to ensure tolerance and defensive mechanisms towards exogenous antigens.

An imbalance of microbiota is called dysbiosis; this condition dysregulates its beneficial activities, facilitating inflammatory and infectious disorders (12). Dysbiosis represents a common pathogenic mechanism in many diseases, including cancer, autoimmunity, obesity, allergy, asthma, chronic inflammatory intestinal diseases (13). In this context, a crucial concept is a close relationship between intestinal and respiratory microbiota, the so-called gut-lung axis, fundamental to maintain physiological homeostasis (14). The most common cause of dysbiosis is an infectious disease, mostly viral. Namely, viral infection significantly affects microbiota leading to potentially beneficial or harmful responses (15).

However, detrimental effects are preponderant during viral infections, so perturbation of intestinal and respiratory microbiota is common during and immediately after infections. An important pathway disturbed by viral infections entails the production of type 1 cytokines, namely interferons appointed to fight pathogens (16). In this way, the viral infection interferes with the host defensive mechanisms and creates a vicious circle enhancing aggressive mechanisms. Similarly, considering the SARS-CoV-2 enterotropism, it is conceivable that gastrointestinal symptoms are a consequence of the dysbiosis induced by the coronavirus (17).

As mentioned above, there are no effective and specific treatments for COVID-19; probiotics could be a promising way to contrast SARS-CoV-2. In this regard, probiotics, when administered in sufficient amounts, are microorganisms that confer positive benefits to patients, and their potential clinical utilization has been proposed in several diseases (18). There is convincing evidence, including Cochrane meta-analysis, that probiotics protect against viral infections, mainly concerning acute upper respiratory infections (19, 20). A meta-analysis notably evidenced that probiotics reduced the

number of acute upper respiratory tract infections, the mean duration of disease, antibiotic administration, and cold-related school absences compared to a placebo (21). The mechanisms of action are different, including stimulation of innate immunity, restoration of the intestinal mucosa, enhancement of acquired and regulatory immune response, and anti-inflammatory activity (2). Concerning the last mechanism, SARS-CoV-2 may trigger a hyper-inflammation state consequent the cytokine storm in patients with severe COVID-19 (22). Namely, patients requiring intensive care have very high levels of many pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, the so-called inflammasomes (23). Probiotics could dampen the hyper-inflammatory response reducing cytokine release. At present, there is no robust evidence of effective probiotic activity in patients with COVID-19. However, there is a growing interest from the scientific community around this intriguing topic, as demonstrated by many recent papers (2, 24-28).

Moreover, a preventive strategy could be pursued by prescribing probiotics in healthy subjects, mainly if they are contacts. The rationale is maintaining a state of eubiosis in the host able to contrast the viral penetration into mucosal tissues (29). In this regard, d'Ettorre very recently demonstrated that a specific bacterial formulation (containing *Streptococcus thermophilus* DSM 32345*, L.acidophilus* DSM 32241, *L. helveticus* DSM 32242*, L. paracasei* DSM 32243, *L. plantarum* DSM 32244, *L. brevis* DSM 27961, *B. lactis* DSM 32246, and *B. lactis* DSM 32247) significantly improved the clinical conditions of patients positive for SARS-CoV-2 infection (30). These results underlined the importance of the gut-lung axis in controlling the COVID-19 disease. Moreover, the ongoing three trials explore the capability of probiotics in patients with COVID-19 (2).

Nutraceuticals

In COVID-19 patients, aberrant inflammatory response, impaired innate and acquired immune activity, and oxidative stress are frequently detected. Oxidative stress and inflammation are closely associated as well as coagulative disorders are frequently observed in severe COVID-19. There are many medical devices and food supplements on the market. All claim relevant anti-inflammatory, antioxidant, and immunomodulatory activities, but very few have documented evidence that attests convincing proof of real efficacy. In this regard, we focus the attention on some substances, including selenium, zinc, vitamin D, bromelain, escin, and inulin.

