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REVIEW

Probiotics reduce mutans streptococci counts in humans: a systematic review and meta-analysis

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Abstract
Objectives Systematically review the available literature regarding the caries-preventive effect of probiotics.
Data, sources and study selection An electronic search was conducted in three databases (PubMed MEDLINE, ISI Web of Science and Cochrane Library) to identify all suitable studies. The outcomes had to be presented as the effect of probiotics on the incidence of caries or on the levels of mutans streptococci and/or *Lactobacillus* species. Human studies, written in English, with at least 15 participants, comparing a probiotic product with a placebo/no probiotic were included. Where possible, a meta-analysis was performed to obtain quantitative data.
Results Since only two articles presented useful data on the caries incidence, we focused on the surrogate endpoints: mutans streptococci and/or *Lactobacillus* counts. The meta-analysis showed that when the probiotic and control group are compared after treatment, significantly more patients in the probiotic group had low mutans streptococci ($<10^5$ CFU/ml) counts and significantly less patients had high ($>10^6$ CFU/ml) counts. Regarding the *Lactobacillus* counts, comparing the probiotic and control group at the end of the probiotic use, no significant differences could be observed, neither in low ($<10^4$ CFU/ml) nor in high *Lactobacillus* ($>10^6$ CFU/ml) counts.
Conclusions Within the limitations of the available data, it may be concluded that probiotics decrease the mutans streptococci counts. This suggests that probiotics could have a positive effect in the prevention of caries.
Clinical relevance There is insufficient evidence that probiotics can prevent caries, but they can reduce the mutans streptococci counts.

Keywords Probiotics · Caries · Tooth decay · Cariogenic bacteria · Mutans streptococci · Lactobacilli · *Streptococcus mutans*

Introduction

Dental caries is one of the most common preventable diseases and affects people of all ages [1]. It results from a disturbance in the ecological balance at the tooth surface which ultimately leads to loss of tooth mineral [2]. Endogenous, acidogenic bacteria (largely *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus* spp. [3–7]) are of importance since they produce organic acids which demineralize the hard tissues [1, 4, 8–10]. Besides cariogenic bacteria, a susceptible host and nutrients are considered as essential elements in the aetiology of dental caries [11]. Furthermore, the time factor is important for the production of acids and the subsequent demineralization of tooth structures [11].

Current preventive strategies for dental caries target the host factors, dietary factors and the removal of the plaque biofilm. They encompass mainly the use of topical fluorides, dietary monitoring, and mechanical and chemical plaque control [3]. Recently, the caries preventive effect of probiotics has been suggested [12–14]. Probiotics are defined by the WHO as living microorganisms that confer a health benefit for the

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host when administered in sufficient amounts (www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf) [17]. Although their mechanisms of action are still poorly understood, it is known that probiotics can produce substances such as bacteriocins against pathogenic bacteria [15, 16]. Furthermore, they can stimulate local immunity, modulate the inflammatory response, modify the environment and compete with pathogens for binding sites and nutrients [15, 16].

To date, the effect of probiotics on systemic health and medical disorders is elaborately described [17]. Positive effects have been shown not only in the field of gastrointestinal diseases, e.g. for diarrhoea, inflammatory bowel disease and irritable bowel syndrome, but also for atopic diseases and cancer [17]. Over the recent years, an increasing interest in probiotics from an oral health perspective has emerged. The effect of probiotics on halitosis [18–20], candidiasis [21, 22] and periodontitis [23–31] (for review, see Teughels et al., 2011 [15]) has been investigated. Additionally, several papers have examined the effect of probiotics on caries. Recently, a systematic review showed that probiotics have the capacity to reduce mutans streptococci counts in short-term. However, a meta-analysis evaluating this effect has not yet been carried out. Therefore, this study aimed at systematically evaluating the current literature by means of a meta-analysis. The primary outcome variable of interest was caries incidence and as secondary parameters the surrogate endpoints, mutans streptococci and lactobacilli counts, were analysed.

Materials and methods

This systematic review was conducted in accordance with the guidelines of the Transparent Reporting of Systematic Reviews and Meta-Analyses (PRISMA) [32].

Focused PICO question

What is, in healthy humans, the effect of probiotics compared to a placebo just after its usage on caries incidence and on the level of mutans streptococci and lactobacilli spp. in the oral cavity?

Search strategy

A computerized literature search of PubMed MEDLINE, ISI Web of Knowledge^{SM} and the Cochrane databases was performed in order to identify all studies concerning caries and probiotics regardless of their publication status. These searches were restricted till June 2013. Additional hand searches were performed and included the following: (1) bibliographies of previous reviews on the subject [12–14, 16, 33–35], (2) bibliographies of all publications cited in these articles and (3) cited reference searches of the publications considered using the ISI Web of Knowledge^{SM}.

Search terms

Although there are some differences, no differentiation was made between probiotics and replacement therapy (also known as bacteriotherapy or bacterial interference) given the confusion regarding the use of these terms [15]. The following search was used: "probiotic OR replacement therapy OR bacterial interference OR bacteriotherapy" AND "dental caries OR tooth decay OR cariogenic bacteria OR *Streptococcus mutans*" OR "lactobacilli and dental".

