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Probiotics in the treatment of otitis media. The past, the present and the future.

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Abstract
Otitis media (OM) is one of the most common infectious diseases in children and the leading cause for medical consultations and antibiotic prescription in this population. The burden of disease associated with OM is greater in developing nations and indigenous populations where the associated hearing loss contributes to poor education and employment outcomes. Current treatment and prevention is largely focused on vaccination and antibiotics. However, rates of OM, particularly in indigenous populations, remain high. With growing concerns regarding antibiotic resistance and antibiotic-associated complications, an alternative, more effective treatment is required. Administration of probiotics, both locally and systemically have been investigated for their ability to treat and prevent OM in children. This review explores the theoretical bases of probiotics, successful application of probiotics in medicine, and their use in the treatment and prevention of OM. We conclude that local administration of niche-specific probiotic bacteria that demonstrates the ability to inhibit the growth of otopathogens in vitro shows promise in the prevention and treatment of OM and warrants further investigation.
1 Introduction

Otitis media (OM) refers to inflammation and/or infection in the middle ear and encompasses a continuum of acute and chronic diseases, clinically characterized by fluid in the middle ear (See Table 1 for definitions) [1, 2]. OM is one of the most common infectious diseases in children [3]. There are approximately 709 million cases of acute OM (acute OM (AOM)) per year worldwide, with the highest disease burden in developing nations and indigenous populations [4-6]. Although most episodes of OM resolve quickly without complication, the disease can be associated with significant health and developmental sequelae. It is estimated that approximately 21 000 people die from OM-related complications around the world each year, the highest mortality rate is in the first year of life [4]. Complications can include mastoiditis, cholesteatoma, meningitis, brain abscess and lateral sinus thrombosis [7]. OM often results in conductive hearing loss, which can impact on speech, language and cognitive development [8, 9]. In Indigenous populations, OM and its sequelae contribute to poor education and employment outcomes and greater contact with the criminal justice system [10].

The pathogenesis of OM is multifactorial and involves the adaptive and native immune systems, eustachian tube dysfunction, viral and bacterial load, and genetic and environmental factors [11]. Otopathogens, most commonly *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* and/or respiratory viruses, colonize and proliferate in the nasopharynx (NP), where they travel up the eustachian tube to infect the middle ear and cause OM [1]. Management depends on the type and severity of OM and include watchful waiting, antibiotics and surgery [12, 13]. A Cochrane review which included 1483 otitis-prone children or children at increased risk of OM from around the world showed that long-term antibiotics reduced the number of episodes of AOM, with a number needed to treat of five to prevent one case of AOM [14]. For every 12 months of antibiotic treatment per child, 1.5 episodes of AOM were prevented [14]. None of the included studies reported on AOM with perforated tympanic membrane (AOMwP) or chronic suppurative OM (CSOM). Long-term treatment regimens with antibiotics can be difficult for families to adhere to, leading to increased risk of antibiotic-resistant pathogens. Furthermore, despite liberal use of antibiotics, the prevalence of OM, particularly in Australian Indigenous communities, remain unchanged [15, 16].
Alternatively, attempts to prevent OM via vaccination has been widely trialed with mixed results. Pneumococcal conjugate vaccines demonstrated a modest reduction in episodes of AOM in healthy infants, and no benefit for high-risk infants or older children with a history of OM [17]. Furthermore, disease displacement with non-vaccine *S. pneumoniae* serotypes and *H. influenzae* have been widely reported [1, 18, 19]. The outcomes of vaccination targeting both *S. pneumoniae* and *H. influenzae* (PHiD-CV10) are also equivocal. In Australian Indigenous children PHiD-CV10 has resulted in some reduction in rates of suppurative OM, however these coincided with an increase in OME [20, 21]. In Finnish children there was a non-significant trend towards reduction in the number of AOM episodes [22]. In contrast, implementation of PHiD-CV10 did not change the prevalence of the three main otopathogens in middle ear fluid or NP of New Zealand children with recurrent AOM or OME [23].

