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REVIEW ARTICLE

Effectiveness and safety of *Bifidobacterium* in preventing dental caries: a systematic review and meta-analysis

Siyuan Hao, Jiahe Wang and Yan Wang

State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases & Department of Pediatric Dentistry, West China Hospital of Stomatology, Sichuan University, Chengdu, P. R. China

ABSTRACT

Objectives: The effectiveness and safety of *Bifidobacterium* in dental caries prevention are controversial. Thus, we performed this systematic review and meta-analysis to explore the preventive value of *Bifidobacterium*.

Methods: Eligible studies were identified from several databases, including PubMed, the Cochrane Library, Embase, Web of Science, and Scopus. Hand searches were also conducted in relevant bibliographies. We then extracted and pooled standardized mean difference (SMD) and risk ratio (RR) to analyze the anti-caries effect of *Bifidobacterium* with Stata 16.0 software. If the data obtained was not suitable for meta-analysis, qualitative descriptions were performed.

Results: Compared with the placebo control group, there was no statistically significant reduction in *Streptococcus mutans* and *Lactobacilli* counts in saliva in the test group. Also, there were no significant differences in *Streptococcus mutans* and *Lactobacillus* counts in dental plaque and no significant difference in caries incidence in deciduous teeth. There was no significant difference in the incidence of adverse events between the *Bifidobacterium* and control groups.

Conclusions: Available evidence demonstrates that *Bifidobacterium* is neither effective in reducing *Streptococcus mutans* and *Lactobacillus* counts in the saliva or dental plaque nor in reducing the occurrence of caries in deciduous teeth. Evaluation of its safety requires further investigations. Therefore, *Bifidobacterium* is not a competent probiotic candidate to prevent dental caries.

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Introduction

Dental caries, one of the most common chronic diseases, can lead to chronic and progressive destruction of tooth structure [1]. The disease burden caused by dental caries has become a public health problem, seriously affecting oral and general health [2]. Concerning the pathogenesis of dental caries, the four-factor theory is widely accepted: bacteria, oral environment, host, and time [3]. Among them, the role of bacteria has always attracted researchers' attention [4–6].

The World Health Organization defined probiotics in 2001 as 'live microbial preparations that are beneficial to the health of the host when taken at an appropriate dose' [7]. Studies have shown that probiotics can adjust the balance of the flora, thereby maintaining gastrointestinal, genitourinary, and oral health [8]. Several clinical trials and systematic reviews have been conducted on the anti-caries effect of probiotics [9–11]. Caglar et al. observed that short-term consumption of yogurt containing *Bifidobacterium DN-173010* by young people aged 21–24 reduced *Streptococcus mutans* counts in the oral cavity compared with the baseline [12]. De-Pierro et al. included 76 children with high caries risk in a randomized controlled trial and discovered that the use of *Streptococcus salivarius* M18 could reduce the risk of caries in children [13]. Similarly, Hedayati-Hajikand et al. enrolled 138 children aged 2 to 3 years and reported that new caries in the treatment group receiving tablets containing multiple probiotics decreased significantly compared to the placebo group [14]. Concerning the mechanism, some studies suggested that the effects of probiotics on caries prevention were mainly reflected in the regulation of bacterial plaque microbial community, competing for ecological sites with the main cariogenic bacteria such as *Streptococcus mutans* and *Lactobacillus*, regulating the oral pH and acid production capacity of the plaque [15–17].

As a widely used probiotic, *Bifidobacterium* is one of the most important bacteria in the human digestive system [18]. It plays an active role in regulating the digestive tract, improving and enhancing the host's immunity, anti-oxidation, anti-allergy, anti-tumor ability, and so on [19–21], mainly by secreting exopolysaccharides. Many *in vitro* studies have been undertaken to determine whether *Bifidobacterium* can effectively prevent caries or not. However, the clinical trials are still single-center with small sample size, and the conclusions are controversial. Pinto and Javid reported that it could not prevent caries [22,23], but Nozari and Srivastava argued that it was clinically effective [24,25].

CONTACT Yan Wang wangyan1458@scu.edu.cn State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases & Department of Pediatric Dentistry, West China Hospital of Stomatology, Sichuan University, No. 14, 3rd Section, S. Renmin Road, Chengdu 610041, P. R. China

Therefore, this systematic review and meta-analysis were conducted to explore and verify the effectiveness and safety of *Bifidobacterium* in preventing caries, explore its potential value in clinical application, and guide further clinical research.

Materials and methods

The study protocol was registered on the PROSPERO database (http://www.crd.york.ac.uk/PROSPERO, ID: CRD42020180237). The preferred reporting items for systematic reviews and meta-analyses (PRISMA) were as follows [26].

Search strategies

We searched several electronic databases to identify relevant studies, using the following keywords: Dental Caries, Caries, Dental Decay, and *Bifidobacterium*. The reference lists of the studies were also checked to identify potentially relevant papers. The string of operations and search strategies of databases, including PubMed, the Cochrane Library, Embase, Web of Science, and Scopus, are presented in Table 1. The search time limit was from the establishment of the databases to March 1st, 2020.

Selection criteria

Two reviewers (Siyuan Hao and Jiahe Wang) independently reviewed the titles and abstracts of all the references identified by searching the electronic databases. Then, the full texts of potentially relevant articles were retrieved and further evaluated. Disagreements were resolved by discussion or consulting a third researcher (Yan Wang).

