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Title page

Title: The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

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Running title: Probiotic treatment of halitosis

ABSTRACT

Objectives Halitosis is defined as a foul odor emitted from the oral cavity. Many interventions have been used to control halitosis from mouthwashes to chewing gums. Probiotics have been reported as an alternative method to alleviate halitosis. The present study aimed to investigate the effect of probiotics on halitosis.

Design and methods This is a meta-analysis study. A search was performed in indexed databases up to February 2021. Randomized controlled trials were included that compared probiotics and placebo concerning primary outcomes of organoleptic scores and volatile sulfur compounds levels. Data extraction and quality assessment were conducted independently by two reviewers.

Results Standardized mean difference (SMD) and 95% confidence interval (CI) were calculated to synthesize data. The data were sub-grouped and analyzed in the short term (≤4 weeks) and long term (>4 weeks) based on the follow-up time. Seven articles were included in this review. For primary outcomes in the short term (≤4 weeks), organoleptic scores [SMD= -0.58; 95%CI (-0.87, -0.30), p<0.0001] and volatile sulfur compounds levels [SMD= -0.26; 95%CI (-0.51, -0.01), p=0.04] significantly decreased in the probiotics group compared with the placebo group. However, a significant reduction was observed only in organoleptic scores [SMD= -0.45; 95%CI (-0.85, -0.04), p=0.03] in the long term (>4 weeks). No significant differences were observed in secondary outcomes (tongue coating scores and plaque index).

Conclusions According to the results of this meta-analysis, it seems that probiotics can be used to relieve halitosis in the short term (≤4 weeks). The results of bias assessment and limited data might reduce the reliability of the conclusions.

Strengths and limitations of this study

- This study included larger RCTs involved in halitosis and probiotics.
- The results were rationally analyzed from the follow-up time perspective.
- The included studies had limited patients.
- Some studies reported the outcomes with different forms, increasing the heterogeneity of the results.

INTRODUCTION

Halitosis, also known as "oral malodor," is typically defined as an unpleasant odor emanating from the oral cavity.[1] As a cause of patients' referral to the dentist, halitosis is the third most common disease, only ranking behind dental caries and periodontal disease. [2] According to an epidemiological study, the prevalence of halitosis is approximately 27.5% in the Chinese population.[3] People have a higher demand for social interactions and attach more importance to their personal image in today's society. Halitosis has a significant impact on both patients' daily work and social activities and may even results in frequent psychological problems such as anxiety, depression, and social isolation.[4] Clinically, halitosis is categorized into genuine halitosis, pseudo-halitosis, and halitophobia.[5] The latter two types are related to psychological conditions. Only genuine halitosis is caused by pathological and physiological factors. It includes intraoral halitosis (IOH) and extraoral halitosis, with the former accounting for 80-90% of the cases.[6]

The main etiologic factor of genuine halitosis is the volatile sulfur compounds (VSCs) produced by oral bacteria via complex microbe–substrate and microbe–microbe interactions and putrefaction of organic substrates in the oral cavity, associated with poor oral hygiene, tongue coating, and periodontal disease.[7-10] In particular, hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulphide (C_2H_6S) are considered significant parameters and markers of halitosis.[11] Some microorganisms, such as *Fusobacterium. nucleatum*, *Porphyromonas. gingivalis*, *Prevotella. intermedia*, *Prevotella. nigrescens*, and *Treponema. denticola* not only do participate in periodontal diseases, but they also may facilitate the production of VSC metabolism.[12] Some studies using16S rRNA amplicon sequencing and GC–MS-based metabolite profiling found that the bacterial composition, diversity and metabolites of the halitosis group were different from those of the control group.[13,14] Therefore, anaerobic microbiota might play an important role in the development of halitosis. Consequently, regulating the balance of the oral microbiome to reduce VSC levels is an important method to treat oral malodor.

According to some previous reports, the current treatments for halitosis include mechanical cleaning (scaling and tongue scraping) and chemical therapy (antibiotics, mouthwashes, and other agents).[15, 16] However, mechanical therapy is often uncomfortable, even if carried out by the dentist. In addition, although chemical therapy is generally effective for a short time, it is always associated with various side effects, including the emergence of dysbacteriosis and staining of the tongue and tooth.[17-20] Consequently, new methods with fewer side-effect are constantly suggested to inhibit oral malodor.

As live microorganisms, probiotics confer benefits for the host when administered in appropriate amounts.[21] The beneficial effects of these probiotics are primarily related to regulating the local microenvironment.[22, 23] Recently, probiotics have been widely used in the oral field.[24] There is a growing body of evidence that the administration of probiotics might affect the composition of oral biofilms. They have also been investigated in the treatment of periodontal [25, 26] and peri-implant diseases [27, 28], caries [29], and oral candidiasis.[30, 31] Meanwhile, probiotics have also been reported as an alternative strategy to relieve oral malodor.[32-36] At present, the most common strains of probiotics in clinical studies are *Lactobacillus salivarius* and *Bifidobacterium.*[37] To date, numerous articles have reported the beneficial effects of probiotics on the treatment of halitosis.[38-40] However, a previous systematic review showed that probiotic therapy for oral malodor is associated with insufficient evidence for its recommendation.[41] In this review, only three included articles published during 2012-2016 and the diversity of observation time might affect the reliability and quality of the results. Furthermore,

several new studies on the efficacy of probiotics in the management of halitosis were published in 2020. Thus, it is necessary to carry out a focused analysis of the therapeutic effects of probiotics in the treatment of halitosis.