OM treatment and prevention continues to provide a great challenge for researchers and clinicians. An emerging field of research aims at treating and preventing OM using probiotics. Effective probiotic treatment is based on a comprehensive understanding the microbiota in healthy and OM states, and how this can be manipulated to treat and prevent OM. This review explores the history of successful applications of probiotics in medicine, the development of the upper respiratory tract (URT) microbiota, bacterial interference and probiotics in OM, and finally highlights future opportunities for the use of probiotics in OM.

2 Probiotic success in medicine

Probiotics are “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host”[24]. In 1958, a landmark paper described the use of fecal transplantation to treat patients with *Clostridium difficile* enterocolitis, restoring the dysbiosis caused by antibiotics, and resulting in dramatic health improvement [25]. Despite this success, no further research was conducted on fecal transplantation for another 50 years. Recently, fecal transplantation was tested in a randomized control trial (RCT) for the treatment of *C. difficile* enterocolitis against vancomycin and vancomycin plus bowel lavage [26]. This study was stopped after the interim analysis. Of the 16 patients who received the
fecal transplantation, 13 had resolution of symptoms after the first infusion, another two after second transfusion from a different donor. This compared to resolution of symptoms in just four of the 13 patients receiving vancomycin and three of the 13 receiving vancomycin and bowel lavage [26]. This results has been replicated in a number of RCTs [27], and is now offered as treatment for appropriate patients. This is a striking example of where the use of probiotics has been more effective than antibiotics.

Probiotics have also demonstrated impressive results when used in premature infants. Prophylactic administration of probiotics containing *Lactobacillus spp.* alone or in combination with *Bifidobacterium* spp via enteral feeding has been shown to significantly reduced the incidence of severe necrotizing enterocolitis and all causes of mortality in preterm infants [28]. There were no reports of systemic infection with probiotic species [28]. Probiotic prophylaxis is now routinely used in many neonatal intensive care units around the world.

3 The microbiota and development of respiratory immunity
To determine potential candidates for probiotic therapy in OM, a comprehensive understanding of the development of the healthy URT microbiota is required. The URT is colonized by commensal flora during birth [29, 30]. Soon afterwards the URT microbiota rapidly changes to develop niche-differentiation. Henceforth, one of several microbial profiles develop, defined by dominant key species [30, 31]. Profiles dominated by early colonization with *Moraxella* and *Corynebacterium/Dolosigranulum* are more stable than those dominated by *Haemophilus* or *Streptococcus*, and appear to protect against URT infections (URTI) [31, 32]. The healthy URT microbiota has greater richness and diversity when compared to patients with pneumonia, URTIs and AOM [33, 34]. For example, healthy children had twice (n=15) the number of operations taxonomic units (OTUs) identified in the URT compared to children with pneumonia (n=8) [33].

Resident microbial flora contributes to the protection of the host against pathogen proliferation, but is also integral to the development of a competent immune system [35, 36]. Experiments using germ-free mice have demonstrated the importance of the bacterial
microbiota on mediation of immune cell differentiation and subsequent modulation of inflammation [36]. It is now well established that birth via caesarian section is related to a higher risk of inflammatory disease, where the immune system have difficulties distinguishing self from non-self or where external antigens elicit an over the top response such as seen in food allergy, atopy, allergic rhinitis, and asthma [36]. There is speculation that the respiratory microbiota, shaped by method of delivery, may be contributing to this disruption to the developing immune system [29, 30, 36]. Disruption of the respiratory microbiota can impact local immunity, infection susceptibility and the development of chronic respiratory inflammatory diseases [37]. It is believed that there is a critical period in infancy/childhood when the influence of microbes on the developing immune system establishes a system of homeostasis, this is likely to happen during the first year of life [36]. The local administration of probiotics in the upper airways in infancy/childhood may enable the development of a stable, resilient microbiota, and promote the establishment of a healthy immune system with the ability to distinguish self from non-self as well as deliver a measured response to external antigens.

