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An overview on upper respiratory tract infections and bacteriotherapy as innovative therapeutic strategy

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Abstract. – OBJECTIVE: The aim of this review is to describe the most common recurring and chronic upper respiratory tract infections (URTI) in children and discuss the role of bacterial interference and bacteriotherapy in their prevention and treatment.

MATERIALS AND METHODS: A literature review has been performed on the following topics: acute otitis media, adenoiditis, tonsillitis, rhinosinusitis, microbiotics and the role of bacterial interference, and bacteriotherapy in the prevention and treatment of URTI.

RESULTS: Research studies into the characteristics of the microbiological flora and its role in the pathogenesis of URTI have focused on a single pathogen, on resistance to and ineffectiveness of antibiotic therapies, or on the persistence of bacterial biofilm. Recent evidence supports a central role of the existing microbial ecosystem in the pathogenesis of respiratory disease. In the light of this, new therapeutic approaches include the implantation and persistence within the normal microflora of relatively innocuous "effector" bacteria that can competitively exclude or prevent the outgrowth of potentially disease-causing bacteria. Recently, a retrospective and observational study demonstrated that *S. salivarius* 24SMB and *S. oralis* 89a nasal spray could be effective in the prevention of recurrent otitis media in a real-life setting. Other studies have focused on the role of bacteriotherapy in children with beneficial effects in the prevention of URTI.

CONCLUSIONS: The results of previous studies on the role of bacteriotherapy in paediatric URTI suggest that the use of bacterial interference phenomena through bacteriotherapy is a feasible, safe approach and deserves proper consideration as a promising therapeutic strategy against URTI.

Key Words
OSAS, Children, Snoring, Multidisciplinary approach.

List of Abbreviations

URTI: upper respiratory tract infections; AOM: acute otitis media; RSV: respiratory syncytial virus; OME: otitis media with effusion, SOM: suppurative otitis media.

Introduction

Recurring and/or chronic inflammations/infections of the upper respiratory tract are extremely frequent in the early years of life while children are still developing immune properties.

Immediately after birth, the upper airway rapidly becomes colonized by commensal and potentially pathogenic bacteria: within the first year, 50 to 100% of infants are colonized by *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis*[1]. In general, colonization rises gradually and peaks at 2-3 years of life, then decreases until 15-16 years, eventually increasing again in adults older than 65 years[2]. Although variable, the colonization rates in healthy adults are lower than those in children. Colonization is a dynamic phenomenon varying not only from delivery, through childhood, adolescence, adult and advanced age, but also according to genetic background variables, socioeconomic conditions and geographic areas[3-5]. The upper airway is a major ecological reservoir of bacterial species: as in the gut, the early pharyngeal microbiome influences colonization and respiratory tract health[6]. Protective and potentially pathogenic bacteria have been isolated in populations of healthy children, such as persistent or transient pathogens of the naso- and oropharynx. These are: *Neisseria, Streptococcus pyogenes, Haemophilus influenzae, Staphylococcus aureus, Actinomyces, Bacteroides, Prevotel-*

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la, Porphyromonas, Peptostreptococci, and *Fusobacterium* spp[7,8]. Pathogens enter the host by colonizing the nasopharyngeal mucosal epithelium, but identifiable disease occurs in only a small percentage of persons who are colonized.