Therefore, this meta-analysis was undertaken to investigate the effect of probiotics on managing halitosis from a time perspective. The results could provide some evidence for the administration of probiotics in this field.

METHODS

Patient and public involvement

This is a meta-analysis based on the data in the literature. It is not appropriate to involve patient and the public in our study design and outcome measures.

Study design

This systematic review was based on the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered in the PROSPERO (CRD42021227504).[42] According to the PICOS principle, the following focused question was structured: What is the clinical efficacy of probiotics in patients with halitosis when compared with placebo treatment? The populations were patients diagnosed with halitosis. The intervention was probiotic therapy, representing the test group. The test group was compared with placebo treatment. The considered outcomes were halitosis parameters and other indexes before and after treatment. All study designs were RCTs.

Search strategy

A critical electronic search was conducted in the bibliographic databases, mainly including PubMed, EMBASE, Web of Science, Cochrane Central Register of Controlled Trials up to and including February 2021 to select the published literature. Additionally, gray literature was searched in the database System for Information on Gray literature in European and Google Scholar. The reference lists of the included articles and some related Chinese journals were also searched manually. There was no language restriction.

An initial search strategy was conducted in the PubMed with the combination of Medical Subject Headings (Mesh) terms identified by an asterisk symbol (*) and free text words as the follows:

Probiotic OR Probiotic* OR Probiotic therapy OR Probiotic effect OR Probiotic treatment

AND

halitosis OR halitosis * OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris.

Endnote X7 was used for electronic title management. First, primary screening was performed independently by two reviewers (JJL and NWH) based on the titles and abstracts. Then, the full-text articles were used to assess the eligibility further. Any disagreement was solved by consulting a third reviewer.

Inclusion criteria

Studies meeting the following conditions were considered eligible for this review: 1) study types: randomized controlled clinical trials or randomized controlled cross-over studies; 2) participants: systemically healthy patients diagnosed with halitosis via accepted standards (the organoleptic score and or the concentration of volatile sulfur compounds); 3) interventions: evaluating the efficacy of probiotics with placebo, regardless of the probiotics species and the consumption method; 4) control interventions: placebo treatment; if the control interventions included other measures, the study was not included (e.g., studies comparing tongue scraping plus chlorhexidine plus probiotics and tongue scraping plus chlorhexidine were excluded);[33] 5) clinical data: the measurement values, including halitosis parameters and other indexes before and after treatment.

Risk of bias

The included studies underwent a quality assessment with the Revised Cochrane risk of bias tool for randomized trials (RoB2).[43] This tool assesses the risk of bias in five domain areas, including randomization process, deviations from intended interventions, missing outcome data, measurement of outcome, and selection of the reported result. Each domain assessed bias following several signaling questions. The overall bias was classified as a high risk of bias, some concerns, or a low of risk of bias determined by a validated algorithm. After screening the articles, two reviewers (JJL and NWH) conducted the assessment independently to reach an agreement.

Data extraction

Data were extracted with a researcher-designed data form with the following information: 1) basic information of the included studies (first author's name and the year of publication); 2) study type (RCT); 3) diagnostic criteria for halitosis; 4) characteristics of the participants (sample volume, the age range); 5) treatment (probiotic administration, including the type of bacteria, vehicles, doses, and frequencies); 6) clinical parameters (including the primary and secondary outcomes of final participants); 7) significance and follow-up periods.

Of all these variables, the follow-up periods referred to the duration of probiotic use. If probiotic treatment ceased during the observation period, only the data before ceasing treatment were included. Concerning clinical parameters, organoleptic (OLP) scores and VSC concentrations were considered the primary outcomes, which were directly associated with oral malodor. The secondary outcomes in this review included tongue coating scores (TCS) and plaque index (PI) because they are commonly regarded as halitosis causes.

Statistical analysis

The statistical analysis was performed with Review Manager 5.3. All the data were group-analyzed according to the follow-up time. The time ≤4 weeks was considered the short-term period, and time >4 weeks was considered the long-term period. In one study with three observation periods, the values of 4 weeks were analyzed in the short term to keep consistent with other studies.[44] Study heterogeneity was evaluated using Q statistics and the I^2 test. P-value <0.10 was treated as the standard test. When I^2>50% or p<0.10, there was significant heterogeneity between the studies.

Subgroup or meta-regression was necessary to analyze the sources of heterogeneity. The continuous data on the halitosis parameters of the present studies were expressed with the standardized mean difference (SMD) and 95% CI (confidence interval). A random-effect model was used for analysis. Therefore, the mean difference and standard deviation had to be acquired. If the original text did not provide the related data, the mean difference could be calculated, and the standard deviation was obtained with the formula (r_d= sqrt ($r_1^2/n_1 + r_2^2/n_2$). The excel sheets in the articles were used to convert the values when provided with median and interquartile.[45, 46]

RESULTS

Study selection

In total, 238 articles were potentially identified by electronic and manual searches. After eliminating the duplicates, 14 articles were included by screening the titles and abstracts. Then, these studies were evaluated by reading the full texts, and seven articles met the final inclusion criteria.[40, 44, 47-51] Figure 1 presents the whole process and reasons for exclusion.

Study characteristics

Table 1 presents the main characteristics of the included studies. In this review, all the studies were randomized control trials. The number of participants in the studies ranged between 23 and 68, with an age range of 19 to 70. Halitosis was diagnosed with OLP scores and or VSC concentrations. The probiotics and placebo groups were compared, and the follow-up periods varied from two weeks to 12 weeks.

