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META-ANALYSIS

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Effects of probiotics on preventing caries in preschool children: a systematic review and meta-analysis

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Abstract

This paper systematically evaluate the effects of probiotics on preventing caries in preschool children. The present systematic review was conducted following the Transparent Reporting of Systematic Reviews and Meta-Analyses (PRISMA) guidelines and recorded in the International prospective register of systematic reviews (PROSPERO) database (registration no: CRD42022325286). Literature were screened from PubMed, Embase, Web of Sciences, China National Knowledge Infrastructure (CNKI), Wanfang and other databases from inception to April 2022 to identify randomized controlled trials on the clinical efficacies of probiotics in preventing dental caries in preschool children and extract relevant data. The meta-analysis was performed using the RevMan5.4 software and the Stata16. Cochrane handbook was used to assess the risk of bias. The Grading of Recommendations Assessment, Development and Evaluation (GRADEprofiler 3.6) was used to determine the evidence quality. A total of 17 randomized controlled trials were eligible, of which two trials had certain levels of bias and 15 had a low risk of bias. Evidence quality assessment showed that the included trials were of medium quality. The meta-analysis results showed that *Lactobacillus rhamnosus* was associated with a reduced incidence ($p = 0.005$) and progression ($p < 0.001$) of caries in preschool children. Probiotics could reduce the number of high-level Streptococcus mutans in saliva ($p < 0.00001$) but could not reduce the number of Streptococcus mutans in dental plaque nor the amount of Lactobacillus in the saliva and dental plaque. Current evidence shows that probiotics could prevent caries in preschool children, but *Lactobacillus rhamnosus* was more effective in preventing caries than others. Although probiotics could reduce high levels of Streptococcus mutans in saliva, they could not reduce the amount of Lactobacillus in saliva and dental plaque.

Keywords

Caries; Probiotics; Preschool child; Microorganism; Systematic review; Meta-analysis

1. Introduction

Caries are caused by cariogenic microorganisms in plaque biofilms that ferment dietary carbohydrates to produce acids, resulting in the loss of minerals from the hard tissue of teeth and the formation of cavities. Although researchers have been long looking for ways to prevent caries, the global burden of the disease has not yet been reduced [1]. Caries is a major public health problem worldwide, affecting about 2.43 billion people in varying degrees [2].The results of the fourth National Oral Epidemiological Survey in 2015 showed that the incidence rate of deciduous tooth caries in children aged 5 years was 71.9%, which was higher than that a decade ago [3]. Moreover, the prevalence of dental caries in children is increasing in many countries, making it a serious health problem. With the continuous understanding of the pathogenic theory of caries, it is believed that the occurrence and development of caries are related to the microecological imbalance of dental plaque biofilms [4]. Probiotics are active microorganisms introduced to stomatology after years of use primarily for gastrointestinal diseases and were shown to alter oral microecology and restore microbial populations associated with a healthy oral state [5]. Studies have shown that caries, periodontitis, gingivitis and oral lichen planus are closely related to oral microecological imbalance [6–8]. At present, probiotics can be used as a means to prevent caries based on the principle of affecting the balance of oral flora, inhibiting the growth of cariogenic bacteria and the formation of biofilm, which has become a new method to prevent caries [9].

Several trials have investigated the efficacies of probiotics in the prevention of caries. Cortés-Dorantes *et al.* [10] (2015) studied the effects of daily intake of a probiotic mixture on the number of Streptococcus mutans in the oral cavity of preschool children at high risk of dental caries and found that the number of Streptococcus mutans in the experimental group was significantly decreased ($p < 0.05$). However, Villavicencio *et*

TABLE 1. Search Strategy.

S.No.	Search Strategy
#1	"Dental Caries" (Mesh) OR (Dental Decay) OR (Decay, Dental) OR (Carious Lesions) OR (Carious Lesion) OR (Lesion, Carious) OR (Lesions, Carious) OR (Caries, Dental)
#2	"Probiotics" (Mesh) OR (probiotic) OR (probiotic bacteria) OR (beneficial bacteria) OR (bacteriotherapy) OR (lactobacillus) OR (bifidobacterium) OR (stretococcus)
#3	"Child, Preschool" (Mesh) OR (Preschool Child) OR (Children, Preschool) OR (Preschool Children)
#4	#1 AND #2 AND #3

al. [11] (2018) studied the levels of Streptococcus mutans and lactic acid bacteria after consuming probiotic milk and placebo milk for 9 months and found that the levels of the mutans were significantly lower than in the control group, but the difference was not statistically significant ($p = 0.767$). Pahumunto *et al*. [12] (2018) found that consuming probiotic milk containing *Lactobacillus paracasei* SD1 (10^7 CFU/g) for 3 months reduced caries development and Streptococcus mutans numbers in preschool children compared with placebo. However, not all probiotic interventions positively impacted the oral health of the study subjects. Hasslöf *et al*. [13] (2013) found that supplementation of cereals containing *Lactobacillus paracasei* F19 early in life did not affect dental caries, Streptococcus mutans or the number of Lactobacilli through 9 years of long-term follow-up, and suggested that it might be related to the selected strain.

Currently, there is no consensus on the view of probiotics in preventing caries, and the efficacies of probiotics in preventing caries remain uncertain. Thus, we conducted a systematic review and meta-analysis to evaluate the potential of probiotics in preventing caries in preschool children.

2. Methods and materials

2.1 Protocol and registration

Evidence-based elements of clinical problems were constructed following the PICOS principles. The study was registered on the PROSPERO platform (registration no.: CRD42022325286) and was performed in accordance with the PRISMA guidelines [14].

2.2 Eligibility criteria

Inclusion criteria guidelines according to the PICOS strategy:

(1) Patients/Population (P): Healthy preschool children (<6 years old) with or without caries.

(2) Intervention (I): Probiotic products.

(3) Comparison (C): Placebo (the same products without probiotics).

(4) Outcome (O): The main indicators were the incidence or progression of caries (mainly based on clinical examination), and the secondary indicators were related to microbial measurement results.

(5) Study design (S): Randomized controlled trials (RCTs).

Exclusion criteria: Review, case report, animal study, *in vitro* studies and observational study designs. Gray literature, such as conference papers, textbooks, monographs and thesis, were excluded. Articles without full text were also excluded.

2.3 Search Strategy (Table 1)

Eleven databases were screened for potential studies, including PubMed, Embase, The Cochrane Library, Ovid, Web of Sciences, Scopus, Sinomed, Sciencedirect, CNKI, Wanfang and Chinese Science and Technology Periodicals Database (VIP). The search content was randomized controlled trials assessing the efficacies of probiotics in preventing caries in preschool children. The search time was from the establishment of the database to April 2022. At the same time, the references of the included studies were searched to identify additional relevant literature. Medical subject heading (MeSH) terms and other free terms were used with Boolean operators (OR, AND) to combine searches. The database search was performed using similar keywords and followed the syntax rules of each database.

2.4 Literature screening and data extraction

Two reviewers (NM, QL) independently searched the literature through database searches by assessing the selected literature's title and abstract and reading the full-text articles based on the inclusion and exclusion criteria. Literature meeting the study inclusion criteria were selected. Any disagreements between the two reviewers were resolved through careful discussion or communication with a third reviewer (QD).

The contents of data extraction included: name of the first author, publication year; type of research; sample size of experimental group and control group; age of the subjects; follow-up time; dropout rates; type of selected probiotic strains; dose and frequency of probiotic administration, and; measurement outcome data.

2.5 Quality assessment

The risk of bias was assessed according to the Cochrane Handbook for Systematic Reviews of Interventions [15]. Two reviewers independently assessed the risk of bias in the included studies and cross-checked the results. Disagreements were resolved by mutual discussion.