Eligibility criteria

Following criteria were used for inclusion: studies in the English language conducted in humans. The intervention must comprise the use of a probiotic versus a placebo or no probiotic. For our primary parameter of interest, the results had to be presented as the effect of probiotics on the incidence of caries. For the indirect effect, the outcome measures had to be presented as the levels of mutans streptococci and/or *Lactobacillus* species, which are surrogate endpoints in the development of caries. The evaluation of all parameters had to take place before and just after using the probiotic. Only controlled clinical trials with at least 15 participants for each group were included.

Exclusion criteria

Studies that explicitly mentioned that the patients were wearing fixed orthodontic appliances were excluded, because this may facilitate the establishment and growth of cariogenic streptococci strains [36]. Studies with only a positive control group were excluded.

Risk of bias assessment

A quality assessment was conducted to evaluate the methodological quality of the selected studies. This was based on the randomized controlled trial (RCT) checklist of the Cochrane Center, the CONSORT guidelines [37], the Delphi list [38] and the checklist as proposed by Van der Weijden et al. [39]. Seven criteria from these lists were selected to assess risk of bias, namely random allocation, blinding of the participants and personnel, blinding of outcome assessment, defined inclusion and exclusion criteria, identical treatment between groups except for the intervention, incomplete outcome data and selective reporting.

When all these criteria were assessed as low risk of bias, the article was classified as having a low risk of bias. When one or two of these criteria were assessed as high risk of bias or

unclear, the study was regarded to have a moderate potential risk of bias. The risk of potential bias was high, when three or more criteria had a high or unclear risk of bias. Two reviewers assessed the risk of bias independently (VD, IL).

Data extraction

Two reviewers (VD, IL) independently screened the titles and subsequent the abstracts of all articles found. When there was disagreement or when an abstract contained insufficient information, the full text of the paper was reviewed. The final inclusion of studies was then made by discussion. Thereafter, both reviewers extracted the data separately from the selected papers. This information was transferred to a data extraction sheet. The following characteristics were abstracted from each study: first author, year of publication, age of subjects, study design, length of treatment, number of subjects in each treatment group, vehicle, type and amount of probiotic used, publication bias and original author's conclusion.

Data analysis

Concerning the levels of mutans streptococci and lactobacilli species, the intergroup comparisons after treatment and intragroup comparisons, as described by the authors, were placed in a table. All available microbiological data regarding the mutans streptococci and *Lactobacillus* counts were arranged in groups in analogy with the interpretation charts of these chairside tests. The microbiological results from the studies using specific agars were placed in a table with the baseline and post-treatment counts (expressed as mean and standard deviation) (this tables are available online).

Where possible, a meta-analysis for binary outcomes was performed regarding the number of patients in the probiotic versus the control group in the clusters with the highest and lowest bacterial counts both before and after treatment. Fixed effects were applied. Relative risks were calculated and they were, together with their corresponding confidence intervals, displayed in forest plots. Comparisons were made between placebo control (C) and probiotic groups (P) before and after treatment. In addition, for the control and probiotics group, data collected before treatment were also compared with those from after the treatment. For all studies, the microbiological levels at baseline and at the end of the probiotic usage (post-intervention) were used for this review. When data were missing, incomplete or ambiguous, the authors were contacted.

Results

Search and selection

The electronic searches through the MEDLINE, Cochrane and ISI Web of Knowledge^{SM} retrieved 725 unique articles as summarized in Fig. 1. Of these, 690 were removed after a first selection and 35 articles were read full text for eligibility. Three studies were excluded because they appeared to be in vitro studies [20, 40, 41] Three studies did not have a control group [30, 42, 43]. One study had only a positive control as control group [44]. One study combined the use of a probiotic with the use of fluor [45]. And two studies combined the use of a probiotic with the use of xylitol [46, 47]. One study was conducted in patients wearing fixed orthodontic appliances [48]. And finally, five studies included less than 15 patients in each group [49–53]. This resulted in the retrieval of 19 publications. For two of these studies, we took only the data into consideration from the probiotic and/or test groups that met the inclusion criteria [54, 55].