Table 1 Characteristics of the included studies.

Study	Type	Halitosis criterion	Subjects Age	Clinical parameters	Probiotics Administration (Vehicle, strains and frequency)	Follow-up
Mousquer et al. (2020)	RCT Placebo-double masked, parallel	OLP score≥1	29 ≥18	OLP VSC TCS	A gum including 1 billion colony forming units (CFU) Lactobacillus salivarius G60 taken twice per day	Baseline 2 weeks
Lee et al. (2020)	RCT Placebo-double blind parallel	VSC≥1.5ng/10 mL	68 20-39	OLP VSC (H_2S, CH_3S, C_2H_6S)	An 800-mg tablet contained 1.0×10^8 CFU/g Weissella cibaria taken once per day	Baseline 4 weeks 8weeks
He et al. (2020)	RCT Placebo-double blind parallel	OLP score ≥ 2 VSC ≥ 150ppb	28 23-44	OLP VSC TCS PI	A tablet containing 1×10^9 CFU Streptococcus salivarius K12 taken twice per day	Baseline 4 weeks
Keller et al. (2012)	RCT Placebo-double blind cross-over	OLP score>1	25 19-25	OLP VSC	A chewing gum containing Lactobacillus reuteri DSM 17938 and Lactobacillus reuteri ATCC PTA 5289 -both with a concentration of 1×10^8 CFU taken twice per day	Baseline 2 weeks
Suzuki et al. (2014)	RCT Double-blind placebo-controlled Cross-over	OLP score≥1.5	23 22-67	OLP VSC (H_2S, CH_3S, C_2H_6S) PI TCS	A tablet containing 6.7×10^8 CFU Lactobacillus salivarius WB21 and 280mg xylitol taken 3 times per day	Baseline 2 weeks

Penala et al. (2016)	RCT Placebo-double blind parallel	OLP score > 2	29 25-59	OLP PI	A capsule mixture included Lactobacillus salivarius (2×10^9 CFU) and Lactobacillus reuteri (2×10^9 CFU) dissolved into 10ml distilled water to rinse for 1min, daily twice	Baseline 4 weeks 12 weeks
Kim et al. (2020)	RCT Placebo-double blind parallel	OLP score≥2 VSC≥0.15ng/ml	58 20-70	VSC (H_2S, CH_3S, C_2H_6S) OLP	A bag of powder mixture included Weissella. cibaria CMU (1.0×10^8 CFU) melted in the mouth once per day	Baseline 2 weeks 4 weeks 8weeks

*RCT: randomized controlled trials; OLP: organoleptic; VSC: volatile sulfur compounds; TCS: tongue coating scores; CFU: colony forming units; H_2S: hydrogen sulfide; CH_3S: methyl mercaptan; C_2H_6S: methanthiol; PI: plaque index

Risk of bias

The bias estimation results showed that one study had a low risk of bias, one had a high risk, and five showed some concerns. The reason for a high risk of bias was the incomplete outcome data of the OLP scores. Five articles were identified as some concerns because there were many uncertain factors in their full texts. There were only seven studies in our review; thus, a funnel plot was not performed. Figure 2 presents the concrete data on the risk of bias.

Primary outcomes

Concerning OLP, all the included studies detected the parameter with the 0-5 organoleptic scale by one or two trained and calibrated judges, and five studies contained complete data.[40, 48-51] Studies by Keller et al. (2012) and Penala et al. (2016) reported a significant decrease in OLP in the probiotic group compared to the placebo group after treatment ($p < 0.05$). In the study by Lee et al. (2020) involving different follow-up periods, OLP scores decreased significantly in the test groups at four weeks ($p = 0.002$) but not eight weeks ($p = 0.188$) compared to the baseline. Additionally, the results of the other four studies indicated that the OLP scores did not differ between the two groups.

Concerning VSC, six articles determined VSC concentrations, with three studies detecting the values of VSC and subgroups (H_2S, CH_3SH, and C_2H_6S).[40, 44, 50] According to the results, only two studies[40, 50] reported a significant improvement in VSC levels in experimental groups versus placebo groups.

Secondary outcomes

Concerning TCS, three studies evaluated the changes between the probiotic and placebo groups at four weeks [40, 49, 51]. Although a reduced tendency was observed after treatment compared with baseline values, there was no significant difference between the two groups.

Concerning PI, in the three studies involved,[40, 48, 49] only one study showed a significant reduction in PI in the experimental group compared with the controlled group at 12 weeks.[48]

Quantitative synthesis

A meta-analysis was performed including studies with similar clinical parameters of OLP, VSC, TCS, and PI, according to the follow-up time. Although the detection methods of VSC were different, both of the devices exhibited similar sensitivity and specificity in the detecting of halitosis.[52] Therefore, we

analyzed these values together. Considering the limitations of the included studies and follow-up time, the pooled estimation of TCS and PI was only performed in the short term.

In the short term, the OLP scores significantly decreased in the probiotic group compared to the control group [SMD = -0.58; 95% CI (-0.87, -0.30), $p < 0.0001$] (Figure 3). A similar result was observed in VSC [SMD = -0.26; 95% CI (-0.51, -0.01), $p = 0.04$] and H_2S levels [SMD = -0.73; 95% CI (-1.36, -0.10), $p = 0.02$] (Figure 3 and Figure 5). Other items (TCS, PI, CH_3S, and C_2H_6S) were not significantly different between the experimental and control groups. The heterogeneity of each outcome was low ($I^2 < 50\%$) except for H_2S levels ($I^2 = 75\%$).