To date, research into the characteristics of the microbiological flora and their role in the pathogenesis of recurring and chronic upper respiratory tract infections (URTI) has focused on a single pathogen, on resistance to and ineffectiveness of antibiotic therapies, or, more recently, on the presence/persistence of bacterial biofilm[9]. The worldwide emergency of bacterial resistance to antibiotics has led to the need for new methods of combating bacterial infections[10]. This emergency is aggravated by the long delay in the development of new antibacterial agents. If the design of new agents proves to be in the right direction, such progress would not resolve all current resistance problems; in addition, it should be remembered that the use of antibacterial agents selects not only resistant bacteria but may also negatively affect normal bacterial flora even if with different degree of interference[11]. The administration of antimicrobial agents disrupts normal human flora, further reducing our defense against infections[12]. Modern molecular culture-independent approaches to chronic respiratory diseases have opened a promising scenario on microbial communities associated with the human body and their role in health and diseases, but consensus does not yet exist on the microbial ecology associated with chronic diseases. Last-century theories about the mechanisms involved in infectious episodes viewed the lymphatic vessels and the immune cells of the mucosa grouped in the specific area named Waldeyer's ring as the most important line of defense of our body from external attacks. Lymphoid tissue in Waldeyer's ring structures (namely adenoids, palatine tonsils, tubal tonsils and lingual tonsils) is predominantly characterized by crypts and mucosal irregularities, rendering them more susceptible to antigenic responses through the acquired immunity mechanisms. Nowadays, the upper airway microbiome, including saprophytic, commensal, pathogenic bacteria, fungi, and viruses, is considered the first line of defense of our body; when the inflammatory response to pathogenic stimuli starts, a situation of disequilibrium or dysbiosis opens the door to clinically evident infection and recurring/chronic disease [13]. The aim of this review is to describe the most common recurring and chronic inflammations of the upper respiratory tract in children, such as acute otitis media, adenoiditis and tonsillitis, and rhinosinusitis, and discuss the role of bacterial interference and bacteriotherapy in their prevention and treatment.

Materials and methods

A literature review has been performed on articles retrieved from PubMed and Scopus on the following topics: acute otitis media, adenoiditis, tonsillitis, rhinosinusitis, microbiotics and the role of bacterial interference, and bacteriotherapy in the prevention and treatment of URTI.

Results

Acute Otitis Media

Acute otitis media (AOM) is the most common bacterial infection in young children, with consequences on hearing and associated symptoms that in some cases may also affect behavior and language development[14,15]. Large amounts of antibiotics are prescribed for this pathology, especially for those children with recurring episodes. The peak incidence of AOM is at 12 years of age[15]. The majority of AOM episodes occur concurrently with, or soon after, viral URTI. Chonmaitree et al[16] found a high prevalence of symptomatic viral URTI among young children; >60% of cases were complicated by AOM and/or otitis media with effusion (OME). In addition to young age and immature Eustachian tube function, specific viral infection, such as adenovirus, respiratory syncytial virus (RSV), coronavirus and viral load, are predictors of URTI complicated by AOM.

The risk of developing another episode within one month after the onset of the primary infection is estimated at 35%; in the majority of cases (75%) the new episodes are infections by new bacterial strains, suggesting an underlying susceptibility to recurrent AOM. The remaining 25% are from either re-infection with the same bacterial strain or treatment failure[17].

About 5% of children are "prone" to otitis media, defined as three episodes within a 6-month time span or 4 events within 12 months[18]. More recently, the definition of "stringent otitis prone" was proposed when the clinical diagnosis is confirmed by bacterial culture of the middle ear fluid[19].

Bacterial culture confirmation is of the utmost importance: signs and symptoms are often aspecific and empiric clinical diagnosis doesn't allow

differentiation of OME (i.e. the presence of fluid in the middle ear without signs or symptoms of acute infection) from AOM, which could limit unnecessary antibiotic use and microbial flora alterations. OME often resolves spontaneously, and only rarely may insertion of a tympanostomy tube be required for fluid drainage[20].

Disease etiology and pathogenesis are multi-factorial and begin with colonization of mucosal surfaces in the upper respiratory tract by AOM pathogens[21].

The most common bacteria associated with AOM are *Streptococcus pneumoniae, Haemophilus influenzae* and, less often, *Moraxella catarrhalis* and group A β-streptococci[15]. These bacteria originate and diffuse from the nasopharynx to the middle ear cavity by way of the Eustachian tube.