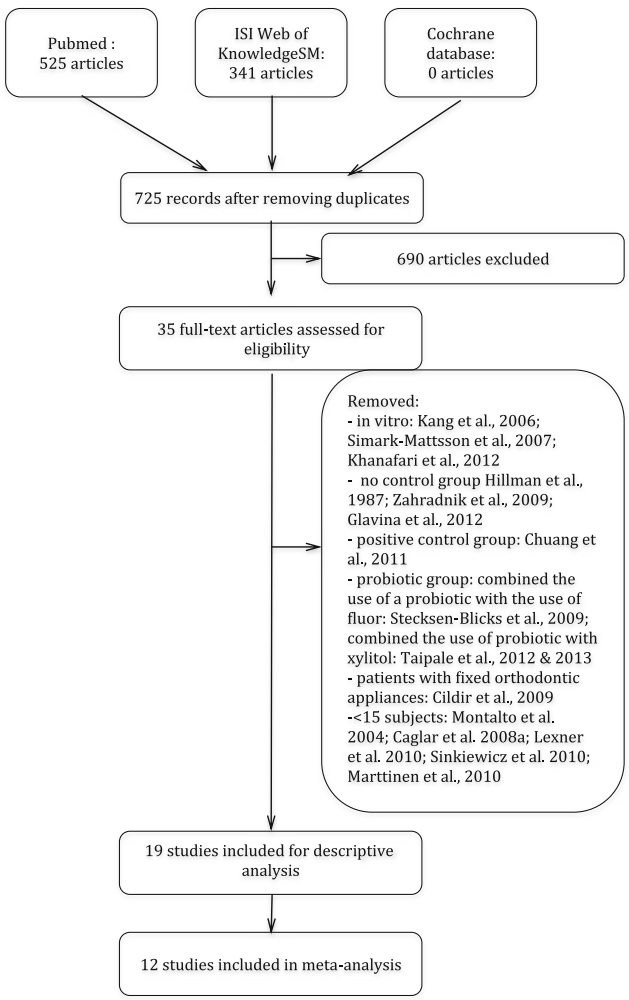

Fig. 1 Search strategy

Table 1 Characteristics of the selected studies

	Age (years)	Design time	Study group	Number of patients	Vehicle frequency	Strain concentration	Publication bias				Original author's conclusion
							Initiated[a]	Supported[b]	Provided[c]	Coauthor[d]	
Nase et al. (2001)	1.3–6.8	RCT 7 months	C	220	Milk 1×/day, 5 days a week	–	x			x	Lower mutans streptococci counts at the end of the study. And probiotic intervention reduced the risk of caries significantly, particularly in the 3- to 4-year-olds
			P	231	Milk 1x/day, 5 days a week	*Lactobacillus rhamnosus GG, ATCC 53103* $5–10×10^5$ CFU/ml					
Ahola et al. (2002)	18–35	RCT 3 weeks	C	36	Edam cheese 16 % fat 15 g/day, preferably after meal/snack	–	x			x	Probiotic intervention might reduce the risk of the highest level of *S. mutans*
			P	38	Edam cheese 16 % fat + probiotic bacteria 15 g/day, preferably after meal/snack	*Lactobacillus rhamnosus GG, ATCC 53103* $1.9×10^7$ CFU/g *Lactobacillus rhamnosus LC 705* $1.2×10^7$ CFU/g					
Nikawa et al. (2004)	20	CO 2 weeks	C	40	Yoghurt with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* 95 g/day at lunchtime	–					Consuming probiotic yoghurt reduced the oral carriage of mutans streptococci significantly compared with a placebo
			P	40	Yoghurt 95 g/day at lunchtime	*L. reuteri* concentration: not mentioned					
Çaglar et al. (2005)	21–24	CO 2 weeks	C	21	200 g yoghurt (Danone Natural ®; Danone, Istanbul, Turkey) 1×/day at noon	–			x		Statistically significant reduction of mutans streptococci counts and a similar but not significant trend for lactobacilli counts after consumption of a probiotic
			P	21	200 g yoghurt (Activia®; Danone, Istanbul, Turkey) 1×/day at noon	*Bifidobacterium DN-173 010* $1×10^7$ CFU/g					
Çaglar et al. (2006)	21–24	RCT 3 weeks	C1	30	200 ml water through a placebo straw 1×/day at noon	–			x		Statistically significant reduction of mutans streptococci counts and a similar but nonsignificant trend for lactobacilli counts after consumption of a probiotic
			C2	30	Ingested one sucking tablet without probiotic bacteria 1×/day at noon	-					
			P1	30	200 ml water through a prepared straw (Life Top straw, Biogaia, Stockholm, Zweden) containing probiotic bacteria 1×/day at noon	*L. reuteri ATCC 55730* minimum 10^8 CFU/straw					
			P2	30	Ingested one sucking tablet (BioGaia ProDenta, BioGaia, Stockholm, Sweden) with probiotic bacteria 1×/day at noon	*L. reuteri ATCC 55730* 10^8 CFU/tab					
Çaglar et al. (2007)	21–24	RCT 3 weeks	C1	20	One placebo gum three times a day (morning, noon, evening)	–			x		Statistically significant reduction of mutans streptococci counts in the probiotic group but no alteration of *Lactabacillus* counts
			P1	20	Probiotic chewing gum (BioGaia, Stockholm, Sweden) three times daily (morning, noon, evening)	*L. reuteri ATCC 55730* $1×10^8$ CFU/gum *L. reuteri ATCC PTA 5289* $1×10^8$ CFU/gum					

Table 1 (continued)

