Prevention of caries with probiotic bacteria during early childhood. Promising but inconsistent findings

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ABSTRACT: **Purpose:** This review summarized the available literature on the prevention of childhood caries through biofilm engineering with probiotic bacteria in early childhood. **Methods:** Three databases (PubMed, Cochrane Library and Trip) were searched through January, 2016 for randomized controlled trials published in English. Out of 144 abstracts, seven studies fulfilled the predetermined inclusion criteria and were quality assessed with respect to risk of bias independently by two examiners. Due to the paucity and heterogeneity, a narrative synthesis was performed. The effect size was estimated from the caries prevalence and expressed as prevented fraction and number needed to treat. **Results:** Probiotic supplements were better than placebo in preventing early childhood caries in all seven studies although the difference was statistically significant in only four of them. The prevented fraction ranged from 11% to 61% with a median of 48%. However, the quality of the evidence was low or very low and further translational research is needed to investigate this preventive approach in the clinic. (*Am J Dent* 2016;29:127-131).

CLINICAL SIGNIFICANCE: Probiotic supplements given to infants and preschool children can modify the establishment and composition of the oral biofilm and may aid the maintenance of dental health.

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Introduction

Early childhood caries (ECC) is a global health problem associated with impaired quality of life for the individual and high costs for families and the society in general.[1] ECC is a complex condition with numerous biological, medical, behavioral, psychological, cultural, and lifestyle factors behind its etiology. It is commonly postulated that ECC is a preventable disease but systematic reviews have shown that preventive measures currently available for ECC are only partly successful.[2,3] Consequently, there is an urgent need of novel and effective strategies to combat the development of caries lesions in young children.

Recent advances in oral biofilm research have provided understanding that the resident oral microbiota is diverse, natural and beneficial to the host.[4] In principle, a stable and diverse biofilm is associated with health (homeostasis) while a stressed destabilized biofilm with reduced diversity is associated with disease (dysbiosis). In this context, the microbial colonization of the oral cavity in infants has gained interest. The composition of the oral biofilm is dictated by the sequence and timing of exposure to microbes on a "first come, first served" basis. According to the "hygiene hypothesis" suggested by Strachan,[5] chronic conditions in childhood such as asthma and eczema, as well as upper respiratory infections, can be prevented by early biofilm interventions with beneficial probiotic bacteria in order to support diversity and stimulate the immune response.[6,7] An emerging strategy within dentistry is therefore to expose newborn, infants and toddlers to such probiotic bacteria in order to maintain oral health.[8] A common definition of probiotics is "live microorganisms which when administered in adequate amounts confer a health benefit on the host"[9] and most utilized strains belong to the *Lactobacillus*, *Bifidobacteria* and *Streptococcus* genera. This paper reviewed and summarized the available literature on the prevention of caries in early childhood through biofilm engineering with probiotic bacteria.

Materials and Methods

The PICO was: Population: preschool children 0-6 years; Intervention: any administration route for live probiotic bacteria; Control: placebo, no treatment or best clinical practice; Outcome: caries prevalence or incidence on initial and cavitated level in primary teeth. Three databases were searched (PubMed; Cochrane Library and the Trip database) through January 2016. Relevant literature published in incidence, increment, progression, and regression, expressed with continuous or categorical data from clinical and/or radiographic examinations was included. Reference lists were hand searched for additional papers. The selected articles were assessed by two independent reviewers and key data were extracted and compiled in tables. Due to heterogeneity and paucity of papers, a narrative synthesis was performed. The effect size was estimated from the caries prevalence figures and expressed as prevented fraction (control event rate minus the experimental event rate, divided with the control event rate, expressed as percent). The number needed to treat (NNT) was calculated as 1/ARR (absolute risk reduction).

The quality of the selected publications was independently assessed according to predetermined criteria for methodology and performance by two authors not involved in the studies. The criteria of Cochrane handbook for interventions[10] was used and the risk of bias for each paper was graded as "low", "moderate" or "high". The quality of evidence was rated with the GRADE tool.[11]

Results

From the full abstract list (n= 145), 11 papers with a clinical caries outcome were identified, but five were excluded since they reported findings from schoolchildren, adolescents and

Table 1. Main characteristics of the included studies.

