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Review

Probiotics Role in Chronic Rhinosinusitis Treatment: an Update of the Current Literature

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Abstract: Chronic rhinosinusitis (CRS) is a significant health problem. It affects 5%–12% of the general population. The causes that underlie the onset of CRS are not yet well known. However, many factors may contribute to its onset, such as environmental factors and the host's condition. Medical treatment mainly uses local corticosteroids, nasal irrigation, and antibiotics. In recent years, a new therapeutic approach that employs the use of probiotics emerged. Probiotics have been extensively studied as a therapy for dysbiosis and inflammatory pathologies of various parts of the body . We aimed to examine the studies in the existing literature to update probiotics' role in rhinosinusitis chronic medical treatment.

Keywords: Chronic rhinosinusitis, probiotics, microbiome, nasal microbiota, microbiome therapy

1. Introduction

Chronic rhinosinusitis (CRS) is a significant health problem. It affects 5%–12% of the general population [1]. Further, it is referred to as chronic when the inflammatory process persists for more than 12 consecutive weeks[2]. The investigation conducted by the Global Allergy and Asthma European Network (GALEN) in 2011 concluded that the prevalence of CRS in Europe amounts to 10.9%—between 6.9% and 27.1% in different European cities [3]. This pathology negatively impacts patients' quality of life. Therefore, it must be correctly identified and treated.

The causes that underlie the onset of CRS are not yet well known. However, many factors may contribute to its onset, such as environmental factors (temperature and humidity, air pollution) and the host's condition (anatomical variants, allergies, local or systemic immune system imbalance, genetic predisposition)[4]. Medical treatment mainly uses local corticosteroids, nasal irrigation, and antibiotics. If medical treatment is insufficient, endoscopic sinus surgery is proposed. In recent years, a new therapeutic approach that employs the use of probiotics emerged [5] . The World Health Organization (WHO) defines probiotics as products containing live microorganisms that, when administered in the right amounts, have a beneficial effect on the host's health [6]. Probiotics have been extensively studied as a therapy for dysbiosis (imbalance of the

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microbial flora of a given habitat—or alteration of its composition or function) and inflammatory pathologies of various parts of the body (digestive system, upper and lower airways) [7-11]. Studies conducted to evaluate probiotics' effect on CRS are still few in number. Furthermore, there are concerns regarding experimental studies on animal models, including in vitro and clinical studies. We aimed to examine the studies in the existing literature to update probiotics' role in rhinosinusitis chronic medical treatment.

2. Microbiota

A microbiota is described as a community of microorganisms that resides in a distinct environment, and the collection of entire genomic elements of a distinct microbiota is the microbiome [12]. In the various microenvironments of our organism, there is a presence of 10–100 trillions colonies of microorganisms that coexist synergistically, constituting a commensal microbiome capable of coexisting within our organism and defending it from insults coming from the external environment. Since childhood, the microbiota composition tends to change to adapt to various internal and external stimuli during the normal physiological growth of tissues [13]. The human being acquires significant quantities of microbiota from the mother during birth by natural means [14]. This microbiota changes in the first three years of life and then become more stable but is always subject to minor changes in the later stages of life [15].

Then each site of the organism is colonized by various microorganisms (microbiota), characterizing the inhabited site, not constant but variable according to the subject's age. With their complex and tortuous anatomy, the nasal cavities define anatomical spaces where we find different microbiota. Consequently, in the nasal vestibule—the nasal cavities and the paranasal sinuses—there exist diverse microbiota residents.

Resident commensal bacteria are involved in the homeostasis of the nasal microbiota, controlling and suppressing opportunistic pathogens through a competition mechanism for spaces and nutrients. Additionally, products with toxic compounds inhibit or directly kill competing microorganisms (lactic acid, antibacterial peptides, hydrogen peroxide) (Fig. 1).