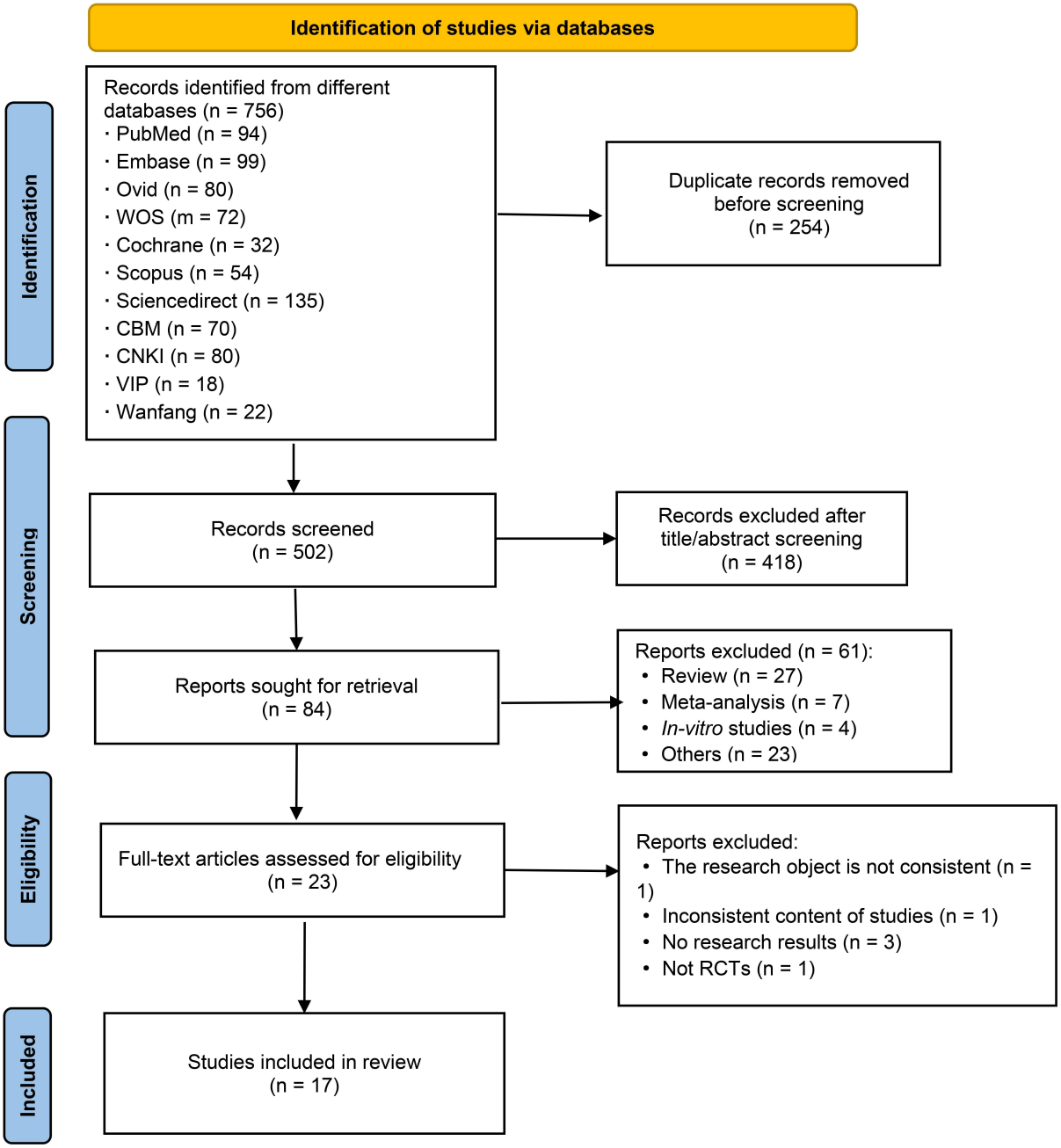

FIGURE 1. PRISMA flow diagram. RCTs: Randomized controlled trials; WOS: Web of Science; CBM: Chinese BioMedical Literature; CNKI: China National Knowledge Infrastructure; VIP: Chinese Science and Technology Periodicals Database.

2.6 Evaluation of evidence quality

The two reviewers used The Grading of Recommendations Assessment, Development and Evaluation (GRADEprofiler 3.6, Jan L. Brozek, Andrew Oxman and Holger J. Schünemann, EUP, Norway) to grade the evidence quality of each outcome index, and disagreements were discussed and resolved. The evidence quality was divided into four grades and comprehensively evaluated according to the risk of bias, inconsistency, indirection, accuracy, and importance of the included studies.

2.7 Statistical analysis

The RevMan5.4 software (The Cochrane Collaboration, EUP, England) was used for data analysis. Standard mean difference (SMD) and 95% confidence interval (CI) were used as effect analysis statistics for continuous variables. Relative risk (RR) and 95% confidence interval were used as effect analysis statistics for binary variables. X^2 test was used to analyze the heterogeneity among the included studies (the test level was $\alpha = 0.1$), and I^2 was used to quantitatively determine heterogeneity in the included studies. The results were analyzed using the random effect model.

Forest plots were used to illustrate the meta-analysis results. The funnel plots, Egger's test and Begg's test were used to analyze the publication bias of the primary outcome measures of the included studies [16]. $p < 0.05$ was considered statistically significant.

Several sensitivity analyses were performed to test for the robustness of the results. We also performed subgroup analyses of main indicators by differences in strains.

3. Results

3.1 Literature search results

Among the 756 potentially relevant publications identified in the databases, 254 duplicates were removed. After reading the titles and abstracts, 418 publications were excluded, resulting in 23 studies for full-text evaluation. A total of 17 studies met the eligibility criteria and were finally included in this systematic review (Fig. 1).

3.2 Basic characteristics of the included studies (Tables 2, 3, 4)

The 17 RCTs [11–13, 17–30] comprised 3781 preschool children divided into an experimental group (n = 2047) and a control group (n = 1734). The basic characteristics of the included studies are shown in Tables 2 and 3. The outcome indicators of the included studies are shown in Table 4. All studies were randomized controlled trials published after year 2000 and lasted from 2 weeks to 24 months.

3.3 Risk of bias and quality assessment of the included studies

3.3.1 Assessment of risk of bias

The risk of bias assessment is shown in Figs. 2,3. Two publications had some levels of bias (Hasslöf, *et al.* [13] 2013; Sandoval, *et al.* [26] 2021), while the remaining 15 had a low risk of bias (Hedayati-Hajikand, *et al.* [27] 2015; Näse, *et al.* [23] 2001; Stecksen-Blicks, *et al.* [28] 2009; Rodriguez, *et al.* [25] 2016; Taipale, *et al.* [29] 2012; Taipale, *et al.* [30] 2013; Pohjavuor, *et al.* [24] 2010; Alamoudi, *et al.* [18] 2018; Almabadi, *et al.* [19] 2020; Pahumunto, *et al.* [12] 2018; Piwat, *et al.* [22] 2020; Manmontri, *et al.* [20] 2020; Wattanarat, *et al.* [21] 2021; MH, *et al.* [17] 2016; Villavicencio, *et al.* [11] 2018).

3.3.2 Evaluation of evidence quality

The quality of each outcome indicator was evaluated using the GRADEprofiler 3.6 to form a summary table of evidence (Table 5). Among the outcome measures, the count of Streptococcus mutans in saliva was of high evidence level, while the others were of medium quality evidence.