Authors/year	No. of children/ study design[A]	Intervention age or age range	Vehicle	Strain	Control	Follow-up age/ duration	Attrition rate
Intervention during infancy							
Taipele et al, 2013[17]	106/DB RCT	3 weeks-2 years	Pacifier/spoon	*B. animalis* BB-12	Xylitol tablets	4 years	35%/11%[B]
Hasslöf et al, 2013[18]	180/DB RCT	4-13 months	Cereals	*L. paracasei* F19	Placebo	9 years	34%
Stensson et al, 2014[19]	188/DB RCT	0-12 months	Drops	*L. reuteri* ATCC55730	Placebo	9 years	40%
Intervention to toddlers and preschool children							
Näse et al, 2001[20]	594/DB RCT	1-6 years	Milk	*L. rhamnosus* GG	Placebo	7 months	24%
Stecksén-Blicks et al, 2009[21]	248/DB CRCT	1-5 years	Milk + 2.5 ppm F	*L. rhamnosus* LB21	Placebo	21 months	25%
Hedayati-Hajikand et al, 2015[22]	138/DB RCT	2-3 years	Lozenges	*Streptococcus*[C]	Placebo	12 months	20%
Rodriguez et al, 2016[23]	261/DB CRCT	2-3 years	Milk	*L. rhamnosus* SP1	Placebo	12 months	22%

A = DB RCT = double-blind randomized controlled trial; DB CRCT = double-blind cluster randomized controlled trial.
B = Originally randomized/started the intervention.
C = A blend of three strains (*S. uberis* KJ2, *S. oralis* KJ3, *S. rattus* JH145).

Table 2. Main results and authors' conclusions.

	Caries prevalence (%) or increment				
Authors/year	Test group	Control group	Statistics[A]	PF/NNT[B]	Authors' original conclusions
Intervention during infancy					
Taipele et al, 2013[17]	31%	35%	NS	11%/25	Early exposure did not affect caries occurrence
Hasslöf et al, 2013[18]	20%	26%	NS	20%/17	Early exposure did not affect caries frequency
Stensson et al, 2014[19]	18%	42%	S	57%/4	Probiotics reduced proximal caries
Intervention to toddlers and preschool children					
Näse et al, 2001[20]	15%	19%	NS	21%/25	Probiotics reduced caries risk
Stecksén-Blicks et al, 2009[21]	Δdmfs 0.3 (1.8)[C]	Δdmfs 1.6 (3.1)[C]	S	48%/5	Probiotics reduced caries increment
Hedayati-Hajikand et al, 2015[22]	Δds 0.2[D]	Δds 0.8[D]	S	49%/4	Probiotics reduced caries increment
Rodriguez et al, 2016[23]	$\Delta ICDAS_{5-6}$ 9.7[E]	$\Delta ICDAS_{5-6}$ 24.3[E]	S	61%/7	Probiotics reduced caries increment

A = NS = not statistically significant; S= statistically significant difference between the groups.
B = PF/NNT = prevented fraction/number needed to treat.
C = dmfs = mean increment of decayed, missed, filled surfaces (standard deviation).
D = ds = mean increment of decayed surfaces.
E = $ICDAS_{5-6}$ = International Caries Detection and Assessment System, score 5-6 = mean increment in percent of obvious dentinal caries.

adults.[12-16] Thus, seven publications, from Sweden, Finland, and Chile, fulfilled the inclusion criteria and were subdivided into two domains:

Intervention during infancy - Three studies examined long-term effects on caries in children who received probiotic supplements during their first year of life.[17-19] The details are summarized in Tables 1 and 2 and the calculated effect size is illustrated in the Figure. In the first study,[17] infants were given probiotic tablets with probiotic *Bifidobacteria* from 1-2 months up to 2 years of age with aid of specific pacifier. Children who did not comply with the pacifier were given crushed tablets with a spoon. The test tablets were sweetened with xylitol and tablets containing either xylitol or sorbitol served as positive controls. The infants were characterized as low-risk children and no significant differences in caries occurrence were seen at 4 years of age. In the second trial,[18] infants were served a cereal diet supplemented with *Lactobacillus paracasei* F19 or placebo cereals during weaning. At the age of 9 years, there was no significant difference in caries experience between the test and placebo group but the caries prevalence was lower in the test group. In the third study,[19] newborn children were given five daily drops containing two strains of the probiotic bacterium *Lactobacillus reuteri* during their first year of life. The infants in the control group were given placebo drops. At the age of 9 years, the children in the probiotic test group displayed a 50% reduction in caries experience compared with the control children. 82% of the children in the probiotic group were caries-free compared to 58% in the placebo group.