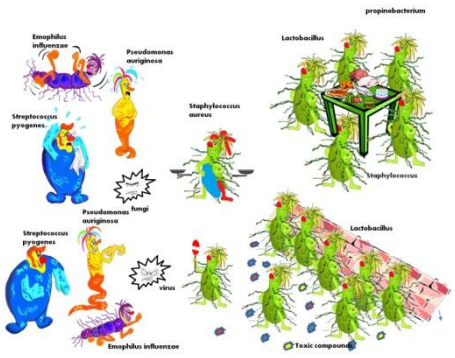

Figure 1. . Resident commensal bacteria are involved in the homeostasis of the nasal microbiota, controlling and suppressing opportunistic pathogens through a competition mechanism for spaces and nutrients. Additionally, products with toxic compounds inhibit or directly kill competing microorganisms (lactic acid, antibacterial peptides, hydrogen peroxide). Opportunistic bacteria (see Staphylococcus aureus) retain the ability to act as commensals or pathogens.

The commensal bacteria are also an essential part of the nasal flora and contribute to maintaining this district's physiological and immune functions. Several studies show that failure balancing and dysbiosis [16] between the microorganisms (due to the use of antimicrobials, lifestyle factors, factors dietary) determine an increase in opportunistic

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pathogens, favoring an alteration of the immune functions and increased infectious processes. The dismicrobism of the upper respiratory tract can cause chronic inflammation of the airways resulting in the spread of microorganisms in the various sections of the airways, thus generating pathological conditions such as allergic rhinitis, rhinosinusitis, chronic otitis media, and asthma [17,18].

3. Nasal Microbiota

Recent studies have shown that microorganisms that make up the microbiota of the nasopharynx are different respect to those present in paranasal sinuses. Therefore, it is evident that there are several varieties of microbiota in the various sections of the upper respiratory tract [13].

As evidenced by Yan M. et al. [19] the nasal cavities constitute a transition zone between the external environment exposed to constant insults, and the protected internal environment. The nose is an environment deficient in nutrients and acid and salty. The higher the acidity and salinity (nostrils), the greater the difficulty of microbial colonization. The nasal mucus, also containing small amounts of nutrients, is restrictive for microbial growth. Therefore, it can be understood how only particular species and microbes have adapted to this environment. In nasal microbiota, are more identified phyla Actinobacteria (corynebacterium and cutibacterium), Firmicutes (Staphylococcus, Streptococcus, dolosigranulum, lactobacillus), and Proteobacteria (Moraxella and Haemophilus), whose abundance varies in different of the nose portion. Among the phyla present in the nasal cavities, Actinobacteria is the predominant one and is present at all stages of life [20]. Furthermore, they have been identified as patterns of microorganisms that tend to change over time. The growth of the individual (typically proteobacteria) and more stable patterns (Staphylococcus epidermidis of the species firmicutes) tend to be maintained over time.

The commensal bacteria represent the majority of the bacteria present in the nasal cavities. Additionally, there are opportunistic bacteria. Opportunistic bacteria retain the ability to act as commensals or pathogens. However, this depends on the integrity of the bacterial flora of the environment where they are found. For example, Staphylococcus aureus can become pathogenic following an alteration of the microbiome due to antibiotic therapies, pharmacological immunosuppression, or radiant therapies.

Staphylococcus Aureus [21] colonizes about 30% of the human population asymptomatically in the nostrils, transiently or persistently. Therefore, it can be considered a human commensal [22].

4. Microbiota and Rhinosinusitis

CRS is a common and widespread inflammatory disease of the upper respiratory tract that significantly impacts the social aspect of life. It worsens the quality of life in everyday life and society, both from an economic view to public health costs.

The nasal microbiome related to CRS has been analyzed in different studies, which revealed a frequent presence of coagulase-negative Staphylococcus, Pseudomonas and Staphylococcus aureus [23]. A recent study [24,25] analyzed the microbiome of patients with nasal polyps by comparing it to that of patients without nasal polyposis. The study established a prevalence of Streptococcus, Haemophilus, and Fusobacterium in patients without nasal polyps versus a predominance of Staphylococcus, Alloiococcus, and Corynebacterium in patients with nasal polyposis.

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In a recent study, by De Boeck et al [26] , Dolosigranulum pigrum was clearly more associated with upper respiratory tract (URT) in healthy subjects, while Corynebacterium tuberculostearicum, Haemophilus influenzae / H. aegyptius and Staphylococcus taxa were found to be more present in CRS patients. Understanding the mechanisms underlying the dysregulation of the nasal microbiome can be instrumental in both the clinical and post-operative evolution of the patient with CRS. In patients with CRS, the microbiome has a reduced bacterial diversity but a higher bacterial load.