Secretory otitis media (SOM) is the most common sequela of AOM; it is characterized by persistence of effusion in the middle ear cavity[20]. One or more of *S. pneumoniae, H. influenzae*, or *M. catarrhalis* are found in approximately 30% of patients with suppurative otitis media (SOM)[15,22]. A strong correlation between the nasopharyngeal flora and middle ear infections has been found. Nasopharyngeal colonization by *H. influenzae, S. pneumoniae* and/or *M. catarrhalis* in the first year of life is associated with an increased risk of recurrent AOM or SOM compared with children who remain free of colonization. The factors that influence colonization are multiple and not entirely clear. There is no doubt that the local host immune response plays an important regulatory role in the trafficking of pathogens in the upper airway. Other factors influencing nasopharyngeal carriage rates are: age, season, type of child day care, number of siblings, acute respiratory illness, diet (breast-feeding *vs.* bottle-feeding), and sleeping position. In adults, other factors are implicated: presence of children at home or at the workplace, chronic obstructive pulmonary disease, obesity, immunosuppression, allergic conditions, and acute sinusitis[2].

In children with recurrent AOM potential pathogens may constitute > 90% of the total bacterial count[15,22]. In contrast, among healthy children viridans streptococci are predominant[23]. Data from Syrjanen et al[24] show that *S. pneumoniae* carriage is lower during health (13-43%) than during AOM episodes, reaching 97-100% carriage in pneumococcal AOM. Otitis-prone children tend to be colonized more often than non-otitis-prone children[25]. In addition, nasopharyngeal colonization with antibiotic-resistant bacteria is associated with an increased incidence of unresolved otitis media [2]. Epidemiologic studies have shown that the risk of *S. pneumoniae* colonization differs according to whether *H. influenzae*, *M. catarrhalis*, and *Staphylococcus aureus* are also present[26-28]. Bacterium-bacterium interaction may also impact AOM incidence. Simultaneous colonization by multiple AOM pathogens is associated with a greater risk of AOM than the risk associated with colonization by a single AOM pathogen [29,30]. Members of the normal flora, such as *alpha-hemolytic streptococci*, inhibit the growth of AOM pathogens *in vitro*[31]. Healthy children are more likely than children with AOM to be colonized by *alpha-hemolytic streptococci*[32-34]. Lower numbers of *alpha-hemolytic streptococci* have been found in the nasopharynx of children who are prone to otitis media compared with those who are not prone and in those with SOM compared with healthy children[15]. Collectively, these data indicate that certain commensals influence the risk of AOM pathogen colonization and the subsequent risk of disease [21].

Adenoiditis/Tonsillitis

The adenoids are believed to play a role in several infectious and non-infectious pathologies of upper airways[35]. Since the 1980s, it has been clear that they are implicated in the etiology of OMA[36-41], rhinosinusitis[36,39,42], adenotonsillitis[43] and chronic nasal obstruction[44,45]. The adenoids in healthy children contain potential respiratory pathogens[43,46]. The nasopharynx and adenoids of healthy individuals, unlike subjects with recurrent URTI, are generally colonized by aerobic and anaerobic microorganisms that are able to interfere with the growth of potential pathogens[47,48].

Exposure to antimicrobial therapy can alter the colonization patterns and select for resistant organisms. Production of β-lactamase is one of the major mechanisms of resistance of these organisms. Maintaining the beneficial effects of normal flora by avoiding unnecessary exposure to antimicrobial therapy may be a useful tool in preventing colonization of the adenoids and/or tonsils by potential pathogens[35].

Adenoids are liable to inflammatory changes (viral, allergic) and frequently are infected concomitantly with the tonsils. Acute adenoiditis may occur alone or in association with rhinitis or tonsillitis. Chronic adenoiditis may result from repeated acute attacks or from persistent infection. Adenoid hypertrophy is defined as an enlargement of the adenoids, which may be simple or inflammatory, and may be linked to infection. Recurring adenotonsillitis is often a bacterial-vi-

ral illness[35]. Along with recurring tonsillitis, adenoid hypertrophy and recurring adenotonsillitis constitute adenotonsillary disease characterized by persistent bacterial infection[35].