4 Bacterial interference of the upper respiratory tract

Bacterial interference as a means of preventing disease has been reported since the late 1800s [38], however, it has struggled to translate into clinical practice. Bacterial interference is defined as “a dynamic antagonistic interaction between at least two different strains of bacteria that affects the life cycle of each”[39]. Microorganisms on the mucosal surface of the URT interact in a multitude of ways, one of which is antagonistically, competing for ecological space and interfering with each other’s growth [40]. It is believed that this ‘bacterial interference’ by normal mucosal flora prevents colonization and proliferation of respiratory pathogens, thereby maintaining respiratory health [40].

As early as the 1970s Viridians Streptococci (part of the alpha hemolytic streptococcus (AHS) group) were shown to inhibit a range of potential pathogens in vitro including Neisseria meningitides, Moraxella spp., Beta-hemolytic Streptococci, Corynebacterium diphtheriae, S. pneumoniae, S. aureus, and Escherichia coli [40, 41]. In vitro studies have confirmed significant bactericidal effects from various strains of AHS against S. pneumoniae, NTHi, and
M. catarrhalis, which are strain specific [39]. In vivo, bacterial interference is thought to be achieved by several mechanisms. These mechanisms include occupying specific sites on the epithelial cell surfaces, thus preventing the adherence of pathogens, changes in the microenvironment by, for example, lowering of pH, production of antagonistic substances, and competition for nutritional substances (Figure 1) [40]. AHS owe their inhibitory potential to their production of bacteriocins (peptide toxins) which have inhibitory activity against gram-positive and gram-negative bacteria [40]. Corynebacterium spp., a URT commensal, is more often found in the URT in children who aren’t colonized by S. pneumoniae [42]. In vitro, Corynebacterium accolens was shown to inhibit the growth of S. pneumoniae by hydrolyzing skin surface triacylglycerols to produce anti-pneumococcal free fatty acids [42]. This is an example how a commensal microbe uses human resources to positively shape the URT microbiota. Local administration of probiotics is the application of bacterial interference to treat and prevent infections in vivo.

5 Probiotics in the upper respiratory tract, past and present evidence for local administration
Roos et al provided the proof of concept that commensal flora from URT of healthy individuals can prevent recurrence of streptococcal tonsillitis [43]. The authors isolated four strains of AHS from the throats of healthy individuals that had a strong ability to inhibit the growth of Group A Streptococcus in vitro. One hundred and thirty children with recurrent streptococcal tonsillitis were treated with antibiotics and then randomized to receive a daily throat spray containing either the probiotic strains or a placebo for 10 days [44]. In the eight weeks post baseline, bacteriologically-verified tonsillitis recurrence occurred in only 2% (1/50) of the children in the probiotic treatment group, whilst it occurred in a much larger proportion, 23% (14/61), of children in the placebo group [44]. Having demonstrated such efficacy in recurrent streptococcal tonsillitis, the authors applied the concept to OM.

There have been several RCTs investigating the local administration of probiotics to treat/prevent OM in otitis-prone children (see Table 2). Roos et al appear to be the first to investigate the use of a probiotic treatment made of bacterial strains with a demonstrated ability to inhibit the growth of otopathogens [45]. Roos and colleagues isolated AHS from
the opening of the eustachian tube of healthy children and identified five strains that were powerful inhibitors of *S. pneumoniae, M. catarrhalis, and S. pyogenes* [45]. They developed a probiotic nasal spray containing two strains of *Streptococcus sanguinis*, two strains of *Streptococcus mitis* and one strain of *Streptococcus oralis*. One-hundred and eight otitis-prone children were randomized to receive either the probiotic or a placebo. All children received 10 days of antibiotics, followed by either the probiotic or placebo nasal spray twice daily for 10 days, then for another 10 days after 55-60 days. There was a significant reduction in the rate of recurrence amongst those who received the probiotic, with 42% (22/53) children in the treatment group experiencing no AOM and having healthy tympanic membranes, compared to 22% (12/55) in the placebo group [45]. Significantly less children without recurrence of AMO in treatment group had OM with effusion (OME) at the last visit (31%; 10/32), compared to those in the placebo group (56%; 15/27) [45].