Furthermore, less stable bacterial species replace more stable bacterial species (Propionibacterium acnes), favoring the colonization of potentially pathogenic bacteria [27,28]. The resulting dysbiosis could cause an alteration of the epithelial barrier, increasing its permeability to pathogens followed by the release of inflammatory factors (cytokines and chemokines) with a consequent compromise of the immune system and chronic inflammation [29]. Moreover, some studies have demonstrated the presence of viruses and fungi in the mucosa of patients with CRS, which would contribute to increased adhesion of bacterial pathogens to the damaged mucosa [27,30 -33]. Of great importance are the bacterial biofilms that are detected on the mucosa of patients with CRS. The development of a microbial biofilm is a complex process. Initially, sessile planktonic bacteria adhere to the mucosal surface and form microcolonies. Once taken root, the bacteria begin to proliferate and secrete an extracellular matrix composed of polysaccharides, nucleic acids, and proteins. This matrix protects the biofilm from harmful factors present in the environment. When bacterial density reaches a critical point, interbacterial cross-talk occurs, triggering a phenomenon known as "quorum sensing" or "communication capacity of bacterial cells." This phenomenon determines the biofilm phenotype that allows bacteria to communicate through small signal molecules to adapt to any change in the environment. The biofilm phenotype is morphologically characterized by the formation of microbial towers, composed of layers of live bacteria embedded within intermediate water channels. Bacteria in biofilms are more resistant to host defenses. The extracellular matrix that makes up most biofilm protects bacteria from antibodies, immune system phagocytosis, antibiotic penetration, and complement binding [34,35].

The biofilm may be pro-inflammatory, through different mechanisms including the release of planktonic organisms and the production of superantigens, which can cause ciliary dysfunction, and inhibition of mucociliary clearance [36].

The mucociliary clearance system represents a defense against inhaled particles. Therefore, its dysfunction favors the colonization of pathogenic bacteria and the establishment of inflammatory processes [37], contributing to the pathogenesis of CRS [28,38].

5. Probiotics

The WHO defines probiotics as products that contain living microorganisms that, when administered in the correct quantity, benefit the host's health [6].

Probiotics should not be confused with prebiotics. Prebiotics are substances derived from foods that cannot be digested, whose beneficial effect on the host is their contribution to the growth, activity, or both types of bacteria.

The products containing prebiotics and probiotics are called symbiotic [39]. The mechanism of action of probiotics has been described mainly in the gastrointestinal system and includes several strategies through which they inhibit the action of pathogenic microorganisms. Probiotics may induce inhibition of adhesion of pathogens to the mucous membranes, the stabilization of tight junctions in the epithelial layer with reduction of the permeability of the mucosa, the competitive inhibition of pathogens, modulation of

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the immune system, and the production of various substances toxic to pathogenic microorganisms [40]. In a 2018 review, Martens et al. [41] described the possible mechanisms of action of probiotics in the respiratory tract, focusing on the positive effect of probiotics on the epithelial barrier and the immune system. They described the action of probiotics in restoring the epithelial barrier through the modulation of tight junctions and adherence junctions and their effect in modulating the host's immune response through their interaction with dendritic cells. This promotes regulatory T cells (Tregs) and downregulates T-helper 1 and T-helper 2. In studies evaluating potential probiotics, the ability to adhere to the nasal epithelium, the ability to survive in aerobic conditions and at low temperatures, must be considered [42]. These conditions are necessary for the probiota to compete with opportunistic bacteria such as S. Aureus.

6. Clinical studies

Habermann et al. in 2002 [43], conducted a double-blind, placebo-controlled, multicenter study on the efficacy of human Enterococcus Faecalis (Symbioflor®1) in reducing the frequency of exacerbations of CRS on a sample of 157 patients. Half of the patients were treated with the oral administration of drops containing the probiotic for six months. The other half of the patients were treated with placebo also for six months. After eight months of follow-up, there were about half of exacerbations in patients treated with human Enterococcus Faecalis compared to patients treated with placebo.