Periodic episodes of fever and upper airway obstruction are the major symptoms of adenoiditis and recurring tonsillitis.

Recurring or chronic inflammation of the adenoids and faucial tonsils leads to chronic activation of the cell-mediated and humoral immune responses, resulting in hypertrophy of the lymphoid tissue. This hypertrophic tissue is the cause for the prominent clinical symptoms: obstruction of the upper airways, snoring, and sleep apnea for adenoiditis or sore throat, dysphagia and halitosis for recurring tonsillitis[49].

In adenoids and tonsils, bacteria, mostly *Staphylococcus aureus*, *Haemophilus* sp., and *Streptococcus* sp., persist predominantly intracellularly and within mucosal biofilms[50]. As with recurring tonsillitis and adenoiditis, the most likely mechanism for pathogenesis is the endogenous reactivation of these persistent pathogenic bacteria[51,52]. In recent research, biofilm formation was identified as playing a pivotal role in the etiology of numerous chronic otorhinolaryngologic infections, such as chronic rhinosinusitis, chronic otitis media, and adenotonsillar disease, which do not respond to conservative antimicrobial treatment[49,53,54].

Bacteria in biofilms are surrounded by a carbohydrate-containing matrix on or within the adenotonsillar epithelium, and communicate with each other by a process called quorum sensing (QS), which responds to the density of cell populations[55,56]. Biofilms in the adenoids may act as reservoir for chronic otitis media and chronic rhinosinusitis[57].

Thus, adenoidectomy is shown to be a plausible therapy for two diseases: as surgical removal of an infectious source in the nasopharynx and as first-line therapy for medically refractory, uncomplicated paediatric rhinosinusitis[58,59].

Conservative treatment of moderate grades of hypertrophy with bacterial compounds must consider these bacterial persistence strategies.

Rhinosinusitis

The upper respiratory tract, including the nasopharynx, serves as a reservoir for pathogens capable of causing URTI, including rhinosinusitis[60]. Potential pathogens can relocate during a viral respiratory infection, from the nasopharynx into the sinus cavity, causing a sinusitis[61]. The nasopharyngeal carriage of upper respiratory tract pathogens, such as *S. pneumoniae, H. influenzae* and *M. catarrhalis*, can, however, occur in healthy subjects; it increases significantly in children during respiratory diseases and in sinusitis[8,34]. The nasopharynx of healthy individuals is generally colonized by relatively non-pathogenic aerobic and anaerobic organisms[48,62], some of which are able to interfere with the growth of potential pathogens[63]. This phenomenon is called bacterial interference[8]. These organisms include, amongst others, aerobic microorganisms such as alpha-hemolytic streptococci (mostly *Streptococcus mitis* and *Streptococcus sanguinis*)[64] and anaerobic bacteria (*Prevotella melaninogenica* and *Peptostreptococcus anaerobius*)[65]. The absence of interference between organisms may explain the greater possibility of recruiting pathogens in these children. The colonization of interfering organisms can play a role in preventing colonization by pathogens and the development of URTI, including sinusitis. Exposure to direct and indirect smoking can increase oral colonization with pathogenic bacteria and decrease the number of organisms that interfere with their growth. The flora of smokers contains fewer aerobic and anaerobic organisms with interfering capability and more potential pathogens as compared with nonsmokers[66]. The high number of pathogens and the low number of interfering organisms found in the nasopharynx of smokers reverts to normal levels after complete cessation of smoking[67].