	Age (years)	Design time	Study group	Number of patients	Vehicle frequency	Strain concentration	Publication bias				Original author's conclusion
							Initiated[a]	Supported[b]	Provided[c]	Coauthor[d]	
Çaglar et al. (2008b)	20	CO 10 days	C	24	Max Star Cup® (Algida Türkiye, Corlu, Turkey), 100 ml 1×/day at noon	–			x		Statistically significant reduction of mutans streptococci counts but unaltered lactobacilli levels after consumption of the probiotic
			P	23	Max Star Cup® (Algida Türkiye, Corlu, Turkey), 100 ml 1×/day at noon	*Bifidobacterium lactis Bb-12®* 1×10^7 CFU/g					
Cogulu et al. (2010)	20–27	RCT 3 weeks	C	34	100 ml milk 1×/day after breakfast	–			x		Statistically significant reduction compared to baseline of mutans streptococci and *Lactobacillus* levels in group P1
			P1	35	100 ml kefir containing 3.5 % fat 2×/day after breakfast and lunch	*Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *diacetylactis*, *Leuconostoc mesenteroides* ssp. *cremoris, Lactobacillus kefyr*, *Kluyveromyces marxianus and Saccharomyces unisporus.* *Lactobacillus* spp. 3.3×10^8 CFU/ml *Streptococcus* spp. 1.9×10^7 CFU/ml					
			P2	35	100 ml kefir containing 3.5 % fat 1×/day after breakfast	*Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *diacetylactis*, *Leuconostoc mesenteroides* ssp. *cremoris*, *Lactobacillus kefyr, Kluyveromyces marxianus and Saccharomyces unisporus.* *Lactobacillus* spp. 3.3×10^8 CFU/ml *Streptococcus* spp. 1.9×10^7 CFU/ml					
Aminabadi et al. (2011)	6–12	RCT 3 weeks	C	35	–	–					Chloorhexidine treatment before probiotic intake induced more stable colonization with LGG strains than probiotic alone
			P1	35	15–20 ml yoghurt, daily at dinnertime	*Lactobacillus rhamnosus GG* 2×10^8 CFU/g					
			P2	35	15–20 ml yoghurt, daily at dinnertime	*Lactobacillus rhamnosus GG* 2×10^8 CFU/g					
Cildir et al. (2011)	4–12	CO 25 days	C	19	BioGaia drops® 5 drops/day	–			x		No statistically significant difference in mutans streptococci and *Lactobacillus* counts, neither after consumption of the probiotic nor after consumption of the placebo
			P	19	BioGaia Reuteri drops® (BioGaia AB, Stockholm, Sweden) 5 drops/day	Freeze-dried *L. reuteri DSM 17938* $\geq1\times10^8$ CFU/5 drops *L. reuteri ATCC PTA 5289* $\geq1\times10^8$ CFU/5 drops daily intake 0.15–0.20 g/5 drops					

Table 1 (continued)

	Age (years)	Design time	Study group	Number of patients	Vehicle frequency	Strain concentration	Publication bias				Original author's conclusion
							Initiated[a]	Supported[b]	Provided[c]	Coauthor[d]	
Jindal et al. (2011)	7–14	CO 2 weeks	C	50	Placebo powder, mixed in 20 ml water. Before swallowing, swish the mixture for 1 min in the mouth. 1×/day, 1 h after lunch	–					Statistically significant reduction of mutans streptococci counts in groups P1 and P2 after consumption of the probiotic
			P1	50	Darolac (Aristo Pharmaceuticals, India) mixed in 20 ml water. Before swallowing, swish the mixture for 1 min in the mouth. 1×/day, 1 h after lunch	*Lactobacillus rhamnosus*, *Bifidobacterium longum* and *Saccharomyces cereviasae* 1 g of powder with 1.25 billion freeze-dried bacterial combination					
			P2	50	Sporalac (Uni-Sankyo Ltd, Inda) mixed in 20 ml water. Before swallowing, swish the mixture for 1 min in the mouth. 1×/day, 1 h after lunch	*Bacillus coagulans* 1 g powder with 150 million spores					
Petersson et al. (2011)	58–84	RCT 15 months	C1	25	200 ml of normal- to medium-fat milk with solution (1.0 ml) (containing skim milk powder) 1×/day	–					Daily intake of milk supplemented with fluoride and/or probiotic bacteria may reverse soft and leathery primary root caries lesions in older adults
			P1	27	200 ml of normal- to medium-fat milk with solution (1.0 ml) (containing skim milk powder and probiotic bacteria) 1×/day	*Lactobacillus rhamnosus LB21* 10^7 CFU/ml					
Singh et al. (2011)	12–14	CO 10 days	C	39	54 g ice cream 1×/day at noon	–		?	?		The probiotic ice cream brought a statistically significant reduction in mutans streptococci counts, but no significant differences in *Lactobacillus* counts
			P	39	54 g ice cream (Amul India Pvt. Ltd., Anand, Gujarat, India) 1×/day at noon	Freeze-dried culture of probiotic strains of *Bifidobacterium lactis Bb-12* and *Lactobacillus acidophilus La-5* 1×10^6 CFU/g					
Mortazavi and Akhlaghi (2012)	18–37	RCT 2 weeks	C	31	50 g white cheese 2×/day with breakfast and dinner meals	–					Statistically significant reduction of *S. mutans* after probiotic consumption. *S. mutans* reduction was not significant between control and probiotic group. No statistically significant inter- nor intragroup changes for lactobacilli counts
			P	29	50 g white cheese 2×/day with breakfast and dinner meals	*L. Casei LAF-TI-L26* 1×10^6 CFU/g					
		CO	C	18	Placebo tablet	–			x		

Table 1 (continued)