Selenium

Selenium is an essential oligo-element for redox metabolism by occurring as selenocysteine in catalytical centers of many selenoproteins (31). Nutritional deficiencies of selenium may impact both the immune response and the pathogenicity of SARS-CoV-2. An association between the cure rate of COVID-19 patients and selenium status has been reported (32). Bioinformatic screening of the SARS-CoV-2 gene signatures, provided further evidence of protein interactions and antisense transcript mRNA–mRNA interactions occurring at selenocysteine-related insertions in RNA viruses (33).

The Keshan disease offers an attractive model: dietary selenium deficiency can alter the Coxsackie 3B viral genome to a highly virulent agent after its entrance into the host (34). The protective effect of selenium because it is an essential cofactor in a group of enzymes, including glutathione peroxidases and the thioredoxin reductases, that with vitamin E diminishes the formation of reactive oxygen species, thus exerting antioxidant activity (35). Consistently, oxidative stress is associated with a sustained inflammatory response. Moreover, selenoprotein K and selenoprotein S regulate immune responses, so selenium protects the respiratory system from viral infections (36). Selenium supplementation also increases interferon production (37). Selenium supplementation increases the response rate to influenza vaccination in old subjects (38). Subjects with suboptimal plasma levels (<100 μg/L) should be supplemented with 100–200 μg Se/day (39). With regards to COVID-19, there is accumulating opinion promoting selenium supplementation also in COVID-19 patients (40-43).

Zinc

Several enzymes contain zinc as fundamental component (44). Zinc is also important for the

development and maintenance of immunocompetent cells (45). Consistently, zinc deficiency results in dysfunction of humoral and cellular immune system, mainly concerning T cells (46). Zinc deficiency is associated with increased release of pro-inflammatory cytokines, including IL-6, IL-8, and TNF-α (47). Therefore, zinc supplementation entails an important source to restore impaired immune response. In this regard, there is evidence that zinc supplementation is clinically relevant in patients with respiratory infections (48). For these reasons, zinc supplementation has been favorably used also in COVID-19 patients (49). Therefore, zinc has been proposed as a potential remedy in the prevention and add-on treatment for COVID-19 (50).

Vitamin D

Cholecalciferol, such as the vitamin D3, is hydroxylated by the liver and the kidneys to 1.2-$(OH)_2$-D3; thus, this is the active form of the vitamin D, and exerts its activities by binding to vitamin D receptors. Vitamin D is a hormone that plays many activities, among which one of the most important is the regulation of the immune system (51). On the other hand, vitamin D deficiency has been associated with impaired immune response and increased susceptibility to infections (52). Consequently, vitamin D supplementation in the prevention of respiratory infections has been positively assessed by meta-analysis (53). Therefore, it has been proposed that vitamin D could play a role in COVID-19, as it has been documented that the rate of infection was higher in countries at higher latitudes and/or lower vitamin D status (54, 55). At present, there are some preliminary experiences that demonstrated an association between vitamin deficiency and COVID-19 severity (56, 57). Consequently, it seems reasonable to consider the vitamin D supplementation as potential preventive remedy also for COVID-19 (58).

Bromelain

Bromelain is a complex mixture of protease extracted from the pineapple plant's fruit or stem (59). Bromelain gained universal acceptability as a phototherapeutic agent due to its history of safe use and lack of side effects. The beneficial effects are due to multiple factors. Bromelain increases bioavailability and reduces the side effects that are associated with various antibiotics. Furthermore, bromelain acts as an immunomodulator and has anti-oedematous, anti-thrombotic, and anti-inflammatory activity. Bromelain has the *in vitro* ability to modulate surface adhesion molecules on T cells, macrophages, and natural killer cells and may induce the secretion of IL-1β, IL-6, and tumor necrosis factor α (TNFα) by peripheral blood mononuclear cells (8). Bromelain influences blood coagulation by increasing the serum fibrinolytic ability and inhibiting fibrin synthesis, a protein involved in blood clotting (60). Therefore, bromelain could be a promising substance able to reduce viral-associated inflammation.