The pattern of many URTI, including sinusitis, evolves in different phases. The initial phase is often a viral infection that usually lasts up to 10 days, after which it manifests a complete recovery in most subjects[68]. However, a small number of patients with viral sinusitis may develop secondary acute bacterial infection[8]. This is generally caused by optional aerobic bacteria (i.e., *S. pneumoniae, H. influenzae,* and *M. catarrhalis*)[8], also called "the infernal trio"[69]. The maxillary sinus has 26% of *S. pneumoniae*, 28% of *H. influenzae* and 6% *M. catarrhalis*, plus 8% of *S. aureus*[70]. If the episode does not resolve, anaerobic bacterial flora becomes predominant over time[8]. The dynamics of these changes in bacterial flora have been demonstrated by performing serial cultures in patients with maxillary sinusitis[71]. It could be assumed that chronic sinusitis is an extension of an acute non-resolved infection[8]. The acute exacerbation of chronic sinusitis is a sudden worsening of chronic sinusitis (worsening of symptoms in progress or the appearance of new symptoms).

Epithelial cells constitute the first barrier to pathogenic agents such as viruses or bacteria.

Goblet cell mucus secretion prevents the microorganisms from sticking to the epithelial cells, thus preventing them from entering the human body. The microorganisms trapped in the mucus are mechanically removed by cilia movements[71]. It may happen that with the progress of the pathogenic process there is a transient increase in pressure in the sinus cavity due to accumulation of mucus. Then subsequently, a "negative pressure" may develop due to an alteration of sinus ventilation with a rapid absorption of oxygen by the mucosa cells[72]. Such an event worsens local congestion, promotes mucus retention, compromises normal gas exchange, reduces both oxygen and pH, prevents infectious material and inflammatory debris from occurring, and increases the risk of a second bacterial infection[73-76].

Discussion

Microbiotics and the Role of Bacterial Interference

Recent evidence supports the hypothesis that the microbiota of the skin and mucous membranes constitutes a "superorganism"[77]. Mutual benefits for the host and microbes are numerous: microbes utilize a nutrient-rich environment, that is of the host, but in return they play a key role in providing a barrier against potential pathogens. Understanding the interactions within the human "superorganism" and their consequences for health and illness requires detailed information on the composition of the microbiome that constitutes the many and distinct ecosystems of the human body.

The upper respiratory tract is the reservoir of a community of potential and pathogenic pathogens including *Streptococcus pneumoniae (pneumococcus), Haemophilus influenzae, Moraxella catarrhalis* and *Staphylococcus aureus*, which under certain circumstances become pathogenic agents responsible for infectious diseases[78,79].

In order to cause respiratory diseases, the bacteria must first colonize the nasopharyngeal niche. The colonization of this niche is a complex dynamic process that involves the acquisition and elimination of species, interactions between microbes and between microbes and host, and interference by environmental factors. In a state of equilibrium, this ecosystem, understood as part of the "human microbiome", is presumed to play an important beneficial role for the person who plays the role of host[80]. However, imbalances in the respiratory microbial community can contribute to the acquisition of new bacterial or viral pathogens, the carriage of more potent pathogenic bacteria, or a viral co-infection.

Subsequently, imbalances in the ecosystem may result in excessive growth and invasion of pathogenic bacteria, causing inflammatory or invasive diseases, particularly in children with immature immune systems[81]. Colonization of this niche is therefore a crucial step in the pathogenesis of respiratory disease[82]. In 1960, Hardin argued that completely competitive species cannot colonize the same ecological niche, indicating that one microorganism has the possibility of fully extinguishing another. However, the concept of colonization is more complex and dependent on several factors. For example, skin and any mucosal surface of the body are colonized directly after birth by a wide range of bacteria[82]. These bacterial communities evolve into a complex ecosystem during the first years of life, varying considerably among individuals and over time[83,84].

Alpha-streptococci dominate the normal flora in the upper respiratory tract and have been the bacteria attracting the most attention in bacterial interference. Most alpha-streptococci have the ability to emit bacteriocins. These are extracellularly released toxins produced by the bacteria [85] that are able to selectively kill other bacteria. Some bacteria release bacteriocins with a broad spectrum, and others release bacteriocins with a very narrow spectrum. Studies have shown that bacteriocin production is a significant ecological determinant in colonization. Nearly all-oral alpha-streptococci release inhibitory substances[86], but bacterial interference can also occur by different mechanisms such as competition for sites at the epithelial cells, competition for specific nutrients, and production of substances that change the pH, like hydrogen peroxide[7,87].