In another prospective, randomized, double-blind, placebo-controlled trial, Mukerji et al. in 2009 [44], used the oral administration of a probiotic strain of Lactobacillus Rhamnosus R0011 (500 million active cells in tablets, twice a day) for four weeks in a group of 38 patients with CRS;

39 CRS patients represented the placebo-treated control group. The authors used the SNOT-20 quality of life test to assess the effectiveness of the treatment. After four weeks, patients treated with the Lactobacillus probiotic reported a better quality of life than the control group. However, this benefit was not confirmed over time, as after eight weeks, there were no significant differences in the responses to the quality of life test between the two groups.

Martensson et al. in 2017 [45], in a randomized, double-blinded, crossover, and sham-controlled study, evaluated the effects of administration through a nasal spray of honeybee lactic acid bacteria (LAB). Honeybee LAB, consisting of various lactobacilli and bifidus bacteria, was administered to 20 patients with CRSsNP for two weeks. The efficacy of the treatment was assessed by considering the trend of the symptoms through the use of the SNOT-22 questionnaire. The impact of the treatment on the microbiome and inflammation products (IL-6, IL-8, and TNF-9) was evaluated using the nasal wash fluid. The treatment proved to be well tolerated. However, it was not effective in reducing symptoms, nor did it affect the microbiota composition. There was no change in the inflammation processes.

Endam et al. [46] conducted a prospective open-label pilot trial of the safety and feasibility study. The authors wanted to verify if topical administration of L. lactis W 136 for 14 days to the nasal and sinus cavities would be safe for patients with CRS refractory to medical and surgical treatment. The evaluation of symptoms was performed with the SNOT-22 test. Simultaneously, an endoscopy nasal was carried out to evaluate the conditions of the mucosa nasal and the UPSIT-40 test to detect the olfactory function. The treatment turned out to be well tolerated for all 24 patients and was found to improve symptoms

that remained 14 days after the end of the course of treatment with the probiotic, while the sense of smell remained stable. (Table 1)

Table 1. Clinical Studies

Author	Type of study	Probiotic	N. patients	Results
Habermann et al. 2002 [7]	Multicenter, randomized, double blind, placebo controlled trial	Enterococcus faecalis	157	Reduction of CRS flare-ups
Mukerji et al. 2009 [8]	prospective, randomized, double-blind, placebo-controlled trial	Lactobacillus rhamnosus	77	Transient improvement in the quality of life
Martensson et al. 2017 [9]	randomized, double-blinded, cross-over, and sham-controlled trial	Honeybee lactic acid bacteria	20	Not effective
Endam et al 2020 [7]	Prospective open-label pilot trial of safety and feasibility.	Lactococcus lactis	24	Transient improvement in CRS symptoms

7. In vivo experimental studies

In 2016, Schwartz et al.[47] evaluated the capacity of the two gram-positive probiotics strains of Lactococcus Lactis (L. lactis) to stimulate the production of IL-10 and TNF on preparations of monocytes peripheral blood (PBMC) was evaluated. Further, the authors assessed the application safety of L. lactis on mucosal cells of the paranasal sinuses of patients with and without rhinosinusitis. These in vitro studies have supported the safety and immunomodulatory capacities of L. Lactis for intranasal use. The cultures of cells of the mucosa of the paranasal sinuses of patients with and without CRS showed no evidence of toxicity when exposed to the supernatant of this strain. Conversely, the preparations of peripheral blood monocytes showed the induction of IL-10 and TNF without evidence of toxicity or excessive Th1-type inflammation. The authors concluded by stating that topical nasal therapy could represent a new therapeutic strategy for patients with CRS. In an in vitro study, Cho et al.[48] 2020, assessed the growth of six strains of Pseudomonas Aeruginosa derived from patients with CRS (three patients with cystic fibrosis and one patient with ciliary dyskinesia)—the first strain of Pseudomonas aeruginosa from the laboratory. These strains were co-cultured with Lactococcus lactis (obtained from commercial probiotic nasal washes) in the presence of mucin. Many Pseudomonas aeruginosa strains were grown without Lactococcus lactis (control cases). No influence on the growth of Pseudomonas aeruginosa colonies was observed in cultures where Lactococcus lactis was present. Growth inhibition of Pseudomonas aeruginosa was observed only in one culture found to be contaminated with Stenotrophomonas maltophilia. The authors concluded that nasal lavage with probi-

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otics (Lactococcus lactis) may not be helpful for all patients. Therefore, further experiments are needed to evaluate the interactions between Pseudomonas aeurigi-
nosa and Lactococcus lactis.