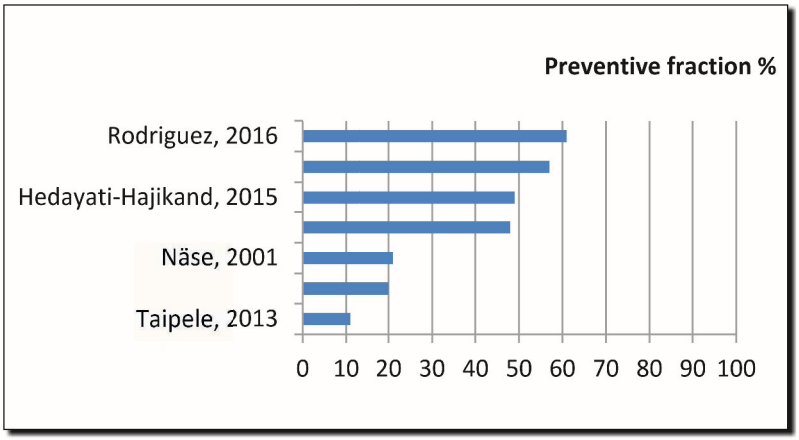

Figure. The effect size expressed as prevented fraction (PF) in the selected papers.

Interventions to toddlers and preschool children - Four studies[20-23] were identified in which the intervention was directed to children between 1 and 6 years (Tables 1 and 2, Figure). The pioneering study was performed in Finland and employed a probiotic bacterium (*Lactobacillus rhamnosus* GG; LGG) which was added to milk and served to children in municipal daycare centers.[20] The children received the probiotic

Table 3. Assessment of risk of bias for the included studies.

Authors/year	Type of bias					Risk level
	Selection	Performance	Detection	Attrition	Reporting	
Intervention during infancy						
Taipele et al, 2013[17]	?	+	+	?	?	Moderate
Hasslöf et al, 2013[18]	?	+	-	-	+	High
Stensson et al, 2014[19]	?	+	?	-	+	High
Intervention to toddlers and preschool children						
Näse et al, 2001[20]	?	+	+	-	-	High
Stecksen-Blicks et al, 2009[21]	+	-	-	-	+	High
Hedayati-Hajikand et al, 2015[22]	-	?	+	-	+	High
Rodriguez et al, 2016	+	+	+	-	+	Moderate/Low

+ = low risk of bias; ? = unclear risk of bias; - =high risk of bias.

milk or placebo 5 days a week during 7 months. The effect of LGG on caries was found to be positive, albeit not statistically significant. This tendency was most obvious among the 3-4 year olds and a subgroup analysis of these children reached statistical significance. The project also evaluated the effects of the intervention on general health.[24] The results showed a modest but consistent reduction of respiratory infections in the group consuming the probiotic milk. The second study[21] had a similar daycare approach and evaluated the effect of daily serving (weekdays) of milk (1.5 dl) containing *Lactobacillus rhamnosus* LB21 on caries incidence over 21 months. In addition, 0.5 ppm fluoride was added to the experimental milk while the control group was given a milk without both fluoride and probiotic bacteria. The caries increment of decayed, extracted and filled surfaces in the test group was significantly reduced and the proportion of caries-free children was 77% in the test group compared with 56% in the control group. Also in this study, a marker of general health was improved; the mean number of days with antibiotic treatment was 60% lower in the test group. However, it should be stressed that the employed study design did not allow separating the potential effects of fluoride and the probiotic supplement from each other. In the third study, probiotic lozenges containing three Streptococcus-derived strains were distributed to a group of children living in a low socioeconomic community in Sweden.[22] The duration of the intervention was 1 year and resulted in significantly fewer new caries lesions in the probiotic group compared with the placebo group. The caries prevalence was 24% in the test group after the intervention compared with 47% in the placebo group. It is important to underline that the clear-cut results were obtained in spite of the fact that around 80% of the families reported supervised tooth brushing twice daily and a far from optimal compliance with the probiotic lozenges.

In a recently published study,[23] socially vulnerable children aged 2-3 years attending nursery schools in Chile were given milk supplemented with the probiotic *Lactobacillus rhamnosus* SP1 or standard milk on weekdays for 10 months. At the clinical examination at follow-up, the number of individuals who had developed new cavitated caries lesions (ICDAS 5-6) was significantly lower in the probiotic group (9.7%) compared with the control group (24.3%).