The microbiome of the upper respiratory tract niche seems to be influenced by the host genetic background, age, and environmental factors such as social status, antibiotic use, vaccination, seasonality, cigarette smoking, social contacts, and number of siblings[2,24]. Studies[88] have shown a decrease in normal flora after antibiotic treatment in URTI.

A decreased number of alpha-streptococci, with interfering efficacy against beta-streptococci, have also been found on the tonsils of patients with recurrent streptococcal pharyngo-tonsillitis, compared with healthy individuals[89]. Grahn and Holm[90], in a study of an outbreak of streptococcal

tonsillitis in an apartment house area, have shown that individuals with interfering alpha-streptococci on their tonsils less often acquired streptococcal pharyngo-tonsillitis than patients lacking these bacteria. Lower numbers of alpha-streptococci have been found in otitis-prone children compared with non-otitis prone children[33], and in patients with secretory otitis media compared with healthy children[34]. Tano et al[31] and Brook and Gober[11] recently found that alpha-streptococci in children with recurrent OMA and SOM had less inhibiting activity against pneumococci and *H. influenzae* compared with alpha-streptococci isolated from healthy children. Additionally, site-specific factors and characteristics of the microorganism itself also play a role. By colonizing a niche, a microbe should be able to survive local clearance mechanisms (i.e., mucus, ciliae, etc.), attach to the epithelium, rely on locally available nutrients, and bypass surveillance by the host immune system[82]. Another essential condition for colonization is to outcompete inhabitants that were already present in the upper respiratory tract[91,92]. *S. pneumoniae, H. influenzae, M. catarrhalis* are etiologic agents commonly recognized in URTI and the colonization by these species is also very common under healthy circumstances, with a high colonization rate especially in children[2,26,93-96]. However, bacterial commensals are thought to play an important role in preventing respiratory and invasive disease[82]. Sanders et al[47] and later Grahn et al[97] showed that some alpha-streptococci may have an inhibiting capacity on group A streptococci *in vitro.* These data support the importance of bacterial interference in maintaining the balance between resident flora and pathogenic microorganisms[2]. Since these colonizing microorganisms share the natural niche with other non-pathogenic microorganisms, it is probable that these species interact with each other even in the absence of pathology. Microorganisms compete for nutrients and space, and when microorganisms overwhelm the native saprophytes of that niche, they can colonize; da Lilja et al[98] documented beta-hemolytic streptococci in tonsillary epithelial cells of patients with acute tonsillitis symptoms, as opposed to those found in asymptomatic patients, where these bacteria did not adhere to the epithelial cells but were found only on the surface mucous layer.

Maintaining normal pharyngeal flora can contribute to preventing or reducing recurrent infections[7] or preventing colonization by potential pathogens[7,14]. Bacteriotherapy, the use of harmless bacteria to counteract pathogenic organisms, is an alternative and promising way of combating bacterial infections[12].

The Role of Bacteriotherapy

The worldwide emergency of bacterial resistance to antibiotics has led to the need for new methods of combating bacterial infections[10,12]. This emergency is aggravated by the long delay in the development of new antibacterial strategies[12]. If the design of new agents proves to be in the right direction,such progress would not solve all current resistance problems[12]. Penicillin-specific antibiotic therapies[99] of streptococcal pharyngeal infections, in line with current therapeutic recommendations and guidelines, fails in about 35% of treatments: alpha-streptococci bacteria are believed to play an important role in prevention, colonization, and subsequent infection as a part of beta-hemolytic streptococci, and their absence in the 3 weeks following antibiotic therapy may favor recurrence of episodes and, therefore, a failure of treatment[100]. While targeting resistant bacteria, antibacterial agents also disrupt normal human flora, further reducing our defenses against infection[12] and, more generally, negatively affecting normal bacterial flora[101].