An attempt to replicate the Roos result by Tano *et al*, whom did not pre-treat participants with antibiotics, was unsuccessful [46]. Specifically, Tano *et al* conducted a smaller RCT (n = 36) testing a probiotic treatment comprised of a mixture of AHS strains that had demonstrated *in vitro* a good ability to inhibit the growth of otopathogens, as well as good adherence to adenoid epithelial cells [46]. The study recruited children with a history of recurrent AOM. Despite four months of treatment, there was no difference in the number of children with AOM, 44% (7/16) in the treatment groups vs. 40% (8/20) in the placebo group) [46]. Prior dosing with antibiotics may be required to reduce the bacterial load, allowing the probiotic strains more ecological space to adhere to the mucosal epithelial cells and proliferate. Testing the NP for the presence and abundance of the probiotic strains after treatment would have informed on whether this contributed to the failure of the treatment.

A noteworthy RCT conducted by Marchisio *et al* [47] provides insight into the ability of a probiotic treatment to colonize the participant. Otitis-prone children were randomized to test a probiotic treatment consisting of *Streptococcus salivarius 24SMB;* a strain obtained from the nose of a healthy child and shown to produce bacteriocin-like substances with activity against otopathogens [48]. The children were treated with antibiotics and then randomized to receive either the probiotic or placebo nasal spray twice daily for five consecutive days each month for three consecutive months. Quantitative PCR of NP swab
samples was used to detect the presence of *S. salivarius* 24SMB. After treatment, the proportion of children who did not experience AOM in the probiotic group (30%; 15/50) was double what it was in the control group (15%; 7/47) (p=0.075) [47]. When the authors limited their analysis to children in the treatment group who yielded an NP swab sample that contained the probiotic, they found that children who were successfully colonized with *S. salivarius* 24SMB were significantly more likely to have no episodes of AOM (43%; 12/28); compared to those who were not colonized with *S. salivarius* 24SMB (14%; 3/22) (p=0.03) [47]. These data suggest that colonization with the probiotic strain may be playing a role in treatment efficacy.

The above studies focused on the prevention of AOM, one study by Skovbjerg *et al*, explored the use of probiotics to treat OME [49]. A double-blinded pilot study was conducted on 60 children with OME, using a nasal spray containing either *S. sanguinis*, *Lactobacillus rhamnosus* or placebo for 10 days; no antibiotics were given prior to the commencement of the treatment [49]. There were significantly more children with complete or significant clinical recovery following treatment with *S. sanguinis* (7/19) compared to placebo (1/17) (p=<0.05), but not for those who received *L. rhamnosus* (3/18) compared to placebo (p=0.60) [49]. Interestingly, there was no evidence of colonization with *S. sanguinis* in the NP following treatment, although culture-bases analysis was used, which may not have sufficient sensitivity. Alternatively, the probiotic may have been exerting its action via the immune system.

5.1 Probiotics in the upper respiratory tract, past and present evidence for systemic administration

These studies have explored local probiotic administration. Other studies have investigated the systemic probiotic administration on the prevention of OM (Table 3). In healthy children, several RCTs used *L. rhamnosus* GG and/or *Bifidobacterium lactis* BB-12 orally to investigate whether OM could be prevented. Milk supplemented with *L. rhamnosus* GG was given to 513 day care children for seven months and showed no significant differences in the number of episodes of AOM in the probiotic (31%; 79/252) compared to placebo groups
(39%; 101/261) (p=0.08) [50]. Stecksen-Blicks et al gave 186 healthy children milk with *L. rhamnosus* GG for 21 months and measured the number of days with OM [51]. They found children who received the probiotic milk (n=110) had fewer days with OM (0.5) compared to placebo (n=76) (1.0) (p=0.003) [51]. In a younger cohort of infants <2 months old, *L. rhamnosus* GG + *B. lactis* BB-12 supplemented infant formula was given for 12 months and shown to significantly reduce the incidence of AOM in the first 7 months of life, 22% (7/32) had AOM in treatment group vs. 50% (20/40) in the placebo group (p= 0.014)) [52]. However, there were no statistically significant differences in the number of children who suffered recurrent AOM (≥ 3 episodes); four (13%) in the treatment group and 10 (25%) in the placebo group (p= 0.183) [52]. Another study gave *B. lactis* BB-12 to infants via slow-release tablets for seven months and showed no significant difference in the incidence of self-reported OM compared to placebo; (26%; 9/34 and 17%; 6/35 respectively (p= 0.455)) [53]. Whether *L. rhamnosus* GG and *B. lactis* BB-12 can prevent OM in healthy children is equivocal. *L. rhamnosus* GG was originally isolated from the healthy gastrointestinal system [54] and *B. lactis* BB-12 was a dairy culture [55]. Therefore, their poor efficacy in preventing disease from respiratory pathogens may be due to them being used beyond their niche environment.