In the long term, there was a significant improvement in OLP scores in the experimental group [SMD = -0.45; 95% CI (-0.85, -0.04), $p = 0.03$] (Figure 4). The results failed to show a significant difference in VSC concentrations and their subgroups levels (Figure 4 and Figure 6). The heterogeneity of VSC concentrations was substantial ($I^2 = 58\%$).

DISCUSSION

Halitosis is a universal phenomenon with a negative impact on people of all ages. Most causes are related to oral health, particularly periodontal diseases and tongue coating.[8, 53] Clinically, organoleptic test and detection of VSC concentrations are two commonly used methods to diagnose and monitor halitosis. Considering VSC generation, the use of probiotics to improve halitosis might be useful by modifying the composition of bacteria. Therefore, this review investigated the efficacy of probiotics in treating halitosis based on symptoms and causes.

This meta-analysis demonstrated that probiotics significantly reduced the OLP scores compared with the placebo group regardless of the duration of observation, confirming the benefits of probiotics for halitosis treatment. The probiotics group exhibited a significant reduction in VSC concentrations in the short term (≤4 weeks), with no noticeable difference in the long term (>4 weeks). Meta-analyses were also performed in the subgroups of H_2S, CH_3SH, C_2H_6S to assess the concrete difference in VSC levels. The results showed that only H_2S levels reduced noticeably in the short term when the probiotic treatment was administered. As for TCS and PI, the meta-analysis estimated the difference based on the data of three included studies, whose observation times were all within four weeks. The results showed no significant differences between the experimental and placebo groups.

Concerning primary outcomes, OLP scores reflecting subjective perception were often treated as the gold standard for diagnosing halitosis clinically and in the research.[54, 55] In the present article, six studies included the identified halitosis criteria of subjects with OLP scores.[40, 44, 47-49, 51] The pooled estimation of this value was in favor of probiotic therapy rather than placebo. The VSC concentration measurement is an objective method, usually using a Halimeter or OralChroma with no significant difference. However, compared with organoleptic evaluation, VSC measurement is a quantitative variable with high sensitivity and reproducibility.[56-58] The short-term results of VSC showed a significant improvement in the probiotic group compared to the placebo groups. These findings mean that probiotics might have a potential beneficial effect on relieving oral malodor symptoms in the short term. The possible mechanism is thought to be related to the oral cavity microbiome. According to some previous studies, odorous compounds are derived from the decomposition of amino acids and proteins by anaerobic bacteria.[7, 59] The principle of probiotic therapy is the competitive inhibition of oral anaerobic bacteria to maintain balance. Based on studies on VSC and bacteria, the significantly lower VSC levels in the short term in the probiotic period might indicate the reduced activity of

anaerobic bacteria. This finding is different from a previous study. One possible reason is the difference in the number of included articles. Besides, the group analysis of the follow-up time might also play a crucial role in assessing the outcomes. Meanwhile, we found that the short-term outcome of H_2S concentration change other than CH_3SH, C_2H_6S was consistent with the total VSC. This might be attributed to the differences in bacterial number and species related to each VSC reduction and mechanism of probiotics [12, 34, 60]. Additionally, the regular VSCs measurement device was reported to be more sensitive towards H_2S than CH_3SH and C_2H_6S,[58] which is also a possible reason for the above result. Because lower sensitivity would have a significant effect on the accurate measurement of the relatively low VSC. However, this specific mechanism is not clear and the high heterogeneity of the assessment reduced the reliability of the findings ($p=0.04$ and $I^2=75\%$).

Regarding the secondary outcomes, based on the present meta-analysis, there was no significant difference between the experimental and placebo groups during the observation time. The possible reason was the short observation time in the included studies because one study included in the analysis showed a significant improvement in PI at 12 weeks.[48] Tongue coating and periodontitis are often regarded as the leading causes of halitosis[49, 61]. In the original articles, the TCS and PI showed a pronounced decline after using probiotics compared with the baseline, with no decrease in the placebo group. This phenomenon might be related to the type of probiotics, some of which were reported to boost salivary flow by interacting with the oral microbiome.

Considering the inconsistency in the results of organoleptic scores and VSC concentrations in the long term, time is likely to be the primary reason due to its significant effect on community diversity. Additionally, it is also associated with the interaction of probiotics and anaerobic bacteria related to VSC. In the present review, four articles used probiotics consisting of *Lactobacillus salivarius* as the intervention treatment, while two articles selected *Weissella cibaria*, a bacterium isolated from *Lactobacillus*, and one article with *Streptococcus*. The bacterial species related to VSC production mainly included *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Treponema denticola.*[53, 62-64] *Lactobacillus salivarius* reduces the production of VSC mainly by inhibiting the growth of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*. However, *Streptococcus* has been reported to inhibit various bacteria, including *Streptococcus mutans*, *Actinomyces naeslundii*, and *Rothia mucilaginosa.*[34] When oral bacteria vary over time, the efficacy of probiotics, especially VSC concentrations, changes based on the number of anaerobic bacteria. Moreover, along with VSCs, various other malodor gases are often present in bad mouth air, such as indoles, skatole, pyridine, picolines and polyamines. The oral microbiota included not only VSCs-producing bacteria, but also other bacteria being able to produce malodor compounds.[65] Therefore, the long-term results may attribute to the inhibition effect of probiotics on other bacteria. Therefore, the data about microorganisms changing in different periods are significant for the evaluation of probiotic effects. However, only three included articles mentioned microorganism detection. The differences in detection methods and bacterial species and insufficient data in the included studies limited the microorganism statistical analysis in this review.