3.4 Meta-analysis

3.4.1 Incidence of caries (Fig. 4)

Pooled effect estimates for 10 studies [11–13, 23–25, 28–30] showed that probiotics could significantly prevent the incidence of dental caries with a pooled RR of 0.70 (95% CI, 0.54–0.91) (p = 0.009). The heterogeneity between the studies was low (I^2 = 3%). Subgroup analysis based on the different strains showed that the probiotic mixture group had high heterogeneity (I^2 = 85%), while no heterogeneity was observed in the remaining three groups. The results of subgroup analysis showed that the incidence of caries in the *Lactobacillus rhamnosus* group was significantly lower than in the control group (p = 0.006).

3.4.2 Progression of caries on the tooth surface (Fig. 5)

Pooled effect estimates for 8 studies [11–13, 22, 25–28] showed that the development of dental caries was significantly lower in the probiotic group than in the placebo group, with a pooled (SMD = −0.24, 95% CI (−0.39, −0.10)) (p = 0.001). Subgroup analysis according to different strains showed that caries progression was significantly lower in the *Lactobacillus rhamnosus* group than in the control group ($p < 0.0001$), while no statistically significant differences were observed in the remaining three groups.

3.4.3 Streptococcus mutans count in saliva (continuous variable) (Fig. 6)

Pooled effect estimates for 2 studies 11, 20 showed that the number of S. mutans in saliva could not be decreased despite consuming probiotics, and the difference between the two groups was not statistically significant (p = 0.06, RR = −0.16, 95% CI (−0.33, 0.01)).

3.4.4 Streptococcus mutans count in saliva (dichotomous variable) (Fig. 7)

The normal value of S. mutans count is 10^5 CFU/mL, and a value $> 10^5$ CFU/mL indicates a higher risk of caries. Therefore, patients with S. mutans count $>10^5$ CFU/mL in the experimental and control groups were recorded. Pooled effect estimates for 4 studies [18, 19, 23, 24] showed that the number of S. mutans $>10^5$ CFU/mL decreased significantly after consuming probiotics ($p < 0.00001$, RR = 0.62, 95% CI (0.51, 0.74)), with low heterogeneity (I^2 = 20%). MH *et al.* [17] (2016) observed the changes in S. mutans in saliva after taking probiotics and placebo at levels $<10^3$ CFU/mL, 10^3–10^4 CFU/mL, and $>10^4$ CFU/mL and found that the count of S. mutans in the probiotics group was significantly decreased (p = 0.04), while there was no significant change in the placebo group.

3.4.5 Streptococcus mutans count in dental plaques (Fig. 8)

Pooled effect estimates for 3 studies [28–30] showed that the number of Streptococcus mutans in dental plaques could not be decreased despite consuming probiotics. Although the difference was not statistically significant (p = 0.38, RR = 0.77, 95% CI (0.43, 1.38)), the heterogeneity was considerable (I^2 = 80%).

3.4.6 Lactobacillus counts in saliva (Fig. 9)

A total of 4 studies were included. Pooled effect estimates for 2 studies [11, 20] showed no significant difference in the number of Lactobacillus in saliva after eating probiotics (p = 0.63, RR = −0.12, 95% CI (−0.61, 0.37)), but the statistical heterogeneity was considerable (I^2 = 88%). Alamoudi *et al.* [18] (2018) and Almadadi *et al.* [19] (2020) randomly assigned 178 healthy preschool children to receive a probiotic lozenge containing *Lactobacillus reuteri* or a placebo without probiotics, respectively. After 4 and 8 weeks, the results showed that the consumption of probiotic lozenges could significantly reduce the high lactobacillus count.

TABLE 2. Characteristics of included studies.

First author, year	Sample size (T/C)	Age	Intervention	Control	Duration	Dropout
MH [17], 2016	30/23	3–6 yrs	Probiotic drops	Placebo drops	2 wk	0%
Alamoudi [18], 2018	90/88	3–6 yrs	Probiotic lozenges	Placebo lozenges	4 wk	0%
Almabadi [19], 2020	90/88	3–6 yrs	Probiotic lozenges	Placebo lozenges	8 wk	20%
Pahumunto [12], 2018	62/62	1.5–5 yrs	Probiotic milk	Placebo milk	3 mon	17%
Manmontri [20], 2020	182/86	1–5 yrs	Probiotic milk	Placebo milk	6 mon	0%
Wattanarat [21], 2021	89/86	1–5 yrs	Probiotic milk	Placebo milk	6 mon	0%
Piwat [22], 2020	312/157	1–5 yrs	Probiotic milk	Placebo milk	6 mon	25%
Näse [23], 2001	231/220	1–6 yrs	Probiotic milk	Placebo milk	7 mon	24%
Pohjavuor [24], 2010	228/222	3–6 yrs	Probiotic juice	Placebo juice	7 mon	15%
Hasslöf [13], 2013	84/87	4 mon	Probiotic cereals	Placebo cereals	9 mon	31%
Villavicencio [11], 2018	136/227	3–4 yrs	Probiotic milk	Placebo milk	9 mon	12%
Rodríguez [25], 2016	150/111	2–3 yrs	Probiotic milk	Placebo milk	10 mon	22%
Sandoval [26], 2021	21/21	2–3 yrs	Probiotic milk	Placebo milk	10 mon	0%
Hedayati-Hajikand [27] , 2015	71/67	2–3 yrs	Probiotic chewing tablets	Placebo chewing tablets	12 mon	20%
Stecksen-Blicks [28], 2009	133/115	1–5 yrs	Milk of fluoride and probiotic	Placebo milk	21 mon	25%
Taipale [29], 2012	38/37	1–2 mon	Probiotic tablets	Placebo tablets	24 mon	11%
Taipale [30], 2013	38/37	1–2 mon	Probiotic tablets	Placebo tablets	24 mon	13%

TABLE 3. Probiotic strains and dose frequency of included studies.

First author, year	Probiotics	Dose/Frequency
MH [17], 2016	Mix of L. rhamnosus ATCC 15820 (1×10^{10} CFU[1]/mL), L. reuteri ATCC 55730 (2×10^{9} CFU/mL) and Bifidobacterium longum subsp. infantis ATCC 15697 (1.5×10^{9} CFU/mL).	Five drops/nightly
Alamoudi [18], 2018	Lactobacillus reuteri DSM 17938, Lactobacillus reuteri ATCC PTA 5289	2 lozenges/twice daily
Almabadi [19], 2020	Lactobacillus reuteri DSM 17938, Lactobacillus reuteri ATCC PTA 5289	2 lozenges/twice daily
Pahumunto [12], 2018	Lactobacillus Paracasei SD1	50 mL milk powder (10^{7} CFU/g)/daily
Manmontri [20], 2020	Lactobacillus Paracasei SD1	50 mL milk powder (1.8×10^{7} CFU)/daily
Wattanarat [21], 2021	Lactobacillus Paracasei SD1	50 mL milk powder (1.8×10^{7} CFU)/daily or triweekly
Piwat [22], 2020	Lactobacillus Paracasei SD1	50 mL milk powder (10^{7} CFU/g)/daily or triweekly
Näse [23], 2001	Lactobacillus rhamnosus GG	Fresh milk ($5–10 \times 10^{5}$ CFU/mL)/weekdays
Pohjavuor [24], 2010	Lactobacillus rhamnosus GG	200 mL juice[2] (5×10^{6} CFU/mL)/weekdays
Hasslöf [13], 2013	Lactobacillus paracasei F19	Cereals (1×10^{8} CFU) /daily
Villavicencio [11], 2018	Equal mix of Lactobacillus rhamnosus and Bifidobacterium longum	200 mL milk powder (8×10^{6} CFU/mL)/weekdays
Rodríguez [25], 2016	Lactobacillus rhamnosus SP1	150 mL milk powder (10^{7} CFU/mL)/weekdays
Sandoval [26], 2021	Lactobacillus rhamnosus SP1	150 mL milk powder (10^{7} CFU/mL)/weekdays
Hedayati-Hajikand [27], 2015	Streptococcus uberis KJ2, Streptococcus oralis KJ3, Streptococcus rattus JH145	1 tablet (10^{8} CFU)/daily
Stecksen-Blicks [28], 2009	Lactobacillus rhamnosus LB21	150 mL fresh milk[3] (10^{7} CFU/mL)/weekdays
Taipale [29], 2012	Bifidobacterium animals/lactis BB-12	2 tablets[4]/spoon (5×10^{9} CFU)/daily
Taipale [30], 2013	Bifidobacterium animals/lactis BB-12	2 tablets[4]/spoon (5×10^{9} CFU)/daily

[1]*CFU: colony-forming units;* [2]*Add calcium lactate gluconate;* [3]*Supplemented with 2.5 mg/kg fluor;* [4]*Containing xylitol.*

TABLE 4. Outcome indicators of included studies.