Escin

Escin is the primary active principle from *Aesculus hippocastanum* (Hippocastanaceae), such as the horse chestnut tree (61). Escin is a natural mixture of triterpene saponins. Escin effectively prevents the formation of edema in models of inflammation that reproduce the initial exudative phase. The mechanism of the anti-oedematous effect, in addition to the earlier described sensitization to Ca ions, resulting in a 'sealing effect' on small vessels permeable to water, has also been related to reduced hypoxia-induced activation of human endothelial cells. Escin can well antagonize the reduction in ATP content and increased phospholipase A2 responsible for releasing precursors of inflammatory mediators.

Furthermore, there is a reduced neutrophil adherence/activation, all resulting in the protection of veins and reduced edema. Escin also exerts antiviral activity (62). Consequently, escin has been proposed as a potential substance to contrast SARS-CoV-2 infection (63). In particular, it has been reported that β-escin reduces the degree of lung injury and improves the function of gas exchange in LPS-induced lung inflammation in mice by inhibiting both the lipid peroxidation and the expression of pro-inflammatory factors, for example, NO, TNF-α, IL-1β, and IL-6 (64). Interestingly, sodium aescinate exerted anti-inflammatory activity in SARS-infected patients (65).

Inulin

Inulin is a fructan-type plant polysaccharide, with the structure constituted with a variable number of fructose units (66). It was first isolated in 1804 from the roots of compositae plant *Inula helenium*, and, therefore, named as inulin. Inulin is widely distributed in nature in the form of storage carbohydrate and has been found in more than 30,000 plants, mostly in chicory, Jerusalem artichoke, and dahlia. Inulin is used as a prebiotic. Prebiotics are non-digestible substances naturally contained in some foods which promote the growth, in the colon, of one or more bacterial species useful for the development of probiotic microflora. Therefore, inulin can be fruitfully associated with probiotics, thus constituting a synbiotic product, to enhance their positive effects in many diseases, including infections (67). Inulin has also antioxidant mechanisms (68) and modulates the immune system, mainly concerning the enhancement of a tolerogenic immune response (69).

Nasal washing

The WAO recommended a series of public health measures, including hand hygiene, respiratory etiquette, respirator masks and environmental cleaning, to protect individuals, their families, and others against COVID-19. As the nose is the first passage for the virus into the host, saline nasal washing is prescribed as a preventive strategy to clean the nasal cavities by removing antigens, inflammatory mediators, and microorganisms such as bacteria and viruses, also reducing the viral load (70). In this regard, Ramalingam reported that after the onset of viral illness, volunteers who were practicing hypertonic nasal saline irrigation and gargles had 36% lesser use of over the counter medications, 35% lesser transmission, and viral shedding (71). Therefore, there is increasing consensus about the treatment option with nasal washing in COVID-19 patients (72-74).

Hyaluronic acid

Hyaluronic acid (HA) is a fundamental component of the connective tissue. HA can modulate the inflammatory response, cellular proliferation, and remodeling of the extracellular matrix (75). High molecular weight HA exerts important anti-inflammatory and immunomodulatory activity, mainly concerning airways diseases (76). In the pandemic COVID-19 era, there is an urgent need to discover a therapeutic strategy to combat COVID-19, as there are no specific treatments nor vaccines at present.

In this context, the use of non-pharmacological remedies are welcome by the general population. Many products are available, but the choice should be oriented toward compounds that can boast adequate studies. In this regard, there is a medical device that contains bromelain, escin, and selenium and has been evaluated in patients with inflammatory disorders involving upper airways (77). Another medical device for nasal washing, containing thermal water and hyaluronic that has been tested in patients with acute, chronic, or relapsed upper airways disorders (78-80). A food supplement containing a probiotic mixture has also been positively tested in patients with gastrointestinal diseases (81). Soon, a new symbiotic compound containing inulin, prebiotic mixture, zinc, and vitamin D will be available to reinforce immune system.

However, methodologically robust clinical research data are required to support any such claim. In conclusion, we believe that preventive immune-stimulant courses with probiotics and other natural substances as well as early treatment of upper airway infection with anti-inflammatory and antioxidant compounds and medicated nasal washing could be a promising strategy to potentially prevent COVID-19 in the general population and overall in at risk subjects.

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