Assessment of study quality - Five of the studies were assessed with high risk of bias while the studies of Taipale et al[20] and Rodriguez et al[23] displayed a moderate and moderate/low risk, respectively (Table 3). The most common concern was high attrition rates (≥ 20%) followed by performance bias and selection bias. Thus, the quality of evidence was rated as low or very low.

Discussion

Probiotic supplements performed better than placebo in preventing or reducing early childhood caries in all seven studies although the difference was statistically significant in only four of them. This clearly supports the hypothesis of Devine & Marsh[25] that it may be beneficial to modify or alter the caries-associated oral microbiota in early childhood in order to combat ECC. However, the quality of the evidence was low since only few of the included publications appeared to have a low risk of bias.

There are a number of factors that can contribute to explain the partly diverging results. First of all, host benefits to probiotic exposure are strain specific and findings from one strain cannot be extrapolated to another.[26,27] Five of the present studies were conducted with lactobacilli-derived probiotics but unfortunately different strains were utilized. It has also been speculated that a mix of probiotic strains could perform better than a single strain.[28] No comparative studies were identified however, so this issue remains a knowledge gap. Secondly, the mechanisms of probiotic action are still enigmatic. It is generally believed that there is a direct effect in the oral biofilm/oral mucosa and a systemic effect through immunomodulation.[29] For early childhood caries, the dominating effects are most likely co-aggregation (adhesion) and competitive exclusion.[27] Most probiotic bacteria are also bacteriocin-producing, a property that can be transferred and augmented in indigenous bacteria through megaplasmids.[30]

Previous research has clearly shown that an early acquisition of *S. mutans* is associated with an increased risk of early childhood caries and further caries development later in life.[31-34] As mentioned above, it can be hypothesized that early exposure to probiotic bacteria can delay or counteract the colonization of caries-associated bacteria (e.g. *S. mutans*). In a simplified way, it seems as a matter of who will win the race to colonize the oral biofilm.[35,36] Two systematic reviews and a meta-analysis[37,38] have concluded that intake of probiotic bacteria affects the mutans streptococci counts in plaque and saliva. It has also been shown that consumption of probiotic strains can induce a shift in the oral microbial composition[39] and reduce biofilm formation in vitro.[40] Although the understanding that probiotic supplements can modulate the composition and function of the

oral biofilm in early childhood is rapidly developing, further translational research is needed to verify this preventive approach in the clinic.

All studies in this review employed an appropriate study design but it should be noted that two of them[18,19] originally explored a medical outcome and that the randomization code was unveiled years prior to the dental examination. Consequently, no a priori power calculations with respect to caries development could be performed. In one study,[19] children from families with a history of allergic diseases were recruited, another[17] relied on children of interested and motivated parents. Likewise, the general parental education level was high in the study of Hasslöf et al.[18] Thus, a certain selection bias cannot be excluded and the external validity remains unclear. Interestingly, the three studies[21-23] with outstanding outcomes were performed in children living in low socioeconomic areas. It is well known that the caries prevalence has a strong socioeconomic gradient[41] and these findings may indicate that the probiotic supplements can be more potent in caries active subjects.

Probiotic supplements are classified as food additives and many different over-the-counter products are today available to the informed consumer. In this review, several vehicles of probiotic supplements with 1-2 intakes per day were described with daily doses ranging from 5×10^5 colony forming units (cfu) to 10^{10} cfu. A daily intake of 10^8 cfu is commonly recommended[28] but it is not clear if a positive dose-response relationship exists concerning caries development. Therefore, this is an interesting topic for future studies.

The effect of a clinical intervention is always dependent on the cooperation of the subjects included. Thus, one could assume that the daycare-based administrations should have a better compliance than those relying on the parents. It should however be noted that the probiotic milk only was served on weekdays at the day-care centers and that the servings were put on hold over the holiday seasons.[21,23] Novel treatment strategies and industrial sponsorships increase the risk of publication bias since positive findings are more likely published than negative. Although too few studies were available for a funnel plot analysis, there is no reason to believe that unpublished data would have altered the conclusions.

In conclusion, the findings of this review illustrate the potential of biofilm modification at early age to maintain dental health and prevent early childhood caries. However, the findings were not entirely consistent and several possible underlying factors could be identified. Further translational research is needed to explore this preventive approach in a clinical context.

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