First author, year	Caries index	Incidence/Mean (SD)		Streptococcus mutans in saliva n/Mean (SD)		Streptococcus mutans in dental plaque (n)		Lactobacillus in saliva Mean (SD)		Lactobacillus in dental plaque (n)	
		T	C	T	C	T	C	T	C	T	C
Hedayati-Hajikand [27], 2015	Δds >0	0%/0.20 (1.20)	20%/0.80 (1.40)	-	-	-	-	-	-	-	-
Pahumunto [12], 2018	Δdt >0	8%/0.76 (1.29)	12%/1.25 (1.64)	-	-	-	-	-	-	-	-
Rodríguez [25], 2016	ΔICDAS 2–6mft >0	16%/1.13 (1.94)	21%/1.75 (2.37)	-	-	-	-	-	-	-	-
Sandoval [26], 2021	ΔICDAS 2–6mft >0	NR/1.29 (1.85)	NR/2.38 (3.11)	-	-	-	-	-	-	-	-
Piwat [22], 2020	Δds >0	79%/3.77 (4.19)	83%/4.44 (5.40)	-	-	-	-	-	-	-	-
Villavicencio [11], 2018	ΔICDAS 2–6mft >0	4%/−0.01 (30.69)	3%/−0.34 (28.98)	0.16 (0.80)	0.42 (2.86)	-	-	4.78 (4.15)	7.03 (7.00)	-	-
Stecksen-Blicks [28], 2009	Δdmfs >0	11%/0.30 (1.80)	25%/1.60 (3.10)	-	-	95	64	-	-	20	10
Hasslöf [13], 2013	Δdmfs >0	20%/0.60 (1.70)	26%/0.70 (2.40)	-	-	-	-	-	-	-	-
Taipale [29], 2012	ΔICDAS 2–6mft >0	0%/NR	0%/NR	-	-	1	10	-	-	8	10
Taipale [30], 2013	ΔICDAS 2–6mft >0	31%/NR	36%/NR	-	-	17	23	-	-	-	-
Näse [23], 2001	Δdt >0	6%/NR	8%/NR	32	36	-	-	-	-	-	-
Pohjavuor [24], 2010	Δdmft >0	5%/NR	8%/NR	4	3	-	-	-	-	-	-
Alamoudi [18], 2018	-	-	-	46	79	-	-	-	-	-	-
Almabadi [19], 2020	-	-	-	39	64	-	-	-	-	-	-
Manmontri [20], 2020	-	-	-	5.82 (3.00)	6.49 (2.74)	-	-	8.08 (2.66)	7.68 (3.40)	-	-

[1] *SD:Standard Deviation*
[2] *Countless values "-". NR: Not reported.*
[3] *Δds: increment of decayed surfaces; Δdt: increment of decayed teeth.*
Δdmft: increment of decayed, missed and filled teeth;
Δdmfs: increment of decayed, missed and filled surfaces.
ΔICDAS2-6mft: International Caries Detection and Assessment System (codes 2 to 6).

TABLE 5. Evidence quality evaluation chart.

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considera-tions	Probiotics	Placebo	Relative (95% CI)	Absolute		
Caries prevalence (follow-up median 9 mon)												
10	Randomized trials	No serious risk of bias	No serious inconsis-tency	No serious in-directness	Serious[1]	None	84/974 (8.6%)	117/977 (12.0%) 15.6%	RR 0.65 (0.5 to 0.84)	42 fewer per 1000 (from 19 fewer to 60 fewer) 55 fewer per 1000 (from 25 fewer to 78 fewer)	⊕⊕⊕O Moderate	Critical
Caries progression (follow-up median 9 mon; Better indicated by lower values)												
8	Randomized trials	Serious[2]	No serious inconsis-tency	No serious in-directness	No serious impreci-sion	None	777	672	-	SMD 0.22 lower (0.33 to 0.11 lower)	⊕⊕⊕O Moderate	Critical
Number of Streptococcus mutans in saliva (continuous variable) (follow-up 6–9 mon; Better indicated by lower values)												
2	Randomized trials	No serious risk of bias	No serious inconsis-tency	No serious in-directness	Serious[3]	None	301	288	-	SMD 0.16 lower (0.33 lower to 0.01 higher)	⊕⊕⊕O Moderate	Important
Streptococcus mutans counts in saliva (dichotomous variable) (follow-up 1–7 mon)												
4	Randomized trials	No serious risk of bias	No serious inconsis-tency	No serious in-directness	No serious impreci-sion	None	121/587 (20.6%)	182/572 (31.8%) 48.9%	RR 0.63 (0.54 to 0.75)	118 fewer per 1000 (from 80 fewer to 146 fewer) 181 fewer per 1000 (from 122 fewer to 225 fewer)	⊕⊕⊕⊕ High	Important
Streptococcus mutans counts in dental plaque (dichotomous variable) (follow-up 21–24 mon)												
3	Randomized trials	No serious risk of bias	No serious inconsis-tency	No serious in-directness	Serious[2]	None	115/174 (66.1%)	99/144 (68.8%) 72.7%	RR 0.77 (0.45 to 1.3)	158 fewer per 1000 (from 378 fewer to 206 more) 167 fewer per 1000 (from 400 fewer to 218 more)	⊕⊕⊕O Moderate	Important

TABLE 5. Continued.

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Probiotics	Placebo	Relative (95% CI)	Absolute		
Number of lactobacilli in saliva (continuous variable) (follow-up 6–9 mon; Better indicated by lower values)												
2	Randomized trials	No serious risk of bias	No serious inconsistency	No serious indirectness	Serious[3]	None	301	288	-	SMD 0.12 lower (0.61 lower to 0.37 higher)	⊕⊕⊕O Moderate	Important
Lactobacillus counts in dental plaque (dichotomous variable) (follow-up 21–24 mon)												
2	Randomized trials	No serious risk of bias	No serious inconsistency	No serious indirectness	Serious[2]	None	28/142 (19.7%)	20/111 (18.0%) 20.9%	RR 1.16 (0.68 to 1.95)	29 more per 1000 (from 58 fewer to 171 more) 33 more per 1000 (from 67 fewer to 199 more)	⊕⊕⊕O Moderate	Important

[1] *Taipale's study had a small sample size;* [2] *Sandoval has no specific random method;* [3] *Hasslöf's study had a small sample size. CI: confidence interval; SMD: Standard mean difference; RR: Relative risk.*

Author(s): NM, QL, QD, JQG, YB.

Date: 18 June 2022.

Question: Should probiotics vs placebo be used for caries?

Settings:The effects of probiotics on preventing caries in preschool children.

Bibliography: Probiotics for caries. Cochrane Database of Systematic Reviews (Year), Issue (Issue).