In 2012, Abreu et al. [49] conducted a study to determine whether Corynebacte-
rium tuberculostearicum exhibited pathogenic potential and whether this could be affected by the resident microbiota. They developed a mouse model of sinus infection using goblet cell hyperplasia and mucin hypersecretion as markers of pathology.

Nasal inoculation of large numbers of Corynebacterium tuberculostearicum in the presence of a complete (healthy) sinus microbiota resulted in an increase in the number of mucin-secreting goblet cells. Animals treated with both an antibiotic (to reduce the bacterial load in the microbiota) and Corynebacterium tuberculostearicum showed profound goblet cell hyperplasia. To demonstrate that goblet cell hyperplasia and mucin hypersecretion were explicitly induced by Corynebacterium tuberculostearicum, they repeated the experiment by adding a group of antibiotic-treated murine before nasal inoculation of Lactobacillus sakei, which is present in abundance in healthy mucosal samples and significantly reduced in CRS patients. Sinus mucosal histology demonstrated that the group treated with antibiotics and inoculated with Corynebacterium tuberculostearicum showed significant increases in goblet cell hyperplasia and mucin hypersecretion. However, mice that received identical numbers of Lactobacillus
sakei demonstrated epithelial physiology comparable to that of control animals (no significant difference in the number of goblet cells), thus confirming that the observed sinus histopathology was explicitly due to Corynebacterium tuberculostearicum. Furthermore, species such as Lactobacillus sakei protect the epithelium of the rhino-sinus mucosa through competitive inhibition of Corynebacterium tuberculostearicum. In 2014, Cleland et al. [50] investigated the probiotic properties of Staphylococ-
cus epidermidis against Staphylococcus aureus in murine sinusitis models. They demonstrated that Staphylococcus epidermidis exerts a probiotic effect by producing a serine protease that inhibits biofilm production and Staphylococcus aureus colonization. Even in this case, the hypertrophy of muciparous cells and their hypersecretion, and characteristics of the CRS were considered markers of inflammation. This study showed that Staphylococcus epidermidis can be a potential probiotic having induced, in a murine sinusitis model, reduced counts of goblet cells in a group of co-inoculated mice Staphylococcus epidermidis + Staphylococcus aureus compared to those who receive only Staphylococcus aureus (Table 2).

Table 2. Experimental Studies

Author	Type of study	Probiotic	Conclusions
Schwartz et al. 2016	In vitro study	Lactococcus lactis	Absence of cellular toxicity, induction of IL-10 and TNF .
Cho et al. 2020	In vitro study	Lactococcus lactis	Lactis nasal washes may not be helpful for all CRS patients
Abreu et al. 2012	In vivo study (mouse)	Lactobacillus sakei	Treatment with L.sakei could counteract the action of C. tuberculostearicum
Cleland et al. 2014	In vivo study (mouse)	Staphylococcus epidermidis	S. epidermidis inhibits the colonization of S. aureus

8. Conclusions

In the literature, there are still few and conflicting studies on the efficacy of probiotics in acute inflammatory diseases of the upper airways and, in particular, in CRS. The studies available to date are also based on small sample sizes. Only three of the studies described above, of which two are in vivo and one in vitro, described a beneficial effect of treatment with probiotics on CRS. Animal studies highlight the ability of some probiotics to reduce inflammatory phenomena of CRS on the mucosa. To the best of our knowledge, no other data in the literature can illustrate the long-term effect of probiotics on CRS. Further efforts will undoubtedly have to be made to evaluate the potential of probiotics on CRS. Indeed, the study of the microbiota of affected patients and bacterial biofilm will have to continue.

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