The accessible surfaces of the skin, spleen cavities, upper respiratory tract, gastrointestinal tract and vagina of healthy vertebrates are colonized by microbes immediately after birth[102]. The process is a dynamic, orderly succession of microbial acquisitions and site-specific eliminations[102]. Collectively known as the normal microflora or indigenous microbiota, these microbes are the body's first line of defense against pathogenic invasion[102].

The basis of replacement therapy is the implantation and persistence within the normal microflora of relatively innocuous "effector" bacteria that can competitively exclude or prevent the outgrowth of potentially disease-causing bacteria, without significantly disturbing the balance of the existing microbial ecosystem[102].

An early attempt to recolonize the high respiratory tract ecosystems was documented by Sprunt and Leidy[103], who reported that a single inoculation of intensive-care infants with alpha-streptococci isolated from normal flora, in order to avoid excessive growth of pathogens, helped restore the balance of microflora of most of the infants within 48-72 hours; the authors concluded that this approach was safe in the neonatal population. Further studies[104-106] evaluated the use of inoculation of the pharyngeal niche with an alpha-streptococ-

ci pool, in order to prevent relapsed streptococcus pharyngitis. Alpha-streptococci are significantly reduced in subjects suffering from pathologies in the pharyngeal district[107] and, conversely, the presence of this bacterial family is associated with a significant reduction in the risk of recurrent infectious diseases in the pharyngeal district[108]. The inoculation agent of alpha-streptococci was used in these studies because patients treated with antibiotics often have pharyngeal flora that becomes completely deregulated with the likely deficiency of alpha-streptococci, which have an interfering activity against beta-hemolytic streptococci.

Roos et al[104] conducted a randomized, double blind, placebo-controlled study that enrolled 36 patients with relapsing streptococcal tonsils, who first underwent antibiotic therapy for 10 days; 19 patients were then treated with an oral spray comprising 4 strains selected from alpha-streptococci, and 17 patients received placebo. None of the patients in the intervention group presented a new episode of tonsillitis during the 3-month follow-up period, while 7 of 17 patients in the control group had a tonsillitis episode (p <0.05); after 3 months of follow-up, 1 patient in the first group *vs.* 11 in the control group presented an episode of tonsillitis. The authors suggest that recombination with alpha-streptococci offers a new way of lowering the rate of recurrence in streptococcal pharyngeal infections.

Roos et al[105] extended their research with a randomized, double-blind, placebo-controlled, multicenter study involving 130 patients with recurrent episodes of streptococcal pharyngeal tonsillitis. Patients received antibiotics for 10 days, followed by 5 days of topical spray therapy with alpha-streptococci in the intervention group *vs.* placebo in the other group. Clinical recurrences (bacteriologically verified) in the treatment group of patients were 2%, *vs.* 23% of patients treated with placebo (p<0.004). The authors conclude that the spray treatment with alpha-streptococci, used for at least 5 days, significantly reduces the pharyngo-tonsillitis infectious recurrence episodes.

Falck et al[104] conducted a randomized, double-blind, placebo-controlled, multicenter study involving 342 tonsillitis patients treated with antibiotic therapy for 10 days, then segmented into treatment with topical alpha-streptococci (n=189) and placebo control (n=93). At 75-day follow-up, recurrence rates were treatment group 19% and control group 30% (p=0.037). No serious adverse events were reported.

Diagnosis of AOM requires adequate experience and procedure to ensure precise differential diagnosis, particularly with respect to otitis media with effusion. Moreover, AOM therapy is controversial; many guidelines suggest watchful waiting for mild-to-moderate episodes in children aged >2 years. However, in clinical practice, antibiotics are frequently prescribed, ignoring guidelines and their underlying precepts[109]. Prevention of recurrent AOM is even more debated. At present, no evidence on proposed treatments, conventional and otherwise, is definitively convincing.