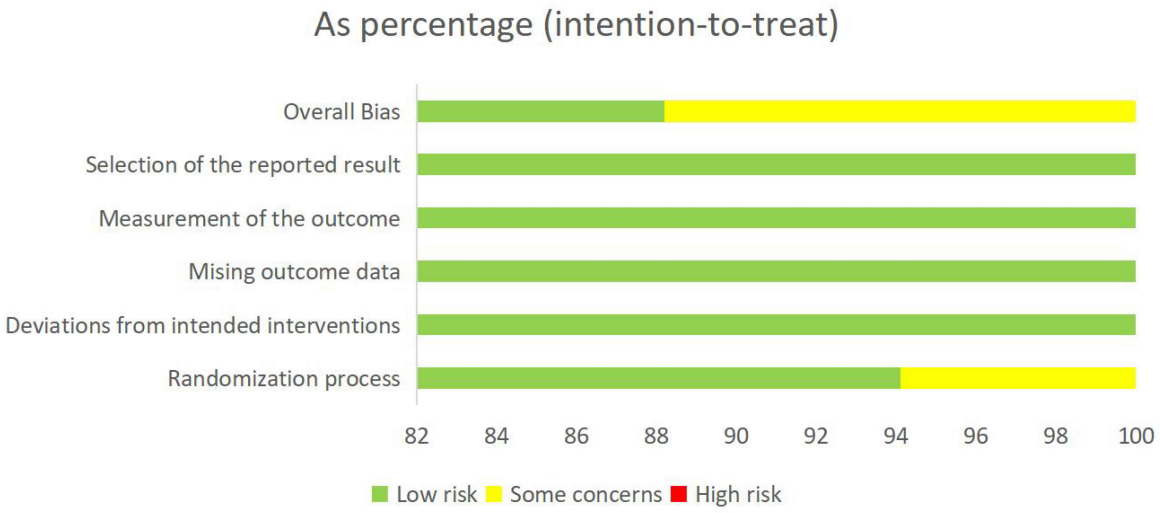

FIGURE 2. Risk of bias graph.

3.4.7 Lactobacillus counts in dental plaques (Fig. 10)

Pooled effect estimates for 2 studies [28, 29] showed that the Lactobacillus count in dental plaques could not be significantly decreased after consuming probiotics (p = 0.64, RR = 1.13, 95% CI (0.67, 1.92)), and there was no heterogeneity in the included data (I^2 = 0%).

3.5 Sensitivity analysis

By eliminating the studies that may affect the results, sensitivity analysis was conducted on the combined analysis results. Most of the combined analysis results did not change significantly, indicating that the results obtained were stable and reliable. However, the Lactobacillus count in saliva might be unstable and unreliable because only two studies were included, and the follow-up time and probiotic strains differed; thus, the results should be treated with caution.

3.6 Bias analysis

The results showed that there was no publication bias. The number of included studies for secondary outcome analysis was small; thus, unsuitable for bias detection.

(1) Incidence of caries.

The funnel plot (Fig. 11) showed that all studies were symmetric and within the 95% confidence intervals. The Egger's and Begg's test results were z = 1.11, p = 0.266 > 0.05; t = −1.46, p = 0.195 > 0.05, indicating no obvious publication bias in the included studies.

(2) Progression of caries on the tooth surface.

The Egger's and Begg's test results were z = 0.52, p = 0.602 > 0.05; t = −1.04, p = 0.332 > 0.05, indicating no obvious publication bias in the included studies.

4. Discussion

Oral biofilm is one of the most complicated microbes in nature, of which bacterial biofilm microecological imbalance is one of the causes of oral diseases. Multiple types of oral biofilm interact with saliva, diet, and host immunity, making it difficult for disease treatment. Meanwhile, this also indicates a new direction for anti-biofilm strategies targeting host microbial-diet interactions [31]. Caries prevention traditionally relied on thorough oral hygiene and antimicrobial measures with dietary fluoride exposure [32]. Traditional fluoride toxicity or antibacterial drugs may cause microecological damage, resulting in secondary opportunistic pathogen re-colonization and other negative clinical consequences. Thus, safer methods were needed to effectively prevent caries without significant adverse events. Probiotics are active microorganisms used to regulate the imbalance of microbial flora and are considered beneficial to health when ingested in sufficient quantities [5]. At present, the action mechanism of probiotics in oral health is mainly divided into three stages: attachment, adhesion and colonization [33]. Its effects on pathogenic bacteria in biofilms are multiple, complex, and vary according to the probiotics strains [34, 35]. It mainly plays its role by competing with pathogens for binding sites to produce antimicrobial substances or changing the protein composition of dental plaque to affect the oral microecological environment [36–38]. The anti-biofilm activity of probiotics has been detected in various *in vitro* caries models [39]. *In vitro* studies (2020) showed that probiotics *Streptococcus Sialis* K12 and M18 had antibacterial effects against Streptococcus mutans, among which M18 exhibited better antibacterial activity [40].

A previous meta-analysis by Hao *et al.* [41] (2021) on the efficacy and safety of *bifidobacterium* in preventing caries concluded that *bifidobacterium* could not reduce the incidence of caries and the counts of Streptococcus mutans and Lactobacillus in primary teeth. Twetman *et al.* [42] (2021) included 9 articles in their meta-analysis which investigated whether probiotics could effectively prevent caries in young children and concluded that probiotics had a small but statistically significant preventive effect on caries in the young children. However, different probiotic strains may have different effects on caries prevention. In this paper, the occurrence and development of caries were used as the primary outcome indicators, and microbial endpoint was used as the secondary outcome

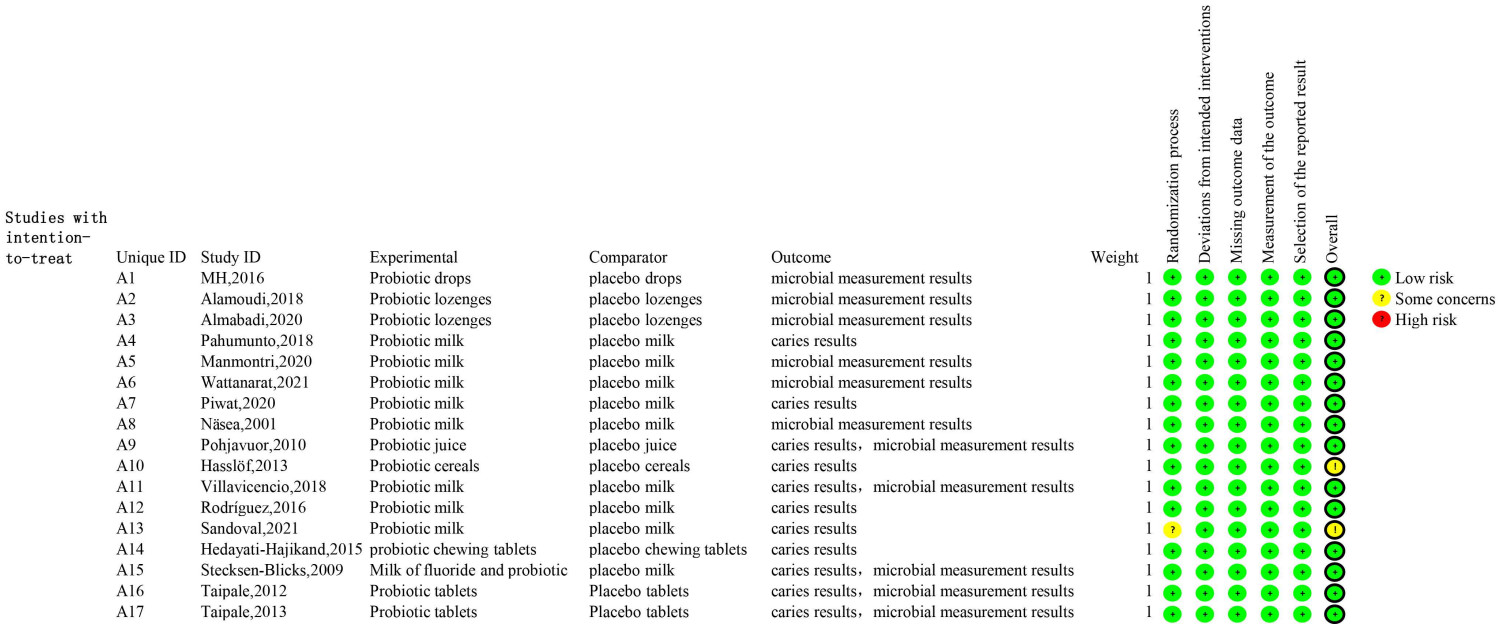

FIGURE 3. Risk of bias summary.