	Age (years)	Design time	Study group	Number of patients	Vehicle frequency	Strain concentration	Publication bias				Original author's conclusion
							Initiated[a]	Supported[b]	Provided[c]	Coauthor[d]	
Keller and Twetman (2012)	Mean age= 26 years	2 weeks									The mutans streptococci counts were not statistically, significantly altered; the *Lactobacillus* increased significantly in the probiotic group
			P	18	Tablet 3×/day (morning, noon and evening)	*L. reuteri* DSM 17938 *L. reuteri* ATCC PTA 5289 1×10^8 CFU/tablet					
Keller et al. (2012)	Mean age= 23 years	RCT 6 weeks	C P	30 32	Placebo lozenge Lozenge 2×/day	– *L. reuteri* DSM 17938 *L. reuteri* ATCC PTA 5289 1×10^8 CFU/tablet			x		Daily oral administration of *L. reuteri* did not seem to affect/ delay regrowth of mutans streptococci after FMD with chlorhexidine
Sudhir et al. (2012)	10–12	RCT 30 days	C P	20 20	200 ml curd 200 ml curd	– *Lactobacillus acidophilus* concentration not mentioned					Short-term consumption of probiotic curds can reduce *S. mutans* counts
Juneja and Kakade (2012)	12–15	RCT 3 weeks	C P	20 20	150 ml milk 150 ml milk 2×/day	– *Lactobacillus rhamnosus htc 70* 2.34×10^9 CFU/day					Statistically significant reduction of mutans streptococci after in the probiotic group post-treatment and post-follow-up
Burton et al. (2013)	5–10	RCT 3 months	C	43	Lozenge 2×/day: after brushing the teeth in the morning and at night	–	x	x	x		Reduced mutans streptococci counts after probiotic use
			P	40	Lozenge 2×/day: after brushing the teeth in the morning and at night	*Streptococcus salivarius* M18					

RCT randomized controlled trial, *CO* crossover trial, ? unclear

[a] Study initiated by manufacturer

[b] Study supported by manufacturer

[c] Products provided by manufacturer

[d] Employee of the manufacturer coauthored

Outcome results

Table 1 summarizes the study characteristics and their outcomes. The selected papers were substantially heterogeneous in their set-up, duration, used probiotics, mode of application and the assessment criteria. The number and the age of the participants varied among the studies.

Characteristics of the study design

Since only two articles that met our inclusion criteria were found that used caries incidence as outcome measure, it was decided to mainly focus on mutans streptococci and *Lactobacillus* counts. All included studies had as outcome measures mutans streptococci and/or *Lactobacillus* counts or the prevalence of patients having low, medium or high counts of either mutans streptococci or lactobacilli. All studies had an evaluation moment immediately post-intervention. Five studies had an extra evaluation moment some weeks later [21, 56–59].

Characteristics of the study population

Three studies did not mention if there were untreated caries lesions present in their study population [21, 56, 58]. This was an exclusion criterion for 13 studies [44, 54, 57, 59–68]. In three studies, caries was present in the study population [55, 69, 70]. Often, specific patient groups were used in the studies. Certain studies focused on specific age groups such as children [56, 58, 64–66, 69, 71] or elderly people [55]. Cildir and coworkers explicitly focused on operated cleft lip/palate children [64]. Other studies targeted patient groups with moderate to high (>10^4 CFU) [56, 58, 68] or high (≥10^5 CFU/ml saliva) [56, 57, 59] salivary mutans streptococci counts. One study selected only female subjects who were studying to become a dental hygienist [60]. Another study recruited their subjects from the University of Helsinki, Helsinki area polytechnic schools and Valio Ltd. personnel in Helsinki [21].

Type of probiotic and way of administration

Nine studies did not report on the time between brushing and the use of the probiotic product [44, 55, 59, 60, 65, 66, 68, 69, 71], but the other authors suggest to wait 1 h after administration of the probiotic. One study [58] suggested using the probiotic lozenges after brushing.

Microbiological changes

Mutans streptococci

Tables 3 and 4 of the online appendix show the raw microbiological data. Table 5 in the online appendix shows the post-intervention microbiological results presented as intergroup comparison after treatment and intragroup comparison.

Twelve studies reported a significant reduction in mutans streptococci when a probiotic was used [54, 56, 57, 60–63, 65–67, 70, 71]. A decrease of the mutans streptococci counts could also be observed in one control group [57]. However, in this study, a pre-treatment with a chlorhexidine mouthwash was performed. In contrast, four studies reported no significant differences in mutans streptococci counts [55, 59, 64, 68], albeit one of them [55] described a tendency to reduced counts. No study reported an increase in mutans streptococci numbers when probiotics were used.

In contrast to the intragroup comparisons, the intergroup comparisons were made in only a few studies. Four authors investigated the intergroup comparison only at the beginning of the study [54, 62, 64, 70], to examine whether they start with similar groups concerning the microbiological counts. Three studies found a significant difference at the end of the study between the mutans streptococci counts in the probiotic versus control group [63, 65, 71], this difference was not noticed at baseline. In contrast, six studies could not detect a statistically significant difference, neither at baseline nor at the end of the probiotic usage [21, 56, 58, 59, 67, 68].