One study by Di Pierro et al investigated whether oral administration of a probiotic strain niche to the URT, *S. salivarius* K12, could prevent AOM in healthy children attending kindergarten [56]. They delivered the probiotics via dissolving oral tablet and found a significant reduction in the incidence of AOM in the treatment (44%; 49/111) compared to placebo group (80%; 89/111) and the number of episodes of AOM, 53 in the treatment group vs 101 in the placebo group [56]. *S. salivarius* has been shown to adhere to the URT and strongly inhibit the growth of the main respiratory pathogens *in vitro*. This study suggests that there may be a role for niche specific probiotic strains in preventing AOM in healthy children and further research is required.

In otitis-prone children a range of probiotic mixtures have been administered systemically to determine whether recurrence of OM can be prevented. Two hundred and sixty-nine otitis-prone children were given a probiotic capsule containing two strains of *L. rhamnosus* GG, *Bifidobacterium breve* 99, and *Propionibacterium freudenreichii* spp. *shermani* JS [57].
There were no differences in the incidence of AOM; 72% (n=135) vs. 65% (n=134) for treatment vs. placebo (OR = 1.48 (95% CI 0.87-2.52)) or recurrent AOM (18% vs 17% respectively; OR = 1.04 (95% CI 0.55-1.96)) [57]. Correspondingly, 166 “high-risk” children were given formula supplemented with *Streptococcus thermophilus*, *S. salivarius*, *L. rhamnosus* LPR and a prebiotic for 12 months and found no difference in the number of episodes of AOM in the treatment (249) (n=83) compared to control (237)(n=83) [58]. Cumulatively, these results demonstrate that ingestion of non-specific systemic probiotics does not prevent AOM in healthy children or reduce the number of episodes in otitis-prone/“high-risk” children, regardless of the duration of treatment. In contrast, local administration using strains isolated from the URT of healthy children showed more promising results in preventing OM in otitis-prone children.

6 The future, opportunities ahead
The use of probiotics to prevent and treat OM shows promise and proof of concept exists. For such a probiotic to be effective it likely needs to be niche-specific, demonstrate *in vitro* bacterial inference against the main respiratory pathogens, and able to colonize the NP. Although, there may also be a systemic effect through activation of the immune system. In otitis-prone children, antibiotics may be required prior to giving the probiotic to reduce the overall bacterial load and facilitate colonization. Further research is required in both the prevention and treatment of all types of OM, and particularly in developing nations and indigenous communities. To facilitate such research agreement on how to define the diagnosis of different middle ear infections is paramount. It is equally important to investigate the complete microbiome in the upper respiratory tract, not only the pathogens as in the past. Infection is a sign of dysbiosis and pathogens in isolation can no longer be considered as solely responsible. Microbiological methods, both culture dependent and molecular methods, need to be aligned across the OM research community. This should be a priority agenda for interest groups such as the International Society for Otitis Media.

Another area for consideration, particularly in the prevention of OM, is the influence that the mother and ingestion of breastmilk may have on the development of the URT microbiota in infants. Breastmilk has demonstrated protection against AOM in children
under two year of age [59]. It may exert at least some of its effect through manipulating commensal flora of the URT and gut as a probiotic and prebiotic [59, 60]. Breastmilk has its own microbiome which may influence the infant’s microbiome, particularly gut microbiome, and confer health benefits without supplementation [61]. Research is beginning to emerge into whether the health of the infant can be influenced by delivering probiotics to the mother prenatally and whilst breastfeeding. In randomized controlled trials, providing probiotics to mothers in their last trimester of pregnancy and through the breastfeeding infants’ first 3-6 months of life has demonstrated reduced risk of atopic dermatitis [62, 63]. However, this effect does not appear to be mediated by the probiotic strains being delivered through the breastmilk [64]. The provision of probiotics prenatally and in breastfeeding mothers in the prevention of OM, particularly in high risk populations should be considered.

There is a significant, ongoing burden of disease caused by OM in developing nations and Indigenous populations, despite vaccinations and antibiotic treatment. The causes are multifactorial and include social, environmental, biomedical and possible immune and genetic factors [65]. A multi-pronged approach to the treatment and prevention of OM is required, however from a biomedical perspective “strategies to reduce the exposure to high doses of multiple OM causing bacteria are urgently needed” [65]. Antibiotics may temporarily resolve OM and reduce the number of otopathogens, however they likely disrupt the normal protective flora and allow for recolonization with otopathogens and recurrent infections. In addition to growing global concerns regarding antibiotics resistance, an alternative, more effective treatment is required. Local administration of a probiotic that is niche-specific for the URT and demonstrates the ability to interfere with the growth of otopathogens needs to be evaluated as an alternative treatment for the refractory problem of OM, particularly in developing nations and Indigenous populations.

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References

Table 1: Otitis Media Definitions

<table>
<thead>
<tr>
<th>Type of OM</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute otitis media (AOM) without perforation</td>
<td>Presence of middle ear fluid with symptoms or signs of suppurative infection, which may include otalgia, fever, irritability, vomiting or diarrhoea.</td>
</tr>
<tr>
<td>AOM with perforation</td>
<td>Acute suppurative infection with recent discharge from the middle ear or through a tympanostomy tube (within the past 7 days).</td>
</tr>
<tr>
<td>Recurrent AOM (rAOM)</td>
<td>Recurrent bouts of AOM — three episodes in 6 months or four to five in 12 months.</td>
</tr>
<tr>
<td>Otitis media with effusion (OME)</td>
<td>Presence of middle ear fluid without symptoms or signs of suppurative infection.</td>
</tr>
<tr>
<td>Chronic suppurative otitis media (CSOM)</td>
<td>A persistent discharge from the middle ear through a tympanic membrane perforation for more than 6 weeks. CSOM may include a chronic perforation with or without acute or chronic otorrhoea.</td>
</tr>
</tbody>
</table>

Adapted from Kong et al [2]
Table 2: Randomized controlled trials investigating local (intranasal) administration of probiotics to prevent/treat otitis media