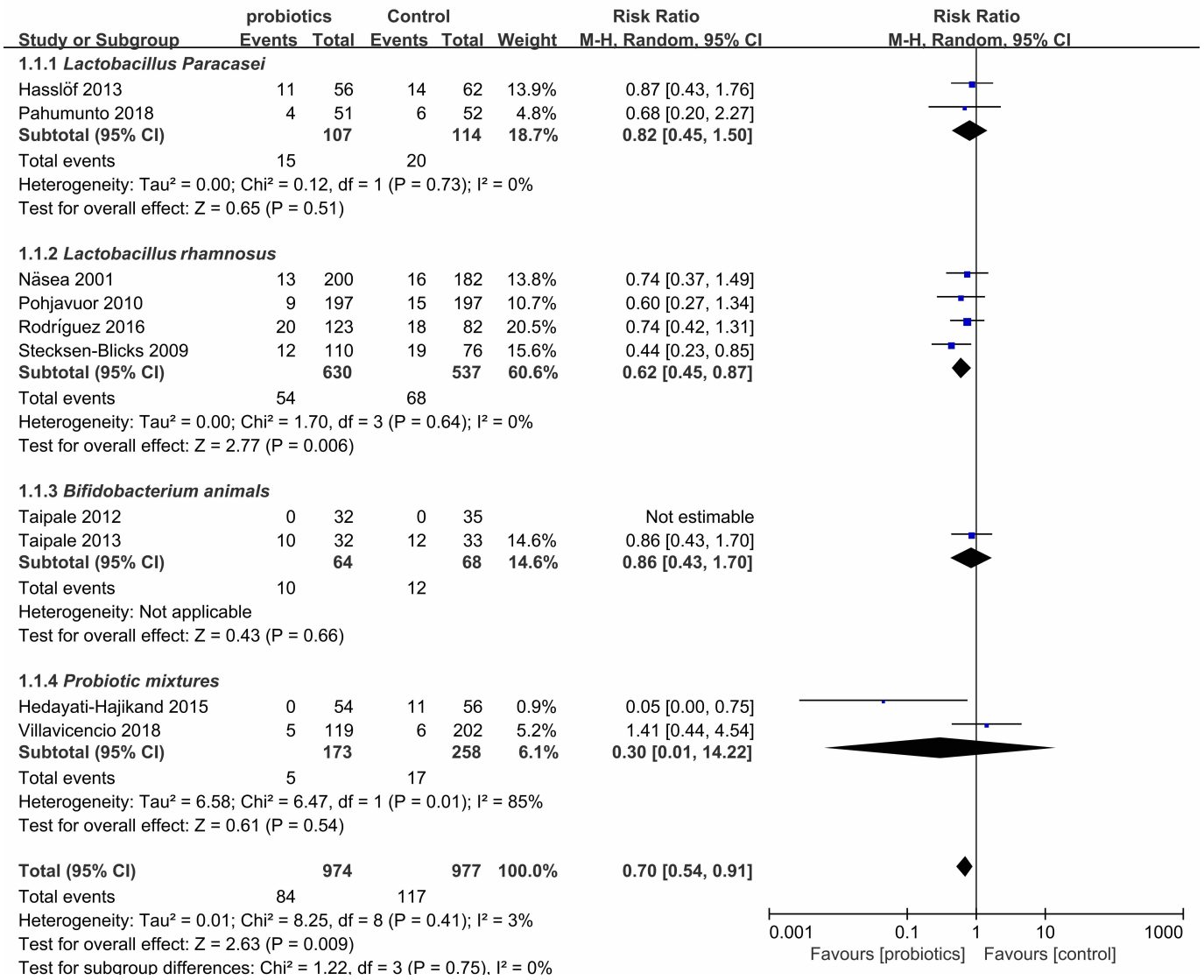

FIGURE 4. Forest plot of incidence of caries in the probiotic and control groups. CI: confidence interval.

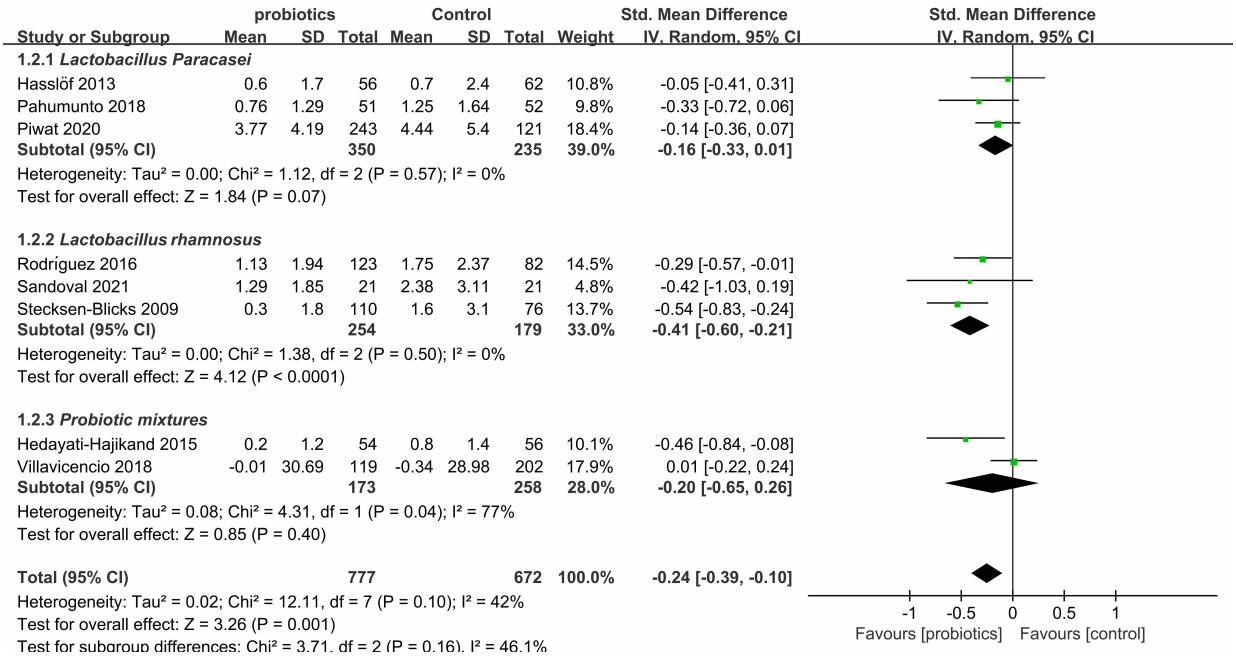

FIGURE 5. Forest plot of tooth decay progression of the probiotics and control groups. CI: confidence interval, SD: Standard Deviation.

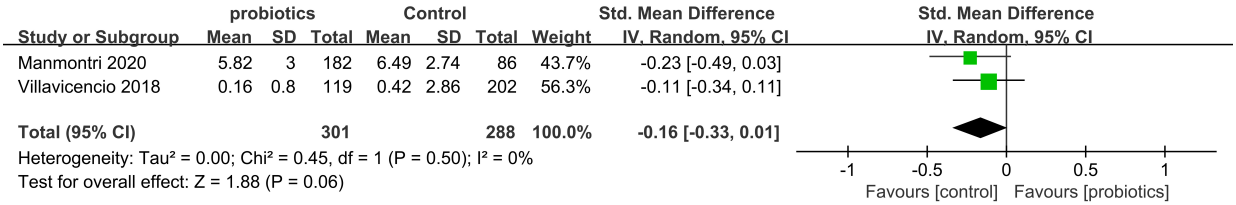

FIGURE 6. Forest plot of streptococcus mutans counts in the saliva of the probiotic and control groups (continuous variable). CI: confidence interval, SD: Standard Deviation.

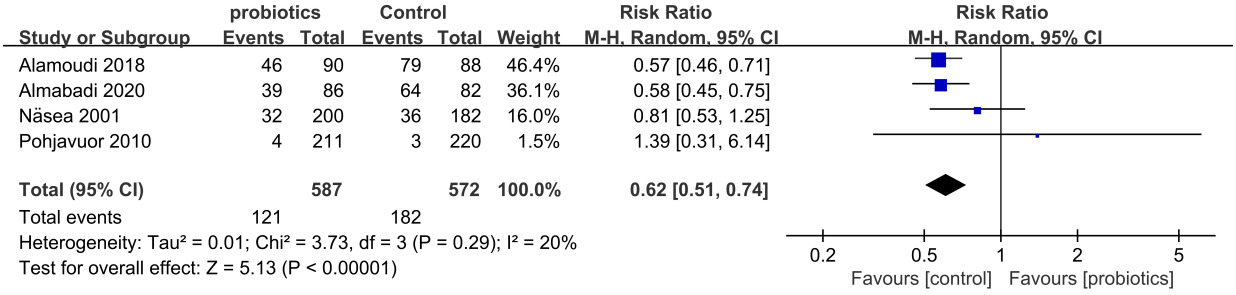

FIGURE 7. Forest plot of streptococcus mutans counts in the saliva of the probiotic and control groups (binary variable). CI: confidence interval.

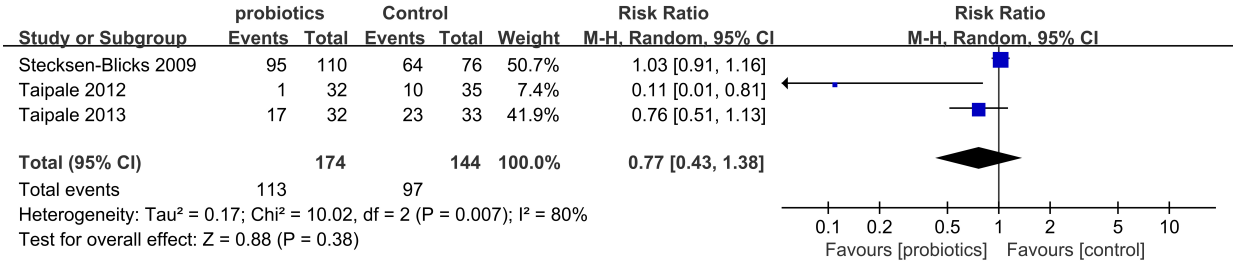

FIGURE 8. Forest plot of S.mutans counts in the dental plaques of the probiotic and control groups. CI: confidence interval.

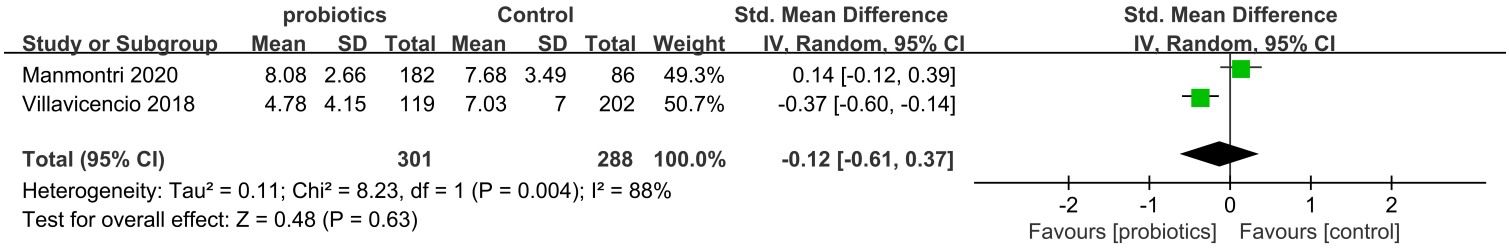

FIGURE 9. Forest plot of lactobacillus counts in the saliva of the probiotic and control groups. CI: confidence interval; SD: Standard Deviation.

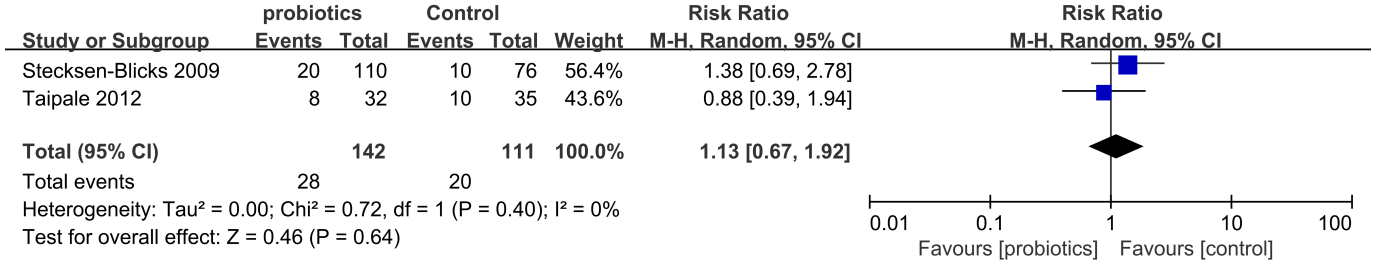

FIGURE 10. Forest plot of lactobacillus counts in the dental plaques of probiotic group and control group. CI: confidence interval.