A randomized, double-blind, placebo-controlled clinical trial on efficacy and tolerability of intranasal administration of *Streptococcus salivarius* 24SMB was conducted in the treatment of acute otitis in children with a history of acute recurrent otitis media [110]. The study recruited 100 patients aged between 1 and 5 years with a history of acute recurrent otitis media (3 episodes in the previous 6 months or 4 episodes in the previous 12 months with the last episode in the previous 2-8 weeks) and intranasally administered *S. salivarius* 24SMB (n=50) or placebo (n=47) twice daily for 5 days per month for 3 consecutive months. Participants were followed for 6 months (3 months each treatment and follow up). Treated children were twice as likely not to manifest acute otitis media at follow up compared to children in the placebo group (30.0% *vs.* 14.9%; p=0.076). In addition, the number of children who received antibiotics during the study period was lower in children treated with *S. salivarius* 24SMB compared to those who received placebo. Compared with children who were not colonized by *S. salivarius* 24SMB after treatment, the number of colonized children who experienced any acute otitis was significantly lower. Similar results were observed considering antibiotic treatment; the number of colonized children using antibiotics was significantly lower compared with non-colonized children. This study, designed in part to evaluate a nasal delivery device, revealed the ability of *S. salivarius* 24SMB nasal spray to reduce the risk of AOM in children[110,111].

Recently, a retrospective and observational study demonstrated that *S. salivarius* 24SMB and *S. oralis* 89a nasal spray could be effective in the prevention of recurrent AOM in a real-life setting. *S. salivarius* 24SMB and *Streptococcus oralis* 89a nasal spray was administered to 159 children after the first AOM episode; 108 children received no bacteriotherapy and served as control. Active treatment consisted of 3 monthly courses: 2 puffs per nostril twice/day for a week. The intervention group showed a significant reduction of AOM episodes in comparison with controls (p<0.0001). No-

tably, all actively treated children with the highest AOM recurrence had a reduction of recurrence, whereas only 50% of the control group children had reduced recurrent AOM ($p<0.0001$)[112].

Interference between normal flora and pathogens is not limited to acute streptococcal pharyngotonsillitis and acute otitis media. Brook and Gober[113] have shown that the nasopharyngeal flora and nasal flora of non-sinusitis-prone children contain more aerobic and anaerobic organisms with interfering capacity compared with the flora of sinusitis-prone children.

The results of these studies suggest that the use of bacterial interference phenomena through bacteriotherapy is a feasible, safe approach and deserves proper consideration as a therapeutic strategy. A basic concept for bacteriotherapy is the persistence in normal microflora of relatively harmless "efficacious" bacteria, which can competitively exclude or inhibit the growth of potentially pathogenic bacteria without significantly disturbing the balance of the existing microbial ecosystem[114]. Large-spectrum antibiotics administered parenterally indiscriminately kill a wide variety of bacterial species associated with the host microflora, causing the formation of an "ecological vacuum" and encouraging the development of superinfections and bacterial resistance to antibiotics[102]. Replacement therapy, i.e. the direct implantation of relatively harmless bacteria known as being highly competitive against potential pathogens, provides favorably cost-effective and long-term protection tailored to the host against specific bacterial infections[102]. In the literature bacteriotherapy has been trialed in dental caries, using *S. mutans* JH 1000[115]; *L. rhamnosus* GG[116]; *S. equi*[117]; *S. salivarius*[118]; *S. faecalis*[119]; in otitis media using *Streptococcus salivarius* 24SMB, *S. sanguinis, S. mitis* and *S. oralis*[15,110,120]; in streptococcal pharyngitis using *S. salivarius* K12[104] or *S. sanguinis* and *S. mitis* together[104,105]; in pseudomembranous colitis after antibiotic therapy to counteract *Clostridium difficile* infection[121]; and in ulcerative colitis by replanting the bacterial flora of the stools of the same patient[122].

Conclusions

The results of previous studies on the role of bacteriotherapy in paediatric URTI suggest that the use of bacterial interference phenomena through bacteriotherapy is a feasible, safe approach and deserves proper consideration as a promising therapeutic strategy in URTI.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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