There were several limitations in the present study throughout the whole review process. First, although both electronic and hand searches were conducted in four primary databases, it was impossible to retrieve all the relevant studies. Second, the number of eligible studies and included subjects was small. Third, the interventions in all the included studies included probiotics, but the strains were different. Moreover, the doses used, frequencies, and administration periods varied greatly. A subgroup analysis was necessary to evaluate the source of efficacy concerning the probiotic

species, but the small size of the included articles prevented further analysis. All these factors would inevitably affect the accuracy of outcomes. Fourth, the detection methods of VSC were different. Although there is not significant difference between them, the combined analysis might still affect the reliability of the results. Fifth, in some included studies, the primary outcomes were presented in different forms, such as percentages or range inter-quartiles. Finally, some important parameters, including the microorganism species and changes, were not presented completely in some articles. The absence of partial original data or the differences caused by data conversion equally impaired the final results though many methods were tried to reduce the bias.

CONCLUSION

The present systematic review and meta-analysis indicated that probiotics might decrease the severity of halitosis in the short term without eliminating pathogens. Considering the heterogeneity and limitations of the study, more high-quality random clinical trials are required in the future to verify the results.

Contributors

JJL collected and analyzed data, and drafted the manuscript; NWH and XHQ helped the literature searching and statistical analysis; YZW and CZW provided help in the literature searching and figure revises; XHQ and YKL critically reviewed the manuscript. LJL designed the experiment and critically reviewed the manuscript; All authors agree to be accountable for the study.

Declaration of competing Interest

The authors declare no conflict of interest

Ethics approval statement

This study does not involve human participants.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author, Longjiang Li, upon reasonable request.

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Figure legends

Figure 1: Flow diagram of literature search and inclusion.

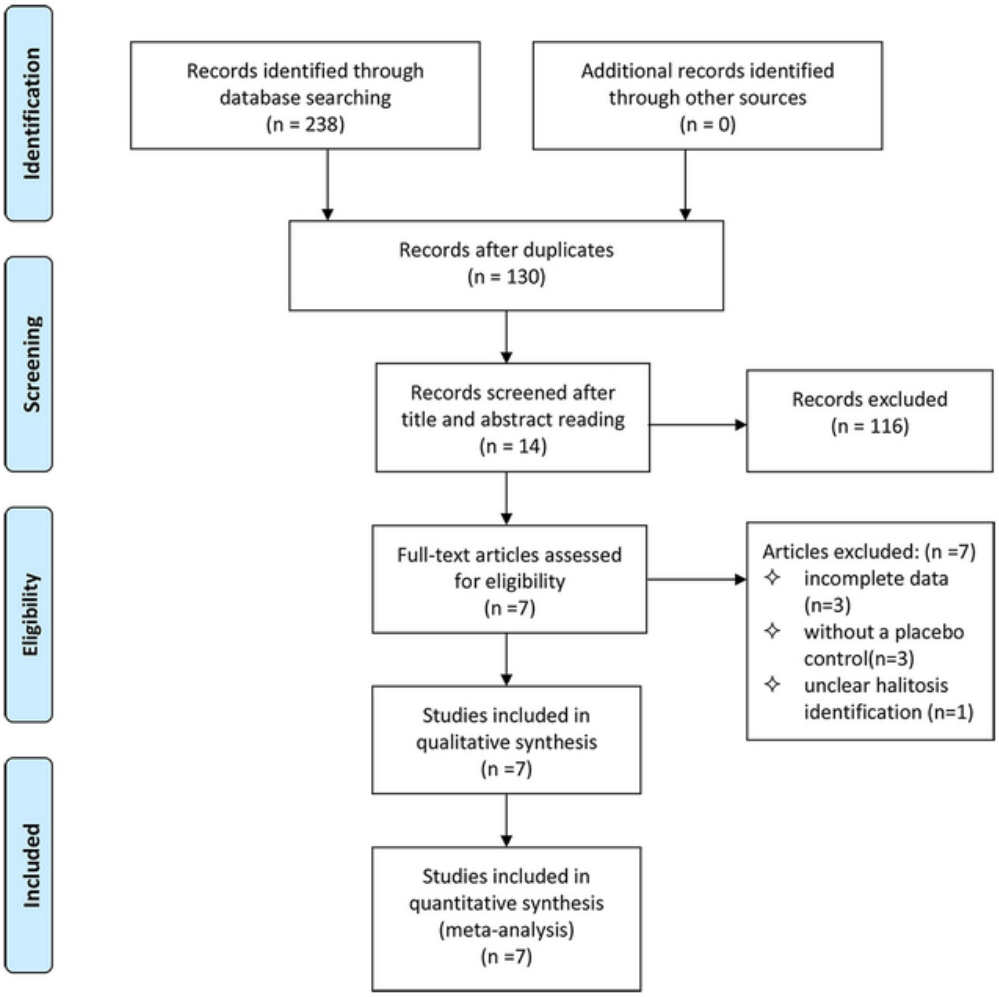
Figure 2: Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represent high risk of bias.

Figure 3: Forest plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

Figure 4: Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

Figure 5: Forest plot of VSC subgroups in short-term (≤ 4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

Figure 6: Forest plot of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.



Flow diagram of literature search and inclusion.

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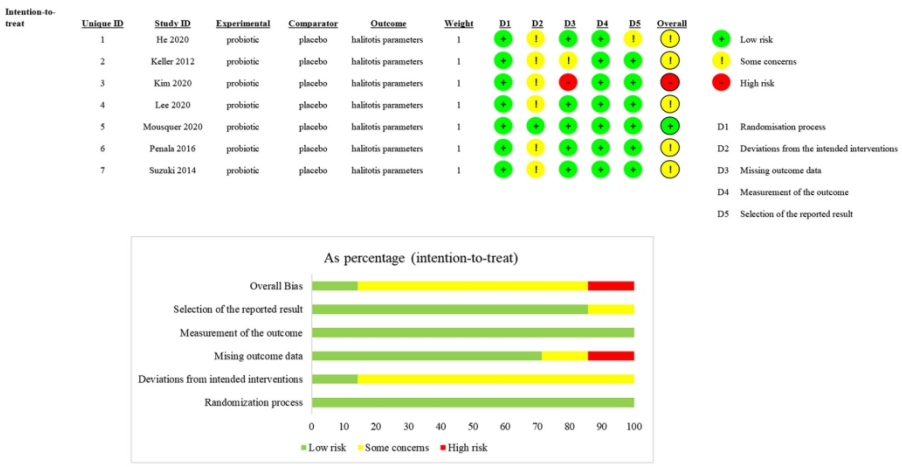

Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represent high risk of bias.

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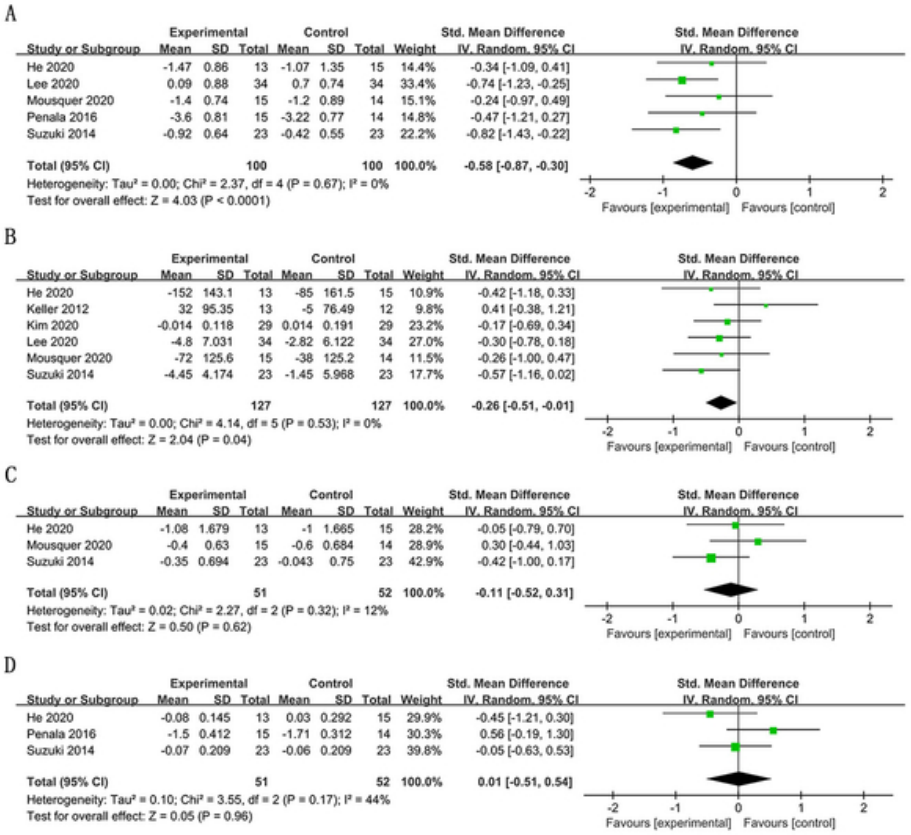

Forest plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

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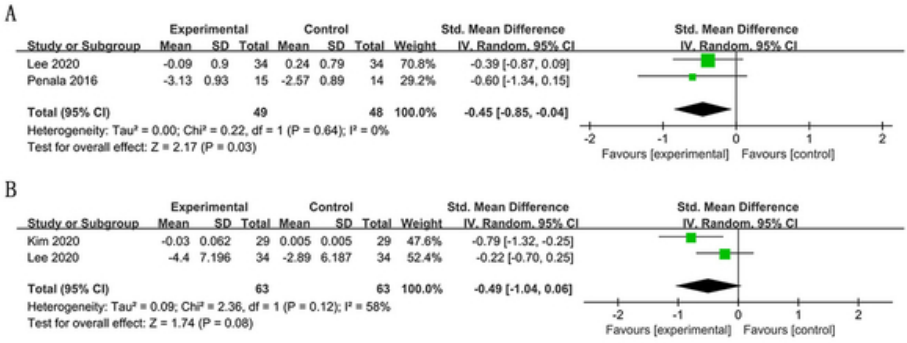

Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

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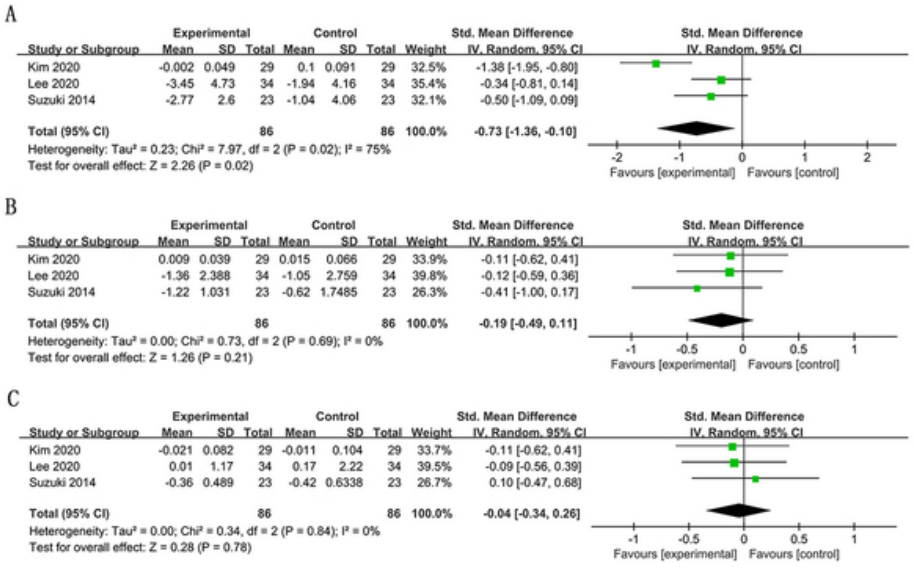

Forest plot of VSC subgroups in short-term (≤ 4 weeks): (A) H2S; (B) CH3S; (C) C2H6S.

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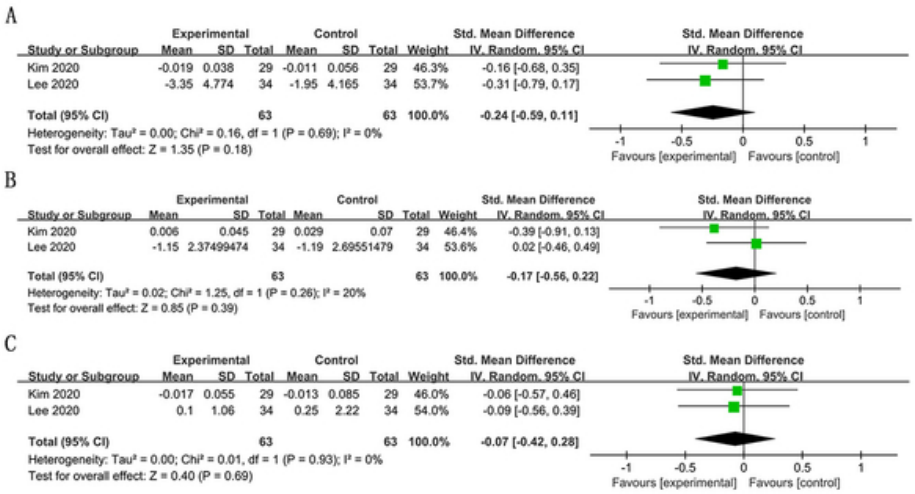

Forest plot of VSC subgroups in long-term (>4 weeks): (A) H2S; (B) CH3S; (C) C2H6S.

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PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2,3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	4
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	4
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	4
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	4
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	5

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	5
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	5
Study characteristics	17	Cite each included study and present its characteristics.	5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	6
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	6
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	6
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7
	23b	Discuss any limitations of the evidence included in the review.	7,8
	23c	Discuss any limitations of the review processes used.	7,8
	23d	Discuss implications of the results for practice, policy, and future research.	8
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	3
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	3
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	3
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	2
Competing interests	26	Declare any competing interests of review authors.	2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	4

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

BMJ Open

The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

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Title page

Title: The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

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[*] Nengwen Huang and Jinjin Li contributed equally to this paper.

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Running title: Probiotic treatment of halitosis

ABSTRACT

Background Halitosis is defined as a foul odor emitted from the oral cavity. Many interventions have been used to control halitosis from mouthwashes to chewing gums. Probiotics have been reported as an alternative method to alleviate halitosis.

Objective The present study aimed to investigate the effect of probiotics on halitosis from a time perspective.

Design and methods This is a meta-analysis study performed in indexed databases up to February 2021. Randomized controlled trials were included that compared probiotics and placebo concerning primary outcomes [organoleptic (OLP) scores and volatile sulfur compounds (VSC) levels)] and secondary outcomes [tongue coating scores (TCS) and plaque index (PI)]. Data extraction and quality assessment were conducted independently by two reviewers. Publication bias and leave-one-out analyses were performed.

Results Standardized mean difference (SMD) and 95% confidence interval (CI) were calculated to synthesize data. The data were sub-grouped and analyzed in the short term (≤4 weeks) and long term (>4 weeks) based on the follow-up time. Seven articles were included in this meta-analysis. Primary outcomes, both OLP scores [SMD =-0.58; 95%CI (-0.87, -0.30), p <0.0001] and VSC levels [SMD =-0.26; 95%CI (-0.51, -0.01), p =0.04], significantly decreased in the probiotics group compared with the placebo group in the short term. However, a significant reduction was observed only in OLP scores [SMD =-0.45; 95%CI (-0.85, -0.04), p =0.03] in the long term. No significant differences were observed in secondary outcomes. There was no risk of publication bias. The leave-one-out analysis confirmed the consistency of the findings.

Conclusions According to the results of this work, it seems that probiotics (e.g., *Lactobacillus salivarius, Lactobacillus reuteri, Streptococcus salivarius, and Weissella cibaria*) may relieve halitosis in the short term (≤4 weeks). The results of the biased assessment, limited data, and heterogeneity of clinical trials included might reduce the reliability of the conclusions.

Strengths and limitations of this study

- This study included larger RCTs involved in halitosis and probiotics.
- The results were rationally analyzed from the follow-up time perspective.
- Subgroup analysis was done to identify the sources of heterogeneity based on the component of VSC.
- The included studies had limited patients.
- Some studies reported the outcomes with different forms, increasing the heterogeneity of the results.

INTRODUCTION

Halitosis, also known as "oral malodor," is typically defined as an unpleasant odor emanating from the oral cavity.[1] As a cause of patient's referral to the dentist, halitosis is the third most common disease, only ranking behind dental caries and periodontal disease.[2] According to an epidemiological study, the prevalence of halitosis is approximately 27.5% in the Chinese population.[3] People have a higher demand for social interactions and attach more importance to their personal image in today's society. Halitosis has a significant impact on both patients' daily work and social activities and may even result in frequent psychological problems such as anxiety, depression, and social isolation.[4] Clinically, halitosis is categorized into genuine halitosis, pseudo-halitosis, and halitophobia.[5] The latter two types are related to psychological conditions. Only genuine halitosis is caused by pathological and physiological factors. It includes intraoral halitosis (IOH) and extraoral halitosis, with the former accounting for 80-90% of the cases.[6]

The main etiologic factor of genuine halitosis is the volatile sulfur compounds (VSC) produced by oral bacteria via complex microbe–substrate and microbe-microbe interactions and putrefaction of organic substrates in the oral cavity, associated with poor oral hygiene, tongue coating, and periodontal disease.[7-10] In particular, hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulphide (C_2H_6S) are considered significant parameters and markers of halitosis.[11] Some microorganisms, such as *Fusobacterium. nucleatum*, *Porphyromonas. gingivalis*, *Prevotella. intermedia*, *Prevotella. nigrescens*, and *Treponema. Denticola*, not only do participate in periodontal diseases, but they also may facilitate the production of VSC metabolism.[12] Some studies using 16S rRNA amplicon sequencing and GC-MS-based metabolite profiling found that the bacterial composition, diversity, and metabolites of the halitosis group were different from those of the control group.[13 14] Therefore, the anaerobic oral condition might play an important role in the development of halitosis. Consequently, regulating the balance of the oral microbiota to reduce VSC levels is an important method to manage oral malodor.

The current treatments for halitosis include mechanical cleaning (scaling and tongue scraping) and chemical therapy (antibiotics, mouthwashes, and other agents).[15 16] However, mechanical therapy is often uncomfortable, even if carried out by the dentist. In addition, although chemical therapy is generally effective for a short time, it is always associated with various side effects, including the emergence of dysbacteriosis and staining of the tongue and tooth.[17-20] Consequently, new methods with fewer side effects are constantly suggested to inhibit oral malodor.

As live microorganisms, probiotics confer benefits to the host when administered in appropriate amounts.[21] Their beneficial effects are primarily related to regulating the local microenvironment through the prevention of adhesion of pathogens and inhibition of growth of pathogens through the production of bacteriocins.[22 23] Recently, probiotics like *Lactobacillus reuteri* and *Bifidobacteria* have been widely used in the oral field.[24] There is a growing body of evidence that the administration of probiotics might affect the composition of oral biofilms. They have also been investigated in the treatment of

periodontal[25 26] and peri-implant diseases,[27 28] caries,[29] oral candidiasis[30 31], and oral mucositis induced by chemo-radiotherapy.[32] Meanwhile, probiotics have also been reported as an alternative strategy to relieve oral malodor.[33-37] However, a previous systematic review showed that probiotic therapy for oral malodor is associated with insufficient evidence for its recommendation.[38] Thus, it is necessary to carry out a focused analysis of the therapeutic effects of probiotics in the treatment of halitosis.

Therefore, this systematic review and meta-analysis was undertaken to investigate the effect of probiotics on managing halitosis from a time perspective to provide some evidence for the administration of probiotics in this field.

METHODS

Patient and public involvement

No patient was involved in the study.

Study design

This systematic review was based on the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered in the PROSPERO (CRD42021227504).[39] According to the PICOS principle, the following focused question was structured: What is the clinical efficacy of probiotics in patients with halitosis when compared with placebo treatment? To answer our research question, we selected clinical trials according to the following study inclusion and exclusion criteria.

Search strategy

A critical electronic search was conducted in the bibliographic databases, mainly including PubMed, EMBASE, Web of Science, and Cochrane Central Register of Controlled Trials up to and including February 2021 to select the published literature. Additionally, gray literature was searched in the database System for Information on Gray literature in European and Google Scholar. The reference lists of the included articles and some related Chinese journals were also searched manually. There was no language restriction.

An initial search strategy was conducted in PubMed with the combination of Medical Subject Headings (Mesh) terms identified by an asterisk symbol (*) and free text words as follows: Probiotic OR Probiotic* OR Probiotic therapy OR Probiotic effect OR Probiotic treatment AND halitosis OR halitosis * OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris. The detailed search strategy for each database is mentioned in supplemental file 1. Endnote X7 was used for electