Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-015-2491-x

ORIGINAL ARTICLE

Streptococcus salivarius 24SMB administered by nasal spray for the prevention of acute otitis media in otitis-prone children

P. Marchisio[1] · M. Santagati[2] · M. Scillato[2] · E. Baggi[1] · M. Fattizzo[1] · C. Rosazza[1] · S. Stefani[2] · S. Esposito[1] · N. Principi[1]

Received: 19 August 2015 / Accepted: 9 September 2015

Abstract This paper reports the results of the first study in which *Streptococcus salivarius* 24SMB, a safe α-haemolytic strain capable of producing bacteriocin-like substances with significant activity against acute otitis media (AOM) pathogens, was intranasally administered in an attempt to reduce the risk of new episodes of AOM in otitis-prone children. In this prospective, randomized, double-blind, placebo-controlled study, 100 children aged 1–5 years with histories of recurrent AOM were randomized 1:1 to receive an intranasal *S. salivarius* 24SMB or placebo twice daily for 5 days each month for 3 consecutive months. Fifty treated children and 47 who received placebo who were compliant with study protocol were followed monthly for 6 months. The number of children who did not experience any AOM was higher among the children treated with the *S. salivarius* 24SMB preparation than among those in the placebo group (30.0 vs 14.9 %; $p=0.076$). Moreover, the number of children who received antibiotics during the study period was lower among the children treated with *S. salivarius* 24 SMB than among those who received placebo (70 vs 83.0 %; $p=0.13$). Compared with the children who were not colonized by *S. salivarius* 24SMB after treatment, the number of colonized children who experienced any AOM was significantly lower (42.8 vs 13.6 %; $p=0.03$). Similar results were observed when the children treated with antibiotics for AOM were analysed (67.8 vs 95.5 %; $p=0.029$). This study revealed the ability of intranasally administered *S. salivarius* 24SMB to reduce the risk of AOM in otitis-prone children.

✉ S. Esposito
susanna.esposito@unimi.it

[1] Pediatric Highly Intensive Care Unit, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Commenda 9, 20122 Milano, Italy

[2] Department of Biomedical and Biotechnological Sciences, MMAR Laboratory, University of Catania, Catania, Italy

Introduction

The prevention of further episodes of acute otitis media (AOM) in otitis-prone children is recommended by all experts to reduce the risk of complications, medical costs, and social and family problems that are strictly related to recurrent AOM [1, 2]. The control of environmental risk factors, antibiotic administration, immunoprophylaxis, vitamin D supplementation, probiotics, surgery, and alternative medicine has been proposed as a series of prophylactic measures [1, 2]. However, none of these measures is completely effective. Compared with controls, the majority of studies have observed reductions in the total numbers of episodes in treated children; however, even when various methods are applied simultaneously, a considerable number of children receive no benefit and continue to experience AOM. Furthermore, the use of some of these measures is widely questioned [1–4]. Long-term prophylactic antibiotic administration is associated with an increased risk of side effects and the emergence of resistant bacteria [3]. Similarly, the safeties and tolerabilities of most alternative medicine remedies are not precisely defined [4].

Some years ago, a number of studies reported evidence that the normal nasopharyngeal flora inhibits the growth of common otopathogens [5] and thus could play a relevant role in the prevention of upper respiratory tract infections, including AOM [6, 7]. Specifically, it has been reported that the numbers of α-streptococci colonizing the nasopharynx are significantly lower in otitis-prone children than in healthy children,

Published online: 18 September 2015

whereas the opposite is true for otopathogens [8]. Moreover, it has been shown that re-colonization with α-streptococci can significantly reduce the risk of new AOM episodes in otitis-prone children [9]. However, despite some encouraging results, the use of pharyngeal re-colonization with normal flora to prevent recurrent AOM has not been further studied, likely due to concerns about the safety of this preventive treatment. Some α-streptococci have been, if only rarely, associated with the development of severe invasive diseases [10].

Recently, an α-haemolytic strain derived from nasopharyngeal swabs obtained from healthy children, termed *Streptococcus salivarius* 24SMB, was selected. This strain was found to be capable of producing bacteriocin-like substances with significant activity against AOM pathogens [11]. An in vitro safety assessment suggested that this strain could be safe for humans [11]. More recently, the colonization, safety, and tolerability of a nasal spray of live *S. salivarius* 24SMB were evaluated in adults who received daily for 3 days the nasal spray containing *S. salivarius* 24SMBc at a concentration of 5×10^9 colony-forming units (CFU)/ml. The study demonstrated the capability of *S. salivarius* 24SMBc to colonize the rhinopharynx tissue in 95 % of the subjects and persist in 55 % of them after 6 days from the last dose of the formulation, maintaining a concentration of 10^5 CFU/ml. The treatment was well tolerated by all healthy subjects and no adverse effect was found [12]. This paper reports the results of the first study in which intranasally administered *S. salivarius* 24SMB was used in an attempt to reduce the risk of new episodes of AOM in otitis-prone children.

Material and methods

Study design

This prospective, randomized, double-blind, placebo-controlled study was performed in the Pediatric Highly Intensive Care Unit of the University of Milan's Department of Pathophysiology and Transplantation between October 1, 2013 and June May 30, 2014. This study was approved by the Institutional Review Board of the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy, and was conducted in accordance with the standards of Good Clinical Practice for trials of medicinal products in humans. The participants' parents or legal guardians provided written informed consent before the children were enrolled.

Study population

The study involved children aged 1–5 years with histories of recurrent AOM (rAOM, defined as at least three episodes in the preceding 6 months or at least 4 episodes in the preceding 12 months with the most recent episode within the previous 2–8 weeks) who were regularly followed up by the outpatient section of the Pediatric Highly Intensity Care Unit. The minimum number of episodes of AOM for inclusion in the otitis-prone group had to be diagnosed by pneumatic otoscopy performed by a trained investigator (P.M., E.B., or M.F.) and documented by medical records, and at least two episodes had be supported by tympanometric findings. At the time of enrolment, the children had to be free of AOM but could be experiencing otitis media with effusion. The exclusion criteria included all factors that could favour the development of AOM, including severe atopy, acquired or congenital immunodeficiency, cleft palate, a chronically ruptured eardrum, craniofacial abnormalities or obstructive adenoids, sleep apnoea syndrome or the placement of tympanostomy tubes. The children were screened before entering the study via physical examinations and careful reviews of their medical histories to assure compliance with the inclusion and exclusion criteria.

In order to favour *S. salivarius* 24SMB colonization and reducing competing nasopharyngeal flora, all of the enrolled children were initially treated with an amoxicillin-clavulanic acid combination at a dose of 80 mg/kg/day (as amoxicillin) in three doses for 10 days. Three days after the end of the antibiotic administration, they were blindly randomized at 1:1 via a random number generator to receive a *S. salivarius* 24SMB preparation or placebo. The suspension of *S. salivarius* 24SMB consisted of a minimum of 100×10^9 colony forming units/mL (CFU/mL) in 5 mL of saline and was delivered with a nasal spray that provided 5×10^9 CFU to each nostril twice per a day. The placebo was based on saline and included colour and taste such that it could not be distinguished from the preparation containing *S. salivarius*. The placebo was administered with the same nasal spray and provided the same saline dose. The study was blinded by labelling the nasal sprays of *S. salivarius* 24SMB and placebo with randomization codes and only revealing the codes to the staff of the data monitoring centre. The staff (C.R.) had no contact with the patients. Similarly, the paediatricians involved in the clinical monitoring (P.M., E.B., and M.F.) were blinded to the treatment assignments.

Procedures

The plan involved all children receiving the *S. salivarius* 24 SMB preparation or placebo in each nostril twice per day for 5 consecutive days each month for 3 consecutive months. Each period of administration had to be spaced from the previous one with 25 days. Both preparations had to be administered by adequately trained parents who received the preparations for the monthly administrations during the first three control visits. The parents were required to keep the preparations in a refrigerator after use and to return them to the centre upon the subsequent control visit. Moreover, it was decided that

children were to be monitored monthly for an additional 3 months after the last *S. salivarius* 24 SMB preparation or placebo administration. Consequently, the study period consisted of a total of 6 months.

To monitor the incidence of new episodes of AOM after study entry, the parents were asked to return to the centre with their child for monthly control visits and each time their child experienced a febrile episode accompanied by symptoms suggestive of AOM, including disturbed sleep, irritability and/or earache. The parents were also asked to complete a diary to record all of their children's clinical problems and the administrations of the study preparations on the planned days. Compliance with the study regimen was verified by examining the parents' diaries and inspecting and weighing the returned nasal sprayers at the control visits. To overcome the potential problem of the underreporting of AOM episodes, all of the families were systematically telephoned weekly to verify the children's status. All of the medical examinations carried out at the centre (i.e., the planned monthly examinations and those following the development of febrile episodes) were performed by trained investigators (P.M., E.B., and M.F.) using standardized questionnaires. At each visit, the details of any medical event that occurred since the previous visit were recorded, and the children underwent complete physical examinations, pneumatic otoscopies (Model 20200, Welch Allyn, Skaneateles Falls, NY) and tympanometries (MicroTymp 3, Welch Allyn). AOM was diagnosed based on the presence of spontaneous otorrhoea from an acute tympanic membrane perforation (TMP) or any combination of fever, earache, irritability and hyperaemia or opacity accompanied by the bulging or immobility of the tympanic membrane. In doubtful cases, tympanometry was performed to establish the presence of effusion or a minimal perforation. The AOM episodes were defined as complicated or uncomplicated based on the presence or absence of spontaneous otorrhoea, respectively. Whenever AOM was diagnosed, amoxicillin (80 mg/kg/day) plus clavulanic acid in three doses was given for 10 days. No other treatments were allowed for AOM with the exception of acetaminophen or ibuprofen in cases of fever. Topical cleaning of the ear canal with saline was also applied in cases of spontaneous otorrhoea.

Cases of bilateral ear involvement were considered single episodes. Relapse and reappearance were defined as the reappearance of the signs and symptoms of AOM within 4 days or within 5–14 days, respectively, of the end of therapy. Relapses were not considered new episodes, but recurrences were considered as such.

Microbiologic analysis of the samples

The microbiologic evaluations were performed with nasopharyngeal swabs obtained after the first antibiotic treatment before enrolment (T_0) and at 6, 60, 120, and 150 days after the end of the first *S. salivarius* 24SMB administration. Each nasopharyngeal swab was plated directly onto Columbia Agar Base (Oxoid, Basingstoke, UK) plus 5 % horse blood to determine the total microflora and onto *Salivarius mitis* agar (Difco Laboratories, Franklin Lakes, NJ), which is a selective medium for α haemolytic streptococci. The cultures were incubated overnight at 37 °C in a 5 % CO_2 air atmosphere. Additionally, all swabs were cultured to determine the presence of other pathogens according to standard laboratory procedures. To evaluate the presence of *S. salivarius* 24SMB, the total DNA was extracted from each nasopharyngeal swab and analysed by quantitative polymerase chain reaction (PCR) using a TaqMan probe designed to identify the *S. salivarius* 24SMB strain [11]. The presence of *S. salivarius* 24SMB was also confirmed with a deferred antagonism test [11] and molecular fingerprinting with randomly amplified polymorphic DNA (RAPD) [13].

Accordingly with the protocol agreed upon, all samples were processed at the LMMAR of the Biomedical and Biotechnological Department of the University of Catania. All nasopharyngeal swabs collected were kept frozen until their processing.

Sample size

We assumed that 90 % of children in the placebo group would have experienced at least one episode of AOM during follow-up. A sample size of 50 children in each group thus achieved 85 % power to detect a difference between the treatment and placebo group proportions of about 24 % (i.e., about 66 % with at least one event in the treatment group). The test statistic used is the two-sided Mantel-Haenszel test, with alpha targeted at 0.05 (PASS v.11 software; NCSS, LLC. Kaysville, Utah, USA).

Statistical analysis

The X^2 and Mann–Whitney tests were used to compare the categorical and continuous variables, respectively, between the treatment and placebo groups at baseline and at the follow-ups. Three types of outcome were considered, i.e., the total number of AOM episodes and the numbers of complicated and uncomplicated episodes. We fit frailty Cox models to calculate the hazard ratios and 95 % confidence intervals of the occurrence of AOM during the study period while accounting for the intraindividual correlations. The data were statistically analysed using Stata 12 Software (StataCorp 2011, College Station, TX, USA).

Results

A total of 100 children (50 in the *S. salivarius* 24SMB treatment group and 50 in the placebo group) were initially

Table 1 Selected demographic and clinical characteristics of the children with recurrent acute otitis media (rAOM) who were treated with *Streptococcus salivarius* 24SMB or with placebo

Characteristic	*Streptococcus salivarius* 24SMB group (N=50)		Placebo group (N=47)		*p*-value
	n	%	*n*	%	
Age at baseline (years)					
<2	15	30.0	10	21.3	
2–3	25	50.0	24	51.1	
4–5	10	20.0	13	27.7	0.52
Mean±SD	2.7±1.1		3.1±1.2		0.07
Family history of AOM					
No	34	68.0	22	46.8	
Yes	16	32.0	25	53.2	**0.03**
Family history of allergy					
No	21	42.0	18	38.3	
Yes	29	58.0	29	61.7	0.71
No. of siblings					
0	20	40.0	19	40.4	
1	25	50.0	18	38.3	
2+	5	10.0	10	21.3	0.25
Gestational age (weeks)					
<37	2	4.0	5	10.6	
37+	48	96.0	42	89.4	0.26
Birth weight (grams)					
<2500	6	12.0	4	8.5	
2500+	44	88.0	43	91.5	0.74
Breastfeeding					
No	8	16.0	9	19.1	
Yes	42	84.0	38	80.9	0.68
Allergy					
No	48	96.0	44	93.6	
Yes	2	4.0	3	6.4	0.67
Pneumococcal vaccination with the 13-valent conjugate vaccine					
No	7	14.0	6	12.8	
Yes	43	86.0	41	87.2	0.86
Passive smoking (from parents)					
No	38	76.0	35	74.5	
Yes	12	24.0	12	25.5	0.86
Day-care attendance					
No	6	12.0	5	10.6	
Yes	44	88.0	42	89.4	0.83
Sniffing					
No	36	72.0	26	55.3	
Yes	14	28.0	21	44.7	0.09
Pacifier use					
No	29	58.0	24	51.1	
Yes	21	42.0	23	48.9	0.49
Hospitalization during the last 3 months					
No	50	100.0	46	97.9	
Yes	0	0.0	1	2.1	0.48
No. of AOM during the last 12 months[a]					
3–4	26	53.1	24	51.1	
5–6	17	34.7	15	31.9	
≥7	6	12.2	8	17.0	0.80
Mean±SD	4.9±1.3		5.2±1.7		0.71

AOM acute otitis media, *SD* standard deviation

[a] The sums may not add up to the total due to missing values

enrolled. Unfortunately, the parents of three subjects in the placebo group refused to continue the study after the first period of treatment. Because no reliable information on disease occurrence and no nasopharyngeal swab were obtained from these children, they were excluded from final evaluation. Consequently, considering that all the remaining patients were considered fully compliant with the study protocol, the comparisons were made between the 50 patients who were treated with the *S. salivarius* 24SMB preparation and the 47 children who received placebo. Table 1 summarizes the general characteristics of the enrolled children according to the randomization arm. The two groups were comparable; no differences were found for any of the studied variables with the exception of family history of AOM, which was significantly more common in the children who received *S. salivarius* 24SMB than in those who were with placebo ($p=0.03$).

Table 2 summarizes the outcomes in terms of AOM recurrences and antibiotic and antipyretic prescriptions among the children treated with *S. salivarius* 24SMB and those who received placebo. During the 6 months of the study period, the

Table 2 Clinical outcomes and prescribed therapies among the children with recurrent acute otitis media (rAOM) who were treated with *Streptococcus salivarius* 24SMB or with placebo

Outcome/therapy	*Streptococcus salivarius* 24SMB group (N=50)	Placebo group (N=47)	*p*-value
Children with at least one episode of AOM			
No	15 (30.0)	7 (14.9)	
Yes	35 (70.0)	40 (85.1)	0.076
Mean AOM episodes±SD	1.78±1.76	1.81±1.47	0.61
Children treated with antibiotic therapy			
No	15 (30.0)	8 (17.0)	
Yes	35 (70.0)	39 (83.0)	0.13

Percentages are shown in parenthesis

AOM acute otitis media, *SD* standard deviation

Table 3 Clinical outcomes and prescribed therapies according to *Streptococcus salivarius* 24SMB colonization status among the children with recurrent acute otitis media (rAOM) who were treated with *Streptococcus salivarius* 24SMB

Outcome/therapy	Colonized by *Streptococcus salivarius* 24SMB (N=28)	Not colonized by *Streptococcus salivarius* 24SMB (N=22)	*p*-value
Children with at least one episode of AOM			
No	12 (42.8)	3 (13.6)	
Yes	16 (57.2)	19 (86.4)	**0.03**
Mean AOM episodes±SD	1.25±1.51	2.13±1.35	**0.03**
Children treated with antibiotic therapy			
No	9 (32.1)	1 (4.5)	
Yes	19 (67.8)	21 (95.5)	**0.029**

The percentages are shown in parenthesis. Values in bold are statistically significant

AOM acute otitis media, *SD* standard deviation

number of children that did not experience any AOM was greater among the children treated with the *S. salivarius* 24SMB preparation than among the placebo group, but this difference did not reach statistical significance (15/50, 30.0 %, *vs* 7/47, 14.9 %; $p=0.076$). Moreover, the number of children who received antibiotics for AOM during the study period was lower among the children who were treated with *S. salivarius* 24 SMB than among those who received placebo, although this difference again failed to reach significance (35/50, 70 % *vs* 39/47, 83.0 %; $p=0.13$).

After the first *S. salivarius* 24SMB administration, colonization by *S. salivarius* 24SMB, not present at baseline in any of the enrolled patients, was evidenced in 25 of the 50 (50.0 %) children who received the probiotic, with bacterial concentrations ranging from 10^2 to 2.3×10^4 CFU/mL. Of these children, 20 (80.0 %) remained colonized prior to the third *S. salivarius* 24SMB administration. Moreover, three patients who were negative after the first *S. salivarius* 24SMB dose exhibited colonization prior to the third administration. Seventeen (34.0 %) and 14 (28.0 %) children remained colonized at 30 and 60 days after the end of therapy, respectively. Antibiotic administration did not influence colonization at T60 but was associated with a significant reduction in colonization rates when it occurred after 120 or 150 days from the end of treatment. In contrast, the agent was not identified in any of the nasopharyngeal swabs of the remaining 23 (46.0 %) patients or in any of the controls. Table 3 shows the clinical outcomes according to colonization by *S. salivarius* 24SMB among the treated children. Compared to the children without colonization, the number of colonized children who did not experience any AOM was significantly lower (12/28, 42.8 %, *vs* 3/22, 13.6 %; $p=0.03$). Moreover, the mean number of AOM episodes was significantly lower among the treated children who were colonized by *S. salivarius* 24SMB than among those who were not treated (1.25±1.51 vs 2.13±1.35; $p=0.03$). Similar results were observed when children who were treated with antibiotics were analysed; 19/28 (67.8 %) of those who were treated and colonized by *S. salivarius* 24SMB received antibiotics, whereas 21/22 (95.5 %) of those who were treated but not colonized received antibiotics ($p=0.029$).

Table 4 summarizes the safeties and tolerabilities of the nasal administrations of *S. salivarius* 24SMB and placebo. The safety and tolerability of *S. salivarius* 24SMB preparation

Table 4 Safeties and tolerabilities of the nasal administration of *Streptococcus salivarius* 24SMB and placebo among children with recurrent acute otitis media (rAOM)

	Streptococcus salivarius 24SMB group (N=50)	Placebo group (N=47)	*p*-value
Local adverse event			
At least one	21 (42.0)	7 (14.9)	**0.003**
Sneezing	13 (26.0)	6 (12.7)	0.07
Burning	8 (16.0)	1 (2.1)	**0.03**
Itching	6 (12.0)	4 (8.5)	0.74
Cough	3 (6.0)	2 (4.2)	1.00
Nasal congestion	2 (4.0)	0 (0.0)	0.49
Epistaxis	2 (4.0)	0 (0.0)	0.49
Rhinorrhoea	1 (2.0)	0 (0.0)	1.00
Systemic adverse event	0 (0.0)	0 (0.0)	1.00
Severe adverse event	0 (0.0)	0 (0.0)	1.00

Values in bold are considered statistically significant

were generally satisfactory, although a significantly greater number of children who received *S. salivarius* 24SMB suffered from at least one local adverse event compared with those who received placebo (21/50, 42.0 %, *vs* 7/47, 14.9 %; $p=0.003$). Among these adverse events, burning was significantly more common among the children who received *S. salivarius* 24SMB than the controls (8/50, 16.0 %, *vs* 1/47, 2.1 %; $p=0.03$), but all of the local adverse events disappeared within 24 h of the last administration of *S. salivarius* 24SMB and did not reappear. Moreover, no systemic adverse events or severe adverse events were observed in either group of children.

Discussion

Several years ago, it was reported that AOM develops more easily when the commensal saprophytic flora of the nasopharynx is reduced, which leads to significant proliferation of asymptomatically carried otopathogens [6, 7]. Re-colonization of the nasopharynx with normal flora, primarily α-streptococci, has been considered to be a possible solution to the problem of recurrent AOM, and some provisional data seem to confirm this hypothesis. However, this method was not definitively developed primarily because its safety was questioned. Recently, *S. salivarius* 24SMB has been identified as an oral probiotic due to safety assessment of strain, its ability to inhibit the most important bacterial pathogens that are responsible for AOM, and the absence of virulence and antibiotic resistance [13]. This paper reports the results of a preliminary study in which the potential ability of the intranasal administration of this agent to reduce the risk of AOM in otitis-prone children was tested for the first time. Global evaluation of the impact of the intranasal administration of this probiotic seems to suggest that it might be useful in the reduction of the risk of new episodes of AOM in otitis-prone children. Although no statistically significant difference between groups was found, data indicate that compared with the children who received placebo, the children treated with *S. salivarius* 24SMB exhibited a clear tendency toward a reduction in the incidence of the disease. The positive effect of *S. salivarius* 24SMB administration seems to be supported by the findings that the number of patients who did not experience any further episode of AOM during the study period and the mean number of AOM episodes were significantly reduced among the patients who were demonstrably colonized by the probiotic compared to those who received it but exhibited no evidence of colonization. More than 40 % of the treated and colonized children did not experience any AOM episodes. This effect is quite similar or even greater than those achieved by other recommended AOM prevention methods, such as the control of negative environmental factors, the use of pneumococcal or influenza vaccines, and vitamin D administration [1–3]. Only long-term prophylactic antibiotic administration achieves better results, but this treatment causes several problems that limit its use to selected cases [4]. On the other hand, the achievement of greater reductions in the incidence of new episodes of AOM among otitis-prone children with the presently recommended methods seems difficult. Recent studies have shown that genetic modifications involving genes that encode factors related to innate or adaptive immunity occur in a non-negligible proportion of subjects with recurrent AOM [13]. In some cases, these genetic variations are associated with significant reductions in host defences that increase the risk of infection; thus, any attempt to reduce AOM cannot lead to the complete elimination of the risk of AOM in all treated subjects.

The *S. salivarius* 24SMB preparation was found to be acceptably safe because the number of significant local adverse events was low, and these events disappeared within a few hours of treatment. Moreover, no systemic adverse events were observed, which confirms the good safety profile that had already been evidenced in adults [12].

However, the suggestions of this preliminary study have to be confirmed with other evaluations that involve greater numbers of subjects. Specifically, because colonization is essential for reducing the AOM risk, the issue of why some subjects were not colonized despite receiving the same treatment as those with evident colonization requires further study. The elimination of factors that prevent colonization might be useful for significantly increasing the real effectiveness of this treatment. The role in this regard of pathogens that commonly cause AOM and frequently colonize nasopharynx, such as *S. pneumoniae* and not-typeable *H. influenzae*, has to be evaluated. Moreover, because *S. salivarius* 24SMB is extremely sensitive to the drugs that are commonly prescribed to treat AOM and other bacterial diseases [11], the utility of additional doses of *S. salivarius* 24 SMB following the use of antibiotics for maintaining colonization and assuring long-term protection against AOM should be defined.

Acknowledgments This study was supported by a grant obtained from DMG Italia S.r.l.

Conflict of interest The author(s) declare that they have no competing interests.

References

1. Grevers G (2010) First International Roundtable ENT Meeting Group. Challenges in reducing the burden of otitis media disease: an ENT perspective on improving management and prospects for prevention. Int J Pediatr Otorhinolaryngol 74:572–577
2. Marchisio P, Nazzari E, Torretta S, Esposito S, Principi N (2014) Medical prevention of recurrent acute otitis media: an updated overview. Expert Rev Anticancer Ther 12:611–620
3. Morris PS, Leach AJ (2008) Antibiotics for persistent nasal discharge (rhinosinusitis) in children. Cochrane Database Syst Rev 2:CD001094

4. Lim A, Cranswick N, South M (2011) Adverse events associated with the use of complementary and alternative medicine in children. Arch Dis Child 96:297–300
5. Tano K, Olofsson C, Grahn-Hakansson E, Holm SE (1999) *In vitro* inhibition of *S. pneumoniae*, nontypeable *H. influenzae* and *M. catharralis* by alpha-hemolytic streptococci from healthy children. Int J Pediatr Otorhinolaryngol 47:49–56
6. Brook I, Gober AE (2000) *In vitro* bacterial interference in the nasopharynx of otitis mediaprone and non-otitis media-prone children. Arch Otolaryngol Head Neck Surg 126:1011–1013
7. Bernstein JM, Faden HF, Dryja DM, Wactawski-Wende J (1993) Micro-ecology of the nasopharyngeal bacterial flora in otitis-prone and non-otitis-prone children. Acta Otolaryngol 113:88–92
8. Marchisio P, Claut L, Rognoni A, Esposito S, Passali D, Bellussi L, Drago L, Pozzi G, Mannelli S, Schito G, Principi N (2003) Differences in nasopharyngeal bacterial flora in children with nonsevere recurrent acute otitis media and chronic otitis media with effusion: implications for management. Pediatr Infect Dis J 22:262–268
9. Roos K, Håkansson EG, Holm S (2001) Effect of recolonisation with "interfering" alpha streptococci on recurrences of acute and secretory otitis media in children: randomised placebo controlled trial. BMJ 322:210–212
10. Doern CD, Burnham CA (2010) It's not easy being green: the viridans group streptococci, with a focus on pediatric clinical manifestations. J Clin Microbiol 48:3829–3835
11. Santagati M, Scillato M, Patanè F, Aiello C, Stefani S (2012) Bacteriocin-producing oral streptococci and inhibition of respiratory pathogens. FEMS Immunol Med Microbiol 65:23–31
12. Santagati M, Scillato M, Muscaridola N, Metoldo V, La Mantia I, Stefani S (2015) Colonization, safety, and tolerability study of the *S. salivarius* 24SMBc nasal spray for its application in upper respiratory tract infections. Eur J Clin Microbiol Infect Dis 34(10):2075–2080
13. Ilia S, Goulielmos GN, Samonis G, Galanakis E (2014) Polymorphisms in IL-6, IL-10, TNF-α, IFN-γ and TGF-β1 genes and susceptibility to acute otitis media in early infancy. Pediatr Infect Dis J 33:518–521