Only studies meeting the following criteria were included: (1) Randomised controlled trial (RCT); (2) The subjects consisted of healthy individuals without caries or with incipient caries; (3) The test group consumed products containing *Bifidobacterium* such as milk, curd, ice cream or tablet; (4) The control group received the same products without *Bifidobacterium*; (5) Duration of the intervention was less more a week. Studies were excluded on account of any of the following reasons: (1) The subjects had a history of smoking; (2) Lactose intolerance; (3) Receiving antibiotics during the intervention; (4) Taking other probiotic products during the intervention; (5) Using mouthwashes during the intervention; (5) Consuming products containing xylitol; (6) Duplicates, and (7) Data on outcome indicators were incomplete and not available after contacting the authors.

Data extraction

Two reviewers (Siyuan Hao and Jiahe Wang) reviewed the included studies and extracted the following data from each included study: authors' names, publication date, study design, sample size, duration of the intervention, baseline characteristics of the study population, and the characteristics of the probiotic. Also, the outcome parameters included: (1) Occurrence of deciduous tooth caries; (2) Streptococcus mutans count in saliva; (3) Streptococcus mutans counts in dental plaque; (4) Lactobacillus counts in saliva; (5) Lactobacillus counts in dental plaque; (6) Adverse events. Since the measurement methods and measurement units of each study were different, the standardized mean difference (SMD) was used instead of the mean difference (MD) for continuous variables. Risk ratio (RR) was used to evaluate the dichotomous variables. SMD and RR with their 95% confidence intervals (CI) were extracted or calculated from the provided data. The authors were contacted if the information could not be obtained from the articles.

Assessment of the risk of bias

Risk of bias, including random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias (Other bias), were evaluated according to the Cochrane guidelines. Two investigators (Siyuan Hao and Jiahe Wang) independently carried out the assessment. Disagreements were settled through discussion or convention. Any unresolved divergence was discussed with a third investigator (Yan Wang).

Statistical analysis

The preventive effect of *Bifidobacterium* on dental caries was evaluated by the pooled standardized mean difference (SMD) and risk ratio (RR) and their 95% confidence intervals (CI). In the meta-analysis, Cochrane's Q statistics and Higgins *I*-squared statistic (I^2) were used to detecting the heterogeneity [27]. A fixed-effect model was selected if the heterogeneity was not statistically significant ($I^2 < 50\%$). On the contrary, in the case of $I^2 \geq 50\%$, a random-effect model was used. All these analyses were performed with Stata 16.0. Qualitative descriptions were performed if the data were not suitable for meta-analysis.

Results

Search results

During the initial search, 64 records were retrieved from PubMed, 5 from the Cochrane Library, 111 from Embase, 151 from Web of Science, and 143 from Scopus. Twelve studies were found through the hand search of relevant bibliographies. In total, 254 records remained after removing the duplicates. Then, 205 records were excluded after the first round of screening the titles and abstracts. The full texts of 49 articles were retrieved for further evaluation, of which 12 studies were excluded because they were non-RCTs, 17 were *in vitro* studies, and 10 were on *Bifidobacterium* mixed with other kinds of probiotics. Ultimately, 10 studies [12,22,23,25,28–33] were included in this systematic review (Figure 1).

Table 1. The string of operations and search strategies of databases, including PubMed, the Cochrane Library, Embase, Web of Science, and Scopus.

Electronic database and search strategy	
PubMed	#1 (Dental Caries[MeSH Terms]) OR (((((((((((((((Dental Decay[Title/Abstract]) OR Caries, Dental[Title/Abstract]) OR Decay, Dental[Title/Abstract]) OR Carious Dentine[Title/Abstract]) OR Carious Dentins[Title/Abstract]) OR Dentine, Carious[Title/Abstract]) OR Dentins, Carious[Title/Abstract]) OR Dental White Spot[Title/Abstract]) OR White Spots, Dental[Title/Abstract]) OR White Spots[Title/Abstract]) OR Spot, White[Title/Abstract]) OR Spots, White[Title/Abstract]) OR White Spot[Title/Abstract]) OR Dental White Spots[Title/Abstract]) OR White Spot, Dental[Title/Abstract]))))
	#2 (Bifidobacterium [MeSH Terms]) OR (Bifidobacterium [Title/Abstract])
	#3 #1 AND #2
Cochrane Library	#1 MeSH descriptor: [Dental Caries] explode all trees
	#2 MeSH descriptor: [Bifidobacterium] explode all trees
	#3 #1 AND #2
Embase	#1 'dental caries'/exp
	#2 'dental decay':ab,ti
	#3 'caries, dental':ab,ti
	#4 'carious dentine':ab,ti
	#5 'dental white spot':ab,ti
	#6 'dental white spots':ab,ti
	#7 'Bifidobacterium'/exp
	#8 'Bifidobacterium':ab,ti
	#9 #1 OR #2 OR #3 OR #4 OR #5 OR #6
	#10 #8 OR #9
	#11 #9 AND #10
Web of Science	(TS = Caries) AND (TS = Bifidobacterium)
	Refined by: DOCUMENT TYPES: (ARTICLE OR CLINICAL TRIAL)
	Timespan: 1975 to March 2020. Databases: WOS, DIIDW, INSPEC, KJD, MEDLINE, RSCI, SCIELO.
	Search language = Auto
Scopus	#1 TITLE-ABS-KEY ('caries') OR TITLE-ABS-KEY ('dental caries') OR TITLE-ABS-KEY ('carious dentine') OR TITLE-ABS-KEY ('dental white spot') OR TITLE-ABS-KEY ('dental white spots')
	#2 TITLE-ABS-KEY ('Bifidobacterium')
	#3 #1 AND #2

The search time limit was from the establishment of the database to March 1st, 2020.

Study characteristics

The characteristics of all the included studies are presented in Table 2. The sample size ranged from 30 to 104 participants. All the studies reported the mean age or age range of the participants, ranging from 0 to 25 years. The intervention duration varied from 10 days to 14.9 months, among which two weeks was the most commonly used intervention time. Seven out of ten studies enrolled healthy participants at baseline, while two studies included orthodontic patients, and one study recruited subjects having initial dental caries. The vehicles used for probiotics comprised yogurt, ice cream, curd, and slow-release pacifier or tablets. Dosage of *Bifidobacterium* taken per day varied from 6×10^7 to 1.1×10^{12} CFU. Outcome measures included: (1) Occurrence of deciduous tooth caries; (2) *Streptococcus mutans* count in saliva; (3) *Streptococcus mutans* counts in dental plaque; (4) *Lactobacillus* counts in saliva; (5) *Lactobacillus* counts in dental plaque; (6) Adverse events.

Risk of bias

Figure 2 presents the assessment of the risk of bias of the selected studies. Out of the ten studies, eight were assessed to have a high risk of bias [12,22,23,29–33], and the remaining two were associated with a moderate risk of bias [25,28]. Out of the ten included studies, five studies explicitly mentioned the double-blind method [12,28–31], four studies claimed that they were double-blinded trials, with no detailed description [23,31–33]. Pinto et al. did not blind the subjects [22]. All the ten included studies were randomized, five of which used the random number table method or used a computer to generate random numbers [22,25,28,32,33], while the remaining five studies did not provide a detailed description of the randomization methods applied [12,23,29–31].

Study outcomes

Streptococcus mutans counts in saliva

Seven studies compared *Streptococcus mutans* counts in saliva after *Bifidobacterium* intervention [12,22,23,25,28,30,31]; four studies reported that continuous variables [22,23,25,31] and three reported that dichotomous variables were included in the meta-analysis [12,28,30].

Continuous variables. A meta-analysis was conducted to compare the difference in *Streptococcus mutans* counts in saliva between the *Bifidobacterium* group and the control group after the intervention, with four included studies containing 208 subjects [22,23,25,31]. The SMD was adopted because of the different measurement methods and measurement units. The pooled result by the random-effect model showed no statistically significant difference (SMD= −0.32, 95% CI [−0.67, 0.04], $p = .08$) (Figure 3(A)).

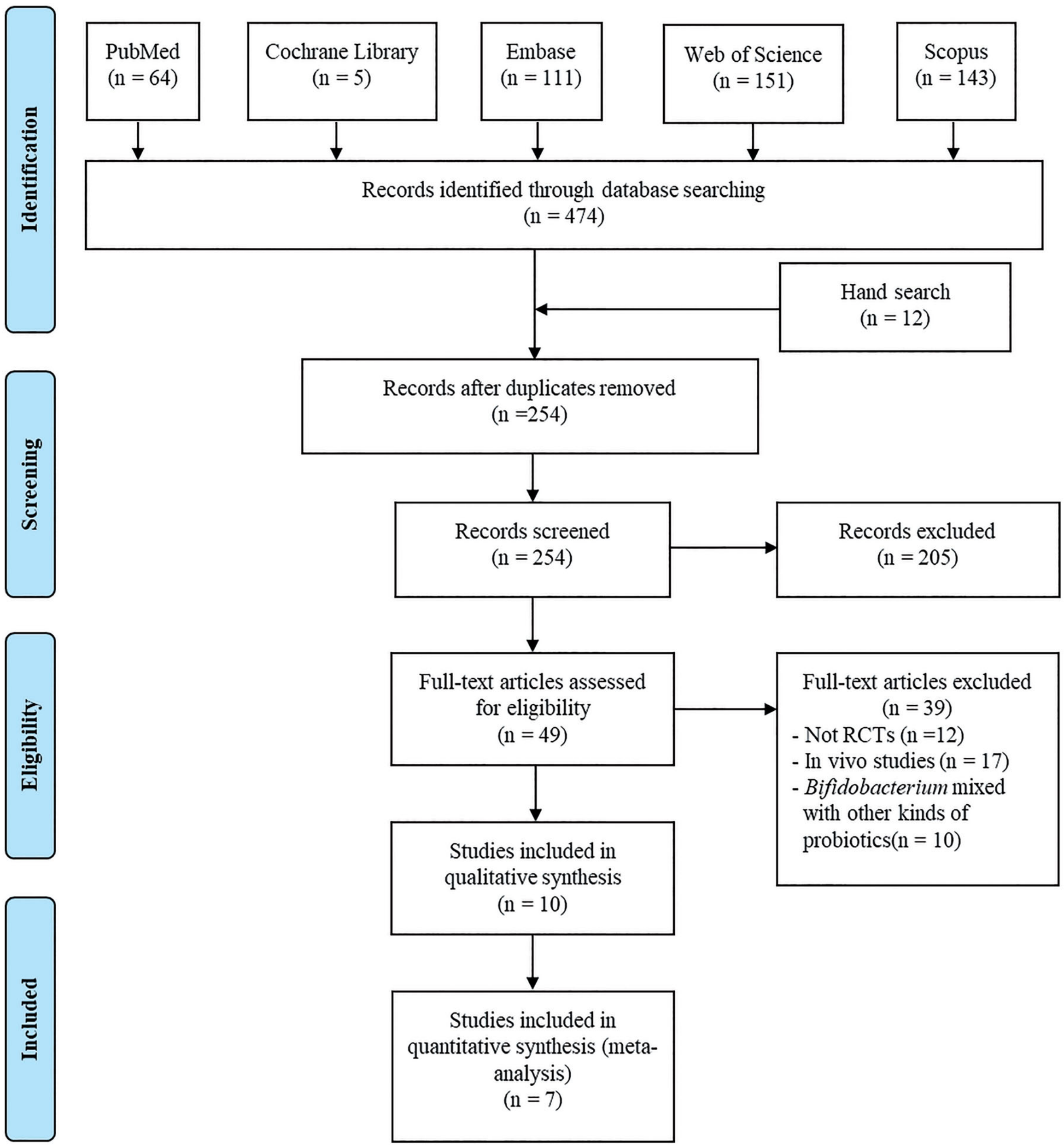

Figure 1. The flowchart of the study selection process.

Dichotomous variables. Then we pooled the RR extracted from three included studies containing 137 subjects to compare *Streptococcus mutans* counts after intervention between the probiotic and control groups [12,28,30]. Since the widely recognized normal value of *Streptococcus mutans* counts in saliva is generally 10^5 CFU/mL, we compared the number of subjects whose *Streptococcus mutans* counts in saliva was more than 10^5 CFU/mL in both the test and control groups. A random-effect model meta-analysis revealed no significant difference between the two groups after *Bifidobacterium* intervention (RR = 0.53, 95% CI [0.17, 1.66], $p = .28$) (Figure 3(B)). Since the heterogeneity was significant, we conducted a sensitivity analysis which indicated that the data from Caglar 2005 (RR = 1.10, 95% CI: 0.60–2.02, $p = .76$) was the source of heterogeneity. After excluding it from the meta-analysis, the result was still not statistically significant (RR = 0.30, 95% CI: 0.07–1.20, $p = .09$), demonstrating that *Bifidobacterium* could not effectively inhibit *Streptococcus mutans in* saliva.

Streptococcus mutans counts in dental plaque

We made a qualitative description for the infeasibility of implementing a meta-analysis. Totally, two studies compared *Streptococcus mutans* counts in dental plaque after the intervention [22,29]. Pinto et al. conducted a cross-design trial on 26 subjects wearing fixed orthodontic appliances with an average age of 15 years [22]. The intervention lasted for two weeks with a four-week interval of elution. Dental plaque samples were then taken around the orthodontic appliances of all the subjects' premolars and canine teeth. The results showed no significant decrease in *Streptococcus mutans* counts in the dental plaque in the probiotic group compared with that before the intervention. Besides, the difference was

Table 2. Characteristics of the included studies.

Studies	Distribution of groups (T/C)	Average age	Intervention time	Baseline characteristics	Vehicle	Intervention (g/d) T	Intervention (g/d) C	Intake (CFU/day)	Outcomes
Caglar 2005 [12]	21/21	22	2 weeks	Heathy	Yogurt	200	200	1.4×10^{10}	(2)(4)
Caglar 2008 [28]	23/24	20	10 days	Heathy	Ice-cream	53	53	5.3×10^{8}	(2)(4)(6)
Caglar 2014 [29]	52/52	8–10	2 weeks	Heathy	Yogurt	110	110	1.1×10^{12}	(3)(4)
Cildir 2009 [30]	24/24	14	2 weeks	Orthodontics	Yogurt	200	200	4×10^{10}	(2)
Javid 2020 [23]	33/33	22	2 weeks	Initial caries	Yogurt	300	300	2.7×10^{8}	(2)(4)(6)
Nagarajappa 2015 [31]	15/15	18–22	18 days	Heathy	Ice-cream	42	42	6×10^{7}	(2)(4)(6)
Pinto 2014 [22]	26/26	15	2 weeks	Orthodontics	Yogurt	200	200	\	(2)(3)(4)(5)(6)
Srivastava 2016 [25]	30/30	20–25	1 week	Heathy	Curd	100^{a}	100^{a}	\	(2)(6)
Taipale 2012 [32]	38/31	0–2	14.9 months	Heathy	Slow release pacifier or tablet	100 or 300^{b}	100 or 300^{b}	5×10^{9}	(1)(6)
Taipale 2013 [33]	32/29	4	14.9 months	Heathy	Slow release pacifier or tablet	100 or 300^{b}	100 or 300^{b}	5×10^{9}	(1)(6)

CFU: colony-forming units.
\: Not reported; [a]mL/d; [b]mg/d.
Outcomes: (1) Occurrence of deciduous tooth caries; (2) *Streptococcus mutans* count in saliva; (3) *Streptococcus mutans* counts in dental plaque; (4) *Lactobacillus* counts in saliva; (5) *Lactobacillus* counts in dental plaque; (6) Adverse events.

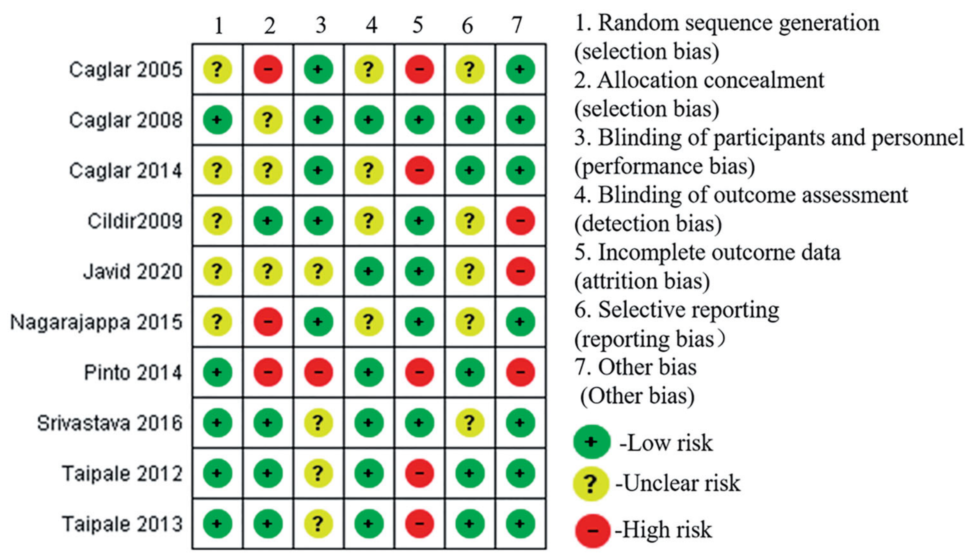

Figure 2. The assessment of the risk of bias of the included studies according to the Cochrane Handbook.

not statistically significant compared with that of the control group. Similarly, Caglar et al. did not report a statistically significant difference in *Streptococcus mutans* counts in the dental plaque after administering placebo or *Bifidobacterium*-containing yogurt to 52 subjects aged 8–10 in a cross-designed trial [29].

Lactobacillus counts in saliva

Six studies compared *Lactobacillus* counts in saliva after the intervention [12,22,23,28,30,31]; three studies reported that continuous variables [22,23,31], and three reported that dichotomous variables were included in the meta-analysis [12,28,30].

Continuous variables. The impact of *Bifidobacterium* on the counts of *Lactobacillus* in saliva with continuous variables was evaluated between three studies with 148 subjects [22,23,31]. The SMD was adopted because of the different measurement methods and measurement units. The results of the meta-analysis of fixed-effect models indicated no significant differences in *Lactobacillus* counts between the two groups after the intervention (SMD = −0.07, 95% CI [−0.39, 0.26], $p = .69$) (Figure 4(A)).

Dichotomous variables. We pooled the RR extracted from three RCTs containing 137 subjects [12,28,30]. The high *Lactobacillus* and low *Lactobacillus* group was divided based on the *Lactobacillus* counts of 10^5 CFU/mL. The results of the

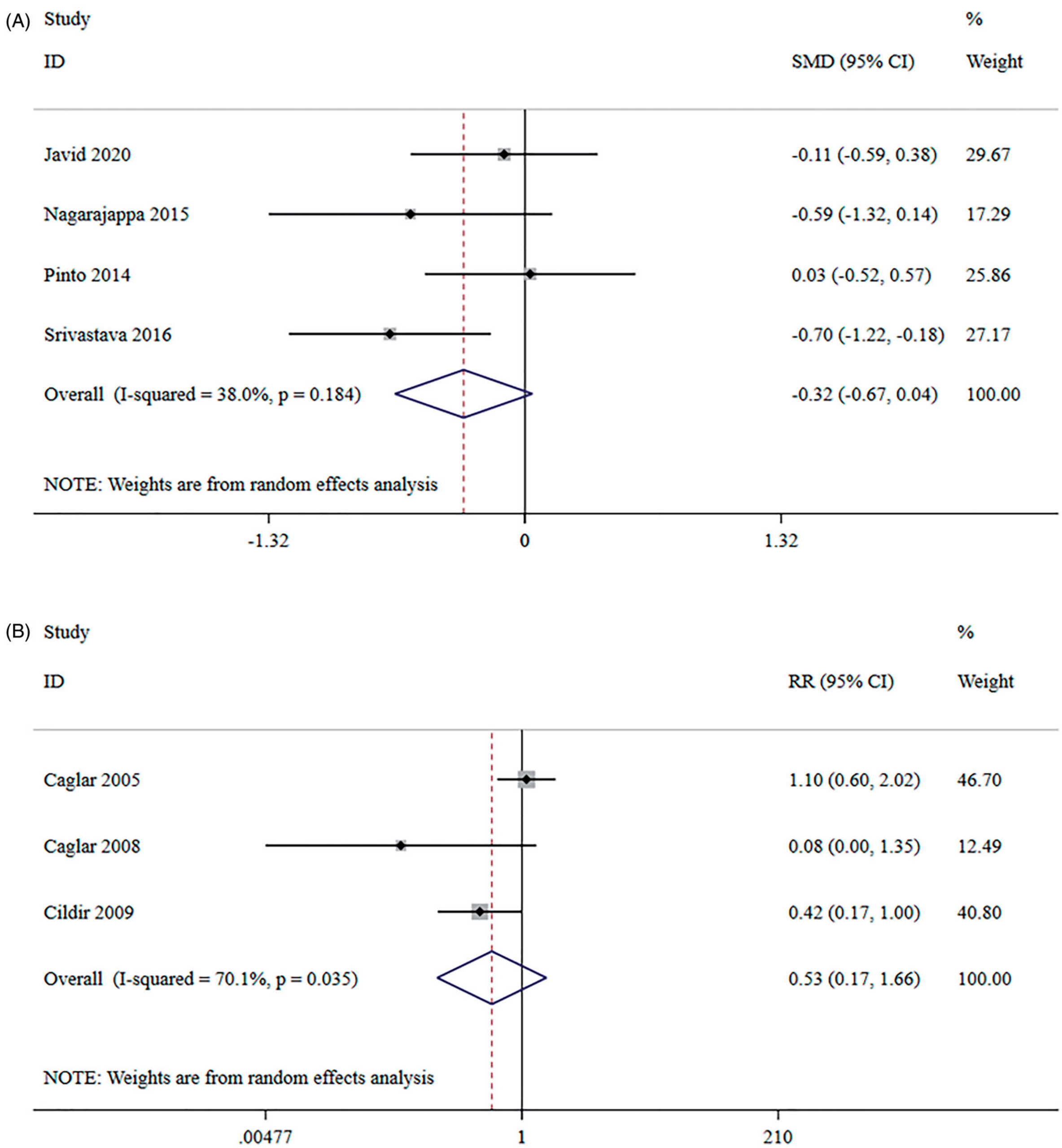

Figure 3. The meta-analysis demonstrated that *Bifidobacterium* could not effectively inhibit *Streptococcus mutans*. (A) Forest plot of pooled standardized mean difference (SMD) of *Streptococcus mutans* counts in saliva showed no statistically significant differences between the intervention and control groups. (B) Forest plot of pooled risk ratio (RR) of *Streptococcus mutans* counts in saliva manifested no significant difference between *Bifidobacterium* and placebo groups.

meta-analysis of the fixed-effect model illustrated that after the intervention, there was no significant difference in the number of patients with *Lactobacillus* counts $>10^5$ CFU/mL in the *Bifidobacterium* group compared with the control group (RR $=$ 0.87, 95% CI [0.59, 1.29], $p = .50$) (Figure 4(B)).

Lactobacillus counts in dental plaque

Pinto et al. determined *Streptococcus mutans* and *Lactobacillus* counts in dental plaque samples [22]. The results suggested that the *Lactobacillus* counts in the dental plaque decreased to a certain extent compared with that before the intervention. However, compared with the placebo control group, the differences were not statistically significant.

Occurrence of dental caries

A qualitative description was made because the two studies conducted by Taipale et al. used DMF (Decayed, Missing, Filled) [32] and International Caries Detection and Assessment System (ICDAS), respectively, for assessments [33]. Taipale et al. respectively administered *Bifidobacterium* or sorbitol (placebo) to 55 and 54 infants with an average duration of 14.9 months, from 1 to 2 months of age, through a sustained-release pacifier or tablet [32]. When examined at 8 months and 2 years of age, the deciduous teeth of both groups had a DMF of 0. Taipale et al. also followed up the children and re-evaluated them when they were four years old, using the ICDAS, to assess the incidence of deciduous tooth decay in 32 children in the test group and 29 in the

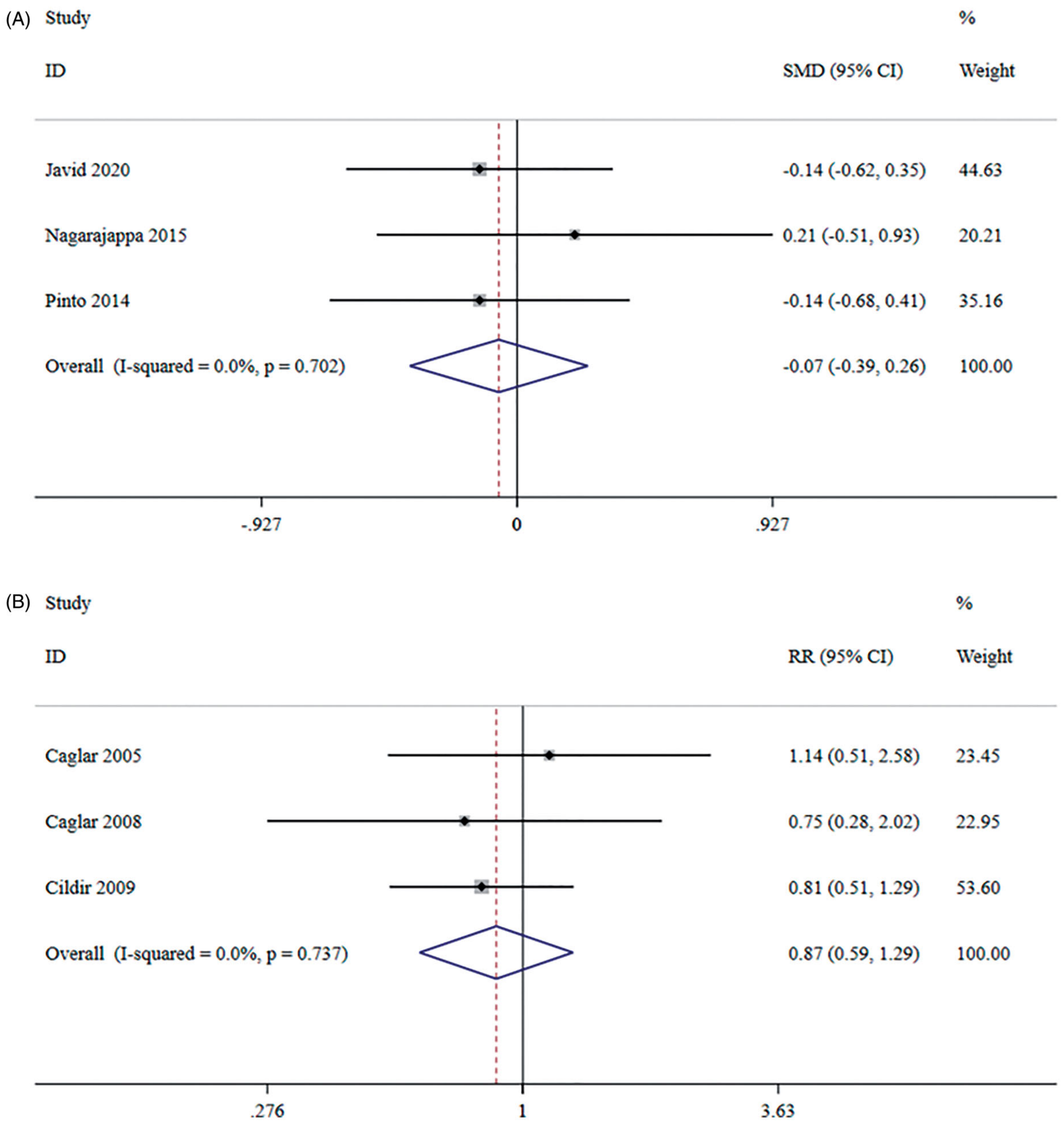

Figure 4. The meta-analysis demonstrated that *Bifidobacterium* could not effectively inhibit *Lactobacillus*. (A) Forest plot of combined standardized mean difference (SMD) of *Lactobacillus* counts in saliva did not show significant differences between the test and control groups. B: No significant difference was detected by combined risk ratio (RR) of *Lactobacillus* counts between the *Bifidobacterium*-intervention and placebo-control groups.

control group [33]. The results revealed that the incidence of enamel caries (ICDAS code 2–3) and significant dentine caries (ICDAS code 4–6) in the test group was even higher than that in the control group, but it did not reach a statistically significant difference.

Adverse events

Five of the ten included studies reported no local or systemic adverse reactions in either the test or the control group [22,23,25,28,31]. Taipale et al. reported that two subjects in the test group suffered from gastrointestinal discomfort, with one in the control group reporting gastrointestinal discomfort [32,33]. There was no significant difference in the incidence of adverse events between the two groups. However, the other three studies did not report whether the adverse events happened or not [12,29,30].

Discussion

To explore the possible use of *Bifidobacterium* as an anti-cariogenic factor, we first evaluated the effect of *Bifidobacterium* on the main cariogenic bacteria, including *Streptococcus mutans* and *Lactobacillus*. Available evidence suggests that *Bifidobacterium* cannot effectively inhibit the *Streptococcus mutans* and *Lactobacillus* in saliva or dental plaque. *Streptococcus mutans* has been recognized as the main cariogenic bacterial species due to its strong acid production and acid resistance ability [4,34]. The RCTs included in this study also used *Streptococcus mutans* counts as a surrogate endpoint for the occurrence of caries. Various studies have shown that *Lactobacillus* is correlated with the initiation and development of caries [35,36]. The metabolism of *Lactobacillus* produces acids, which increases the risk of caries [37]. Shimada et al. discovered that *Lactobacillus* counts in the oral cavity were positively correlated with caries progression, and *Lactobacillus* counts in the oral cavity of children with dental caries were significantly higher than healthy children, especially in the case of dentine caries [38]. Nevertheless, the currently available evidence suggests that *Bifidobacterium* is not effective in inhibiting *Lactobacillus*.

Several studies have supported using the counts of main cariogenic bacteria, such as *Streptococcus mutans*, as a surrogate endpoint. Parisotto et al. reported that *Streptococcus mutans* counts were a risk indicator for early childhood caries (ECC) [39]. Kirthiga et al. also claimed that high levels of *mutans streptococci* were among the strongest factors associated with ECC [40]. However, some studies have raised doubts. Gross et al. and Valdez et al. argued that the accuracy and effectiveness of this surrogate endpoint needed further and more comprehensive investigations and discussions [41,42]. To make the results of the present study convincing, we included studies by Taipale et al., which directly focussed on the incidence of deciduous dental caries. These two long-duration follow-up studies demonstrated no statistically significant difference, revealing that *Bifidobacterium* might not be able to prevent caries of deciduous teeth effectively [32,33].

The safety of *Bifidobacterium* also requires further investigations. Although a systematic review of the available evidence suggests a low incidence of adverse events, the sample size was somewhat limited, and there were still a few RCTs that did not provide information concerning adverse events. Meanwhile, Valdez et al. reported that *Bifidobacterium* could create an acidic environment and promote biofilm formation through *in vitro* experiments, suggesting that *Bifidobacterium* might have cariogenic effects [42]. Haukioja et al. illustrated that *Bifidobacterium* could also produce organic acids, causing demineralization of teeth and reducing dental plaque pH [43]. Zhai et al. explored the distribution of *Bifidobacterium* in children's oral cavity and the correlation between *Bifidobacterium* and caries in children. They found that the rate of *Bifidobacterium* in patients with severe ECC was 47.5%, with 0%, in healthy caries-free infants, indicating that *Bifidobacterium* itself might have a role in caries [44]. The potential risks of *Bifidobacterium* products for the aged, infants, and individuals with immunodeficiency should also be carefully assessed [45–47].

At the same time, we also noticed some inconsistencies between our review and the reviews published previously [11,48]. Nadelman et al. combined the results of several species of probiotics, including *Lactobacillus*, *Bifidobacterium,* and *Streptococcus*, to conduct a meta-analysis and concluded that probiotics prevented dental caries [48]. However, they did not conduct a subgroup analysis of *Bifidobacterium* specifically. Therefore, our research fills the gap in this field.

The meta-analysis of Gruner et al. concluded that *Bifidobacterium* could not effectively reduce *Lactobacilli* counts in saliva, consistent with the present analysis [11]. However, they considered that *Bifidobacterium* could effectively reduce *Streptococcus mutans* counts in both continuous variables and dichotomous variables subgroup, which we regarded as controversial [11]. Firstly, in continuous variables, they included two studies: Cinnappa 2013 [49] and Nozari 2015 [24]. However, on the one hand, Cinnappa et al. reported that their ice cream probiotics consisted of both *Bifidobacterium lactis Bb-12* and *Lactobacillus acidophilus* [49]. This study that mixed two probiotics species, *Lactobacillus* and *Bifidobacterium*, should not have been included in the subgroup of *Bifidobacterium* because it is unclear whether *Lactobacillus* or *Bifidobacterium* contributed or not, and what their interaction was. On the other hand, they included the study by Nozari (2015). However, we thought it was not suitable for inclusion and did not include it in our meta-analysis [24]. At baseline, the *Streptococcus mutans* counts in the control group were already twice that of the test group (129200 ± 97891.1 $n_C = 25$ vs 60083 ± 40696 $n_T = 24$), which failed to meet the criterion of an RCT according to Cochrane handbook [27], indicating that the experiment design was questionable, and it should not have been included in the meta-analysis. After eliminating these two studies that were inconsistent with the review purpose from the synthesis, their data also showed that *Bifidobacterium* could not effectively reduce *Streptococcus mutans* counts. Secondly, in continuous variables, the three studies they included are the same as ours, but the threshold we selected was different. Although they selected *Streptococcus mutans* counts in saliva $>10^4$ CFU/mL as a risk factor for caries, we reviewed the research progress and concluded that choosing 10^5 CFU/mL as a threshold is more appropriate and clinically meaningful. Studies long ago have shown that the *Streptococcus mutans* counts from 10^4 to 10^5 CFU/mL are a normal phenomenon in caries-free individuals. However, at 10^6 CFU/mL, the risk of caries significantly increased [50]. In recent years, investigations have also shown that the counts of *Streptococcus mutans* in saliva in normal caries-free children is $10^4 \sim 10^5$ CFU/mL [51]. Meanwhile, most of the current clinical studies have regarded 10^5 CFU/mL as a threshold [28,52,53]. Gruner et al. claimed that 'the threshold of 10^4 CFU/mL was used as primary analysis for this outcome as it showed greatest differences'. However, we believe that the selection of threshold should not merely consider whether it can show digital differences, but it should consider its practical clinical significance. If we change the threshold to 10^5 CFU/mL, their data will also come to the same conclusion. Therefore, we suggest that Gruner et al.'s conclusions on *Bifidobacterium* are controversial, and we tried to overcome these shortcomings in our meta-analysis to draw more reliable and clinically meaningful conclusions.

There also exist several limitations in the present systematic review and meta-analysis: (1) The number of included studies and the sample size of each study were limited, possibly impacting the test efficiency; (2) There were certain risks of bias in the included studies, such as the unclear implementation of allocation concealment, with a possible bias in measurement; (3) The heterogeneity of these included RCTs in intervention duration, vehicle, and dosage.

Admittedly, it is undeniable that *Bifidobacterium* is critical to human health as one of the main components of daily probiotic products [48,54]. Many studies have verified its positive effect on the human body's digestive system and physiological activities [55–59]. However, from the perspective of evidence-based medicine, we discovered that *Bifidobacterium* plays a limited role in preventing dental caries.

Conclusion

Based on currently available evidence, *Bifidobacterium* is ineffective in reducing *Streptococcus mutans* and *Lactobacillus* counts in the saliva or dental plaque. It is not able to inhibit the initiation of deciduous dental caries either. Its safety also needs further investigations. Therefore, *Bifidobacterium* is not a competent probiotic candidate to prevent dental caries based on present evidence. Further high-quality RCTs using the initiation of dental caries as the endpoint are required to verify these conclusions in the future.

Disclosure statement

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Author contributions

Y.W. and S.H. contributed to the overall conceptualization and design of the study. S. H. and J.W. conducted the literature search, carried out the data extraction and quality assessment, analyzed the data, and drafted and revised the manuscript. Y. W., S. H., and J. W. revised the manuscript. All authors have read and approved the manuscript.

ORCID

Siyuan Hao http://orcid.org/0000-0002-5025-9630

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