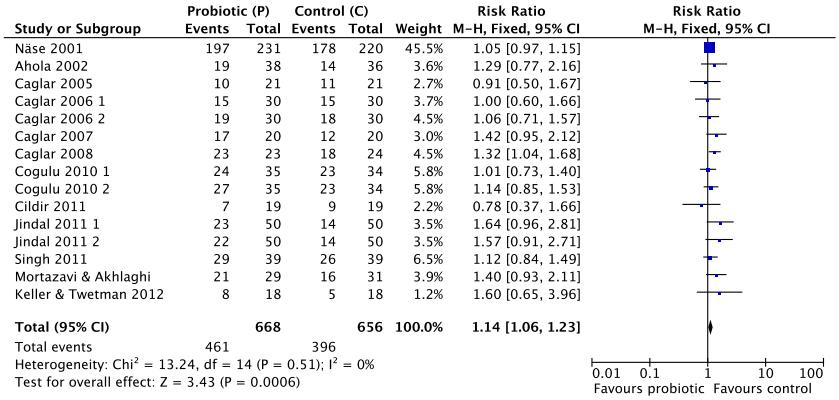

Fig. 2 Forest plot of comparison: probiotic versus control group, outcome <10^5 mutans streptococci

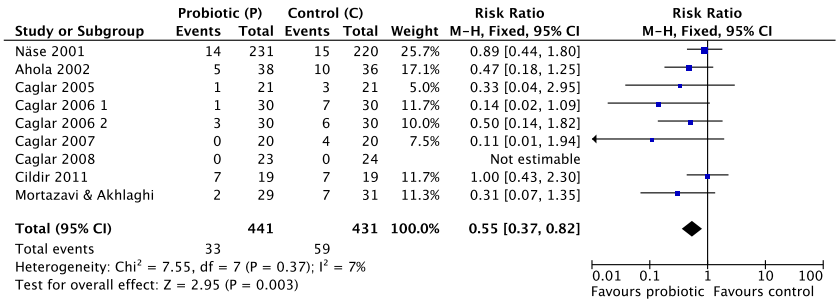

Fig. 3 Forest plot of comparison: probiotic versus control group, outcome >10^6 mutans streptococci

Lactobacillus species

With regard to the *Lactobacillus* counts, the results are even more divergent. One study described decreased lactobacilli counts in one of their two probiotic groups [63]. In contrast, two studies observed a significant increase in lactobacilli counts [57, 68]. Although the majority of studies did not find significant differences in lactobacilli counts between the probiotic group and the control group [54, 55, 61, 62, 64, 66, 67, 70].

Concerning the intergroup comparison, four authors investigated this only at the beginning of the study [54, 62, 66, 70], to examine whether they start with similar groups concerning the microbiological counts. One study found a significant reduction of the *Lactobacillus* counts in the probiotic group compared with the control group [63]. Four studies could not detect a statistically significant difference, neither at baseline nor at the end of the probiotic usage [21, 58, 67, 68].

Meta-analysis

Of these, twelve articles could be used for a meta-analysis since data could be unambiguously extracted regarding the number of patients having low, medium or high counts of either mutans streptococci or lactobacilli.

The variety with which the available data were presented made it impossible to include all the studies in a meta-analysis. Most studies used a chairside test for evaluating the microbial counts, dividing the patients into groups with low, moderate or high microbial counts. However, the study of Petersson et al. (2011) could not be used, because in this paper, there is only mentioned in how much patients' mutans streptococci or lactobacilli are detected [55]. Additionally, two studies which used cultivation methods presented their data accordingly to the data obtained with chairside tests and could be included in the meta-analysis [65, 67]. Of the remaining studies that made use of cultivation methods, only from two studies it was possible to unambiguously extract all necessary data as median and standard deviation, which is too little to perform a meaningful meta-analysis.

The results of this meta-analysis are displayed in Table 6 and 7 of the online appendix, respectively, as the intergroup and the intragroup analysis.

This meta-analysis showed that when the number of patients with the highest mutans streptococci counts for the probiotic group was compared before and after treatment, a significant decrease could be observed (RR=0.37; 95 % CI 0.25 to 0.53). This could not be observed in the control group (RR=0.86; 95 % CI 0.63 to 1.17). When comparing the number of patients with low mutans streptococci counts before and after intervention in the probiotic group, a significant increase was noted (RR=1.33; 95 % CI 1.22 to 1.44). In the control group, a similar but more modest effect could be seen (RR=1.12; 95 % CI 1.02 to 1.22).

Intergroup comparisons showed that when the probiotic and control groups were compared after treatment, significantly more patients in the probiotic groups had low mutans streptococci (<10^5 CFU/ml) counts (RR=1.14; 95 % CI 1.06 to 1.23) (Fig. 2) and significantly less patients in the probiotic group had high (>10^6 CFU/ml) counts (RR=0.55; 95 % CI 0.37 to 0.82) (Fig. 3). This pronounced significant difference was not present at baseline (respectively, RR=0.95; 95 % CI 0.87 to 1.05 and RR=1.35; 95 % CI 1.02 to 1.78).