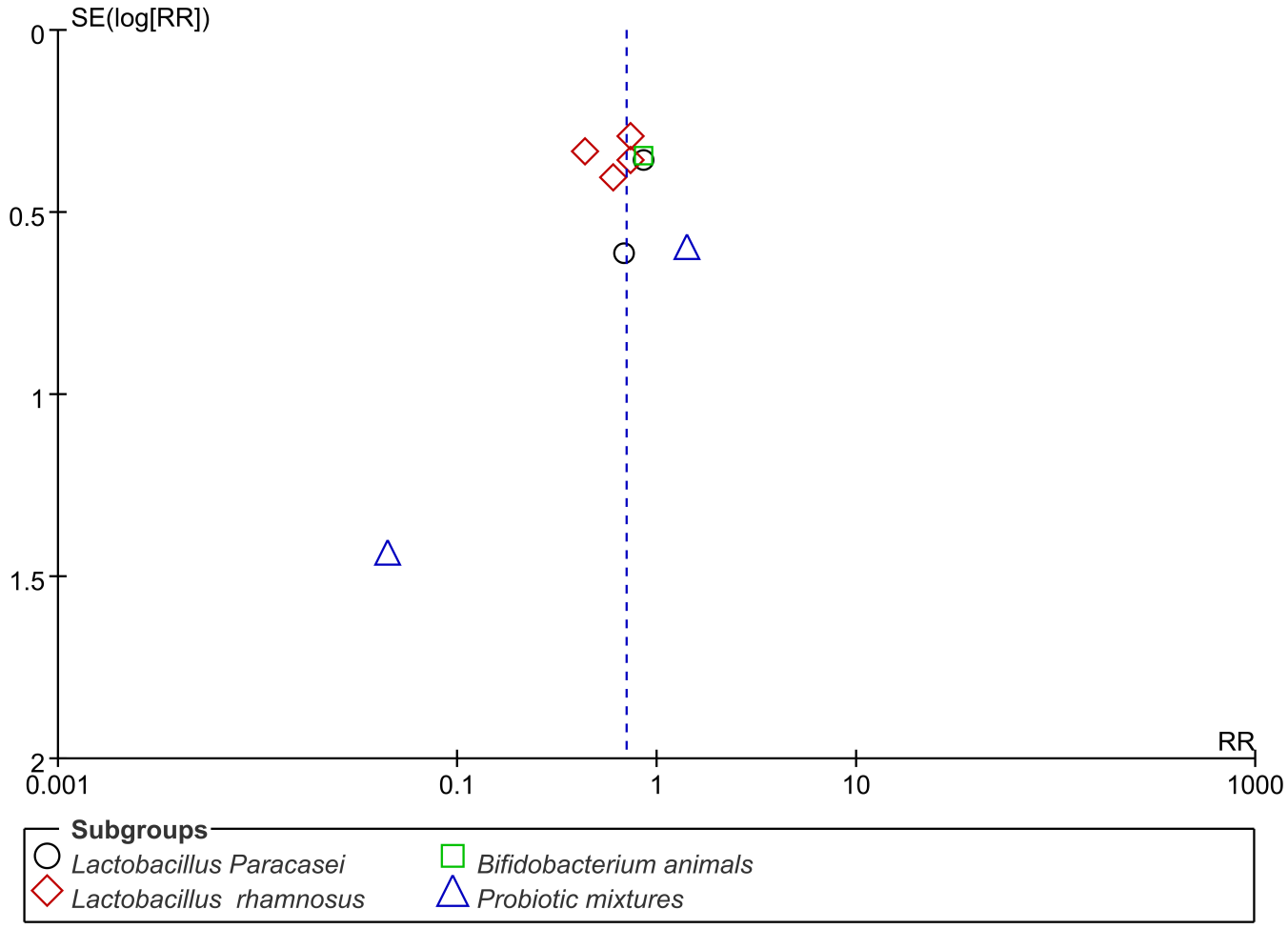

FIGURE 11. Funnel plot of caries incidence analysis for the probiotics and control groups. RR: Relative risk; SE: Standard Error.

indicators to investigate the effects of probiotics as a preventive measure against caries in preschool children. The results showed that probiotics could reduce the incidence and progression of caries in preschool children. According to the results of subgroup analysis, preschool children consuming probiotics containing *Lactobacillus rhamnosus* could significantly reduce the incidence and progression of caries. Even after excluding studies on preschool children at high risk of caries by sensitivity analysis [25], the results were still stable and reliable, which might be explained by the fact that probiotic effects are species- and/or strain-specific, and *Lactobacillus rhamnosus* might stay longer in the mouth [43, 44]. However, other factors such as different probiotic vectors and different carriers of probiotics may have also caused different colonization abilities of probiotics in the mouth [45]. At this point, research focusing on excellent carriers of probiotics seems to be further investigated. *In vitro* studies (2012) showed that probiotic mixtures were more effective in inhibiting pathogens than single strains [46]. Subgroup analysis of this study showed that the probiotic mixtures could not reduce the incidence of caries and the progression of tooth surface caries. However, considering only two studies were included, the results should be treated with caution, and multiple related studies on probiotic mixtures are needed to validate this finding. The high adhesion and colonization ability of probiotics in the oral cavity (such as attaching to tooth tissue and becoming a part of biofilm) is conducive to inhibiting cariogenic bacteria and enhancing their cariogenic effects [47]. Relevant studies (2018) have shown that *Lactobacillus Brevis* BBE-Y52 has a strong adhesion ability to the oral epithelium [48].Therefore, potential oral probiotic candidates should still be explored in the future.

Probiotics can restore dysregulated microbiota and reduce the proportion of caries-associated Streptococcus mutans in dental plaques and saliva [49, 50]. This meta-analysis showed that probiotics could reduce the high count of Streptococcus mutans, but not Lactobacillus, in saliva. Since most of the interventions included in the study were Lactobacillus, the increase in the Lactobacillus population might be related to Lactobacillus colonization. Although Lactobacillus is usually used as a probiotic strain to prevent caries [51], not all Lactobacillus are associated with the incidence of caries [52]. Only some Lactobacillus species, *Lactobacillus salivary*, are strong acid producers [53]. Probiotic strains effective for caries prevention should be selected for clinical application. Apart from *Streptococcus mutans*, various other microorganisms can also produce organic acids, inducing a decrease in the pH value of saliva and dental plaques and leading to caries [54]. By evaluating the saliva buffering capacity and pH value changes in the included studies, we found that probiotics could improve saliva buffering capacity and pH value, but there was no statistical difference in the observed results. Saliva and its components play a crucial role in the homeostasis and prevention of dental caries, and Saliva defensins were shown to possess antibacterial effects [55]. Human neutrophil peptides 1–3 (HNP1–3) are a subfamily of α-defensins. Wattanarat *et al.* [21] (2021) found that the consumption of *Lactobacillus paracasei* SD1 could significantly increase the level of saliva (HNP1–3), thereby reducing the progression of caries. Human β-defensin-3 (HβD-3), one of the β-defensins, was shown to possess powerful antibacterial effects and is widely distributed in the oral epithelial cells of the gingiva, tongue, salivary glands and oral mucosa [56, 57]. The increase in HβD-3 concentration may be related to the decrease of caries. However, Sandoval *et al.* [26] (2021) found that consuming *L. rhamnosus* SP1 reduced the concentration of HβD-3 in saliva, which may be related to the specificity of the strain. More studies are needed to investigate whether probiotics can prevent caries through immune effects.

Existing systematic reviews [58, 59] did not report on the adverse events of probiotics on oral health and the increased risk of caries. Of the 17 trials investigated included in this present study, only one assessed the associated adverse events [29] and reported that the use of probiotics was safe.

All 17 trials mentioned the application of random methods, and most mentioned specific random methods, allocation hiding and the use of double-blindness. Only one trial [26] did not mention specific random methods and allocation hiding, which increased the authenticity and reliability of the research results. Despite the promising results reported, this study still had limitations that should be elaborated. The carriers, frequency and duration of probiotics in the included studies were different, which might have affected the study results. In addition, the reported results could have been affected by the non-inclusion of gray literature. The follow-up time of some included studies was short and differed. Thus, high-quality studies with longer follow-up times are still needed to more accurately observe the effects of probiotics on caries prevention.

5. Conclusions

In conclusion, this current study showed that probiotics could effectively prevent dental caries, of which *Lactobacillus rhamnosus* was more effective than other bacteria in preventing dental caries. Probiotics reduced the high concentration of Streptococcus mutans in saliva but could not reduce the number of lactic acid bacteria in saliva and dental plaques. However, there was still a lack of relevant research on the dose, route of administration, and frequency of probiotic use, suggesting that further RCTs are needed to standardize the use of probiotics to achieve more beneficial effects before they are generally used in clinical practice.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

NM—completed the topic selection, literature search, inclusion, data extraction and analysis and wrote the full text. QL—conducted the literature search and assisted in statistical data analysis. QD—assisted in the statistical analysis of the data. YBY—assisted in chart arrangement. JQG—guided the writing of the article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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