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Age</th>
<th>Population</th>
<th>Treatment</th>
<th>Primary Outcome Measure(s)</th>
<th>Results</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roos 2001[45]</td>
<td>108</td>
<td>6 months – 6 years</td>
<td>Otitis-prone</td>
<td>Local: <em>Streptococcus mitis</em>, <em>S. sanguinis</em>, <em>Streptococci oralis</em></td>
<td>No AOM + normal TM OME</td>
<td>Treatment (n=53) = 42% Placebo (n=55) = 22% Treatment = 31% Placebo = 56%</td>
<td>0.02</td>
</tr>
<tr>
<td>Tano 2002[46]</td>
<td>36</td>
<td>&lt; 4 years</td>
<td>Otitis-prone</td>
<td>Local: <em>S. mitis</em>, <em>S. sanguinis</em>, <em>S. oralis</em></td>
<td>Recurrence of AOM</td>
<td>Treatment (n=16) = 44% Placebo (n=20) = 40%</td>
<td>n.s</td>
</tr>
<tr>
<td>Skovbjerg 2008[49]</td>
<td>54</td>
<td>1-8 years</td>
<td>Otitis-prone</td>
<td>Local: <em>S. sanguinis</em> OR <em>L. rhamnosus</em></td>
<td>Resolution OME</td>
<td><em>S. sanguinis</em> (n=19) = 37% <em>L. rhamnosus</em> (n=18) = 17% Placebo (n=17) = 6%</td>
<td>0.05</td>
</tr>
<tr>
<td>Marchisio 2015[47]</td>
<td>97</td>
<td>1-5 years</td>
<td>Otitis-prone</td>
<td>Local: <em>Streptococcus salivarius</em> 24SMB</td>
<td>Recurrence of AOM</td>
<td>Treatment (n=50) = 70% Placebo (n=47) = 85% NP colonized (n=28) = 57% not colonized (n=22) = 86%</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Note: AOM, acute otitis media; NP, nasopharynx; n.s., non-significant (no p value reported); TM, tympanic membrane.
<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Age</th>
<th>Population</th>
<th>Treatment</th>
<th>Primary Outcome Measure(s)</th>
<th>Result(s)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatakka 2001[50]</td>
<td>513</td>
<td>1-6 years</td>
<td>Healthy</td>
<td>Systemic: <em>Lactobacillus rhamnosus</em> GG</td>
<td>Incidence AOM</td>
<td>Treatment (n=252) = 31% Placebo (n=261) = 39%</td>
<td>0.08</td>
</tr>
<tr>
<td>Hatakka 2007[57]</td>
<td>269</td>
<td>10 mo-6 years</td>
<td>Otitis-prone</td>
<td>Systemic: <em>L. rhamnosus</em> GG and LC705, <em>Bifidobacterium breve</em> 99 and <em>Propionibacterium freudenreichii</em> JS</td>
<td>Incidence AOM</td>
<td>Treatment (n=135) = 72% Placebo (n=134) = 65%</td>
<td>n.s</td>
</tr>
<tr>
<td>Rautava 2008[52]</td>
<td>72</td>
<td>&lt; 2 months</td>
<td>Healthy</td>
<td>Systemic: <em>L. rhamnosus</em> GG &amp; <em>Bifidobacterium lactis</em> BB-12</td>
<td>Incidence AOM 1st 7 months</td>
<td>Recurrent (&gt;3) AOM in 1st year</td>
<td>Treatment (n=32) = 22% Placebo (n=40) = 50%</td>
</tr>
<tr>
<td>Stecksen-Blicks 2009[51]</td>
<td>186</td>
<td>1-5 years</td>
<td>Healthy</td>
<td>Systemic: <em>L. rhamnosus</em> LB21, fluoride</td>
<td>No. days with AOM</td>
<td>Treatment (n=110) = 0.5 Placebo (n=76) = 1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Taipale 2011[53]</td>
<td>69</td>
<td>1-2 months</td>
<td>Healthy</td>
<td>Systemic: <em>B. lactis</em> BB-12</td>
<td>Incidence AOM</td>
<td>Treatment (n=34) = 26% Placebo (n=35) = 17%</td>
<td>0.50</td>
</tr>
<tr>
<td>Cohen 2013[58]</td>
<td>166</td>
<td>7-13 months</td>
<td>Healthy</td>
<td>Systemic: <em>Streptococcus thermophilus</em>, <em>S. salivarius</em>, <em>L. rhamnosus</em> + preB Raftilose/Raftiline</td>
<td>No. episodes of AOM</td>
<td>Treatment (n=83) = 249 Placebo (n=83) = 237</td>
<td>0.80</td>
</tr>
<tr>
<td>Di Peirro 2016[56]</td>
<td>222</td>
<td>3 years</td>
<td>Healthy</td>
<td>Systemic: <em>S. salivarius</em> K12 (BLIS K12)</td>
<td>Incidence AOM</td>
<td>Treatment (n=111) = 44% Placebo (n=111) = 80%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Note: AOM, acute otitis media; n.s., non-significant (no p value reported).
Figure 1: Possible mechanisms in which probiotics/commensal flora maintain a healthy upper respiratory tract: 1) Commensal flora/probiotics bind to respiratory epithelium thereby preventing pathogens from adhering; 2) they change the mucosal environment, for example, by reducing pH; 3) they release bacteriocins and other inhibitory substances which target pathogenic bacteria; 4) they stimulate local mucosal immunity; 5) compete for nutritional substances; and 6) simulate systemic immune response.