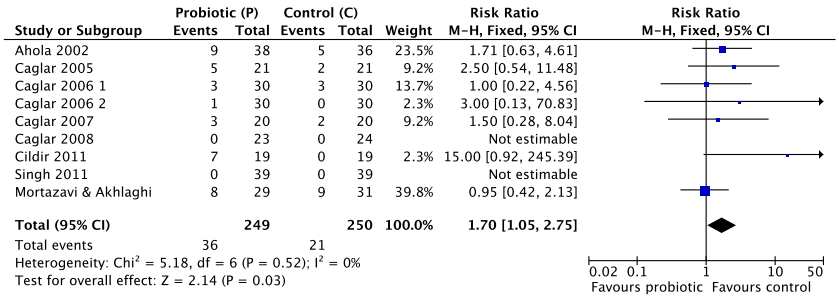

Fig. 4 Forest plot of comparison: probiotic versus control group: outcome <10^4 lactobacilli

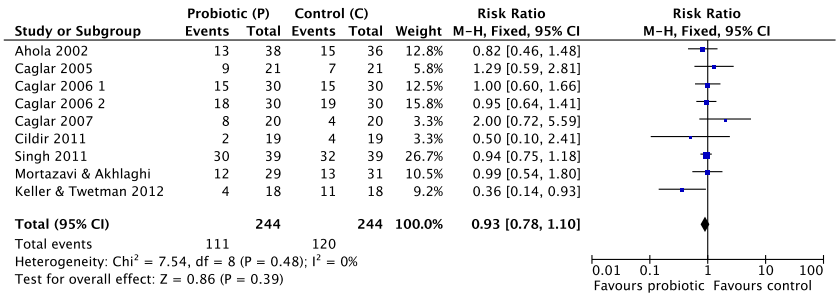

Fig. 5 Forest plot of comparison: probiotic versus control group, outcome >10^6 lactobacilli

When the number of patients with high *Lactobacillus* counts was compared before and after treatment, no significant difference could be noticed (control: RR=0.71; 95 % CI 0.43 to 1.17 and probiotic: RR=0.88; 95 % CI 0.59 to 1.31). Also, for the group with the lowest lactobacilli counts, no significant differences could be noted when comparing the number of patients in this group both before and after treatment (control: RR=0.98; 95 % CI 0.83 to 1.16 and probiotic: RR=1.13; 95 % CI 0.93 to 1.38).

Differences in low counts (<10^4 CFU/ml) of lactobacilli are noted when comparing the probiotic with the control group at baseline (RR=0.80; 95 % CI 0.67 to 0.97); this could not be detected at the end of the treatment (RR=0.93; 95 % CI 0.78 to 1.10) (Fig. 4). When we compare the probiotic and the control group for the patients with high *Lactobacillus* (>10^6 CFU/ml) counts, no statistically significant difference could be observed, neither at baseline (RR=1.24; 95 % CI 0.82 to 1.87) nor after treatment (RR=1.70; 95 % CI 1.05 to 2.75) (Fig. 5).

Risk of bias assessment

An evaluation of the risk criteria showed that two studies had a low potential risk of bias [66, 67]. The estimated risk of bias was moderate for ten papers [54–56, 58, 61, 62, 64, 68–70] and high for seven papers [21, 57, 59, 60, 63, 65, 71]. See Table 2.

Table 2 Estimated risk of bias

	Random allocation?	Blinding participants and personnel?	Blinding of outcome assessment?	Defined inclusion and exclusion criteria?	Identical treatment between the groups except for the intervention?	Incomplete outcome data	Selective reporting	Estimated potential risk of bias
Nase et al. 2001	+	+	+	−	+	+	+	Moderate
Ahola et al. 2002	+	?	?	+	+	+	−	High
Nikawa et al. 2004	?	+	?	−	+	?	−	High
Çaglar et al. 2005	?	+	+	+	+	+	+	Moderate
Çaglar et al. 2006	?	−	+	+	+	+	+	Moderate
Çaglar et al. 2007	?	+	+	+	−	+	+	Moderate
Çaglar et al. 2008b	?	+	+	+	+	+	+	Moderate
Cogulu et al. 2010	?	?	?	+	−	+	−	High
Aminabadi et al. 2011	+	?	?	+	−	−	+	High
Cildir et al. 2011	?	+	+	+	+	+	+	Moderate
Jindal et al. 2011	?	?	?	+	+	+	+	High
Petersson et al. 2011	+	+	+	+	+	+	−	Moderate
Singh et al. 2011	+	+	+	+	+	+	+	Low
Mortazavi and Akhlaghi 2012	+	+	+	+	+	+	+	Low
Keller and Twetman 2012	+	+	−	+	+	+	+	Moderate
Keller et al. 2012	?	?	?	+	+	+	+	High
Sudhir 2012	?	?	?	+	+	+	+	High
Juneja and Kakade 2012	+	?	?	+	+	+	+	Moderate
Burton et al. 2013	?	+	+	+	+	+	+	Moderate

+ low risk of bias, − high risk of bias, ? unclear

Discussion

The aim of this review was to evaluate the effect of probiotics in the prevention of caries. Seeing the multitude of factors that play a role in the aetiology of caries, long-term studies with the incidence of caries as primary outcome measure are needed. However, only two articles that met the inclusion criteria reported on the effect of probiotics just after its usage, reporting the incidence of caries as an outcome measure [55, 69]. On the other hand, it became clear from the initial searches that a wide variety of articles reporting on the effects of probiotics assessed the level of caries-associated bacteria, namely mutans streptococci and lactobacilli. This is probably because caries is a relative slow process and probiotics are often used for a relatively short period of time. Lack of funding for long-term clinical trials involving probiotics seems a reasonable explanation. Although caries incidence should be the preferred hard endpoint of such studies, the lack of studies using this endpoint forced us to rephrase our anticipated focused question "What is the impact of probiotics in healthy humans on the incidence of caries in the oral cavity when compared to a placebo" to "What is the impact of probiotics in healthy humans on the level of the surrogate outcome parameters mutans streptococci and lactobacilli counts in the oral cavity when compared to a placebo" in order to obtain a meaningful result. It should be noted that there exists controversy regarding the value of surrogate endpoints, such as mutans streptococci levels, as a predictor for caries. Some studies report a poor correlation between mutans streptococci levels and risk for caries development [72] while others find correlations [73–76].

Nineteen articles were included in the final, descriptive analysis. These studies often utilized small sample sizes, no follow-up and frequently did not describe how randomization and blinding were performed. Additionally, there was a considerable variation in the study parameters, such as used probiotic strain, mode of application, length of the studies and outcome measures. This caused serious restrictions on reviewing the literature in a quantitative way. Twelve articles could be included into the meta-analysis. Because of the way the available data were presented, it was only possible to perform a meta-analysis concerning the groups with the lowest and highest mutans streptococci and lactobacilli counts. For this, we used the results from research conducted with chairside tests and with conventional cultivation methods on selective agar plates. A significant correlation concerning the mutans streptococci and the lactobacilli counts has already been shown for these two methods [77–79]. However, to date, there are more sensitive and specific techniques available, such as qPCR.

Taking the above-mentioned limitations into account, this meta-analysis showed that when comparing the probiotic and control group, significantly more patients in the probiotic group had low mutans streptococci ($<10^5$ CFU/ml) counts and significantly less patients had high ($>10^6$ CFU/ml) counts. Regarding the *Lactobacillus* counts, comparing the probiotic and control group at the end of the probiotic use, no significant differences could be observed, neither in low counts ($<10^4$ CFU/ml) nor in high *Lactobacillus* ($>10^6$ CFU/ml) counts. The heterogeneity of the used probiotics did not allow a subanalysis concerning the used probiotic strains.

These data suggests that probiotics could have a positive effect in the prevention of caries. These results are in agreement with the three available articles that describe, just after the usage of a probiotic, caries incidence as primary outcome measure. Nase and coworkers (2001) evaluated the children's oral health according to the WHO criteria [69]. They combined these clinical results with the microbiological findings to develop a caries risk index. They claimed that milk containing *Lactobacillus rhamnosus* GG reduced the risk of caries significantly. This effect was particularly clear in the group with the 3–4-year-olds. Stecksen-Blicks et al. (2009) evaluated the effect of milk supplemented with *L. rhamnosus* and fluoride on enamel and dentine caries at the level of the canines and molars [45]. After 21 months, there was a statistically significant difference in caries activity between the two groups, with a preventive fraction of 75 %. Unfortunately, with this study design, one cannot determine if the positive effect is attributable to fluor, the probiotic or the combination of both. Petersson et al. (2011) investigated caries, in particular, root surface caries in older patients [55]. This paper showed that daily milk supplemented with fluoride and/or probiotic bacteria may reverse primary root caries lesions in older adults. The combination of a fluor and a probiotic showed better results than when only one of those two products was administered. However, more long-term studies with caries activity as primary outcome are needed. Besides, it is useful to investigate whether the effect of the probiotic continues after treatment, because it is believed that the effect of the probiotic will disappear when the patient discontinues its use and that the probiotic treatment does not induce a definitive shift towards a less pathogenic microbiota [15]. The currently available literature about the short-term follow-up after probiotic usage is contradicting, in regard to both the mutans streptococci and the *Lactobacillus* counts several weeks after probiotic therapy. However, it is remarkable that Aminabadi and coworkers (2011), when comparing the group that only received a probiotic with the group that received a probiotic and was pre-treated with a chlorhexidine mouthwash, showed in the latter group significantly lower mutans streptococci counts and increased *Lactobacillus* counts post-follow-up. This was not the case in the group that solely had the probiotic yoghurt. These results were not confirmed in the study by Keller and coworkers (2012) nor by Burton and coworkers (2013) [58, 59], yet it can be considered useful to remove the established biofilm before using the probiotic

since probiotics have difficulties exerting their beneficial effects on an already matured biofilm [80]. On the other hand, two recent long-term studies demonstrated that the use of a probiotic in infancy compared to a placebo or the use of xylitol/sorbitol showed no difference in the occurrence of dental caries few years after the cessation of their usage [47, 81]. Furthermore, their microbiological data support the view that probiotic bacteria are only temporary colonizers, even in young children.

Finally, future studies need to focus on the best way of administration, the used bacteria and the optimal concentration.

Conclusion

Within the limitations of the available data, it may be concluded that probiotics can have a positive effect on reducing the mutans streptococci counts as long as they are being used. This may indicate a possible positive effect of probiotics on the development of caries. There is a need for examining the positive effect of these products with caries development as primary outcome and for determining the most appropriate species, treatment time, the ideal concentration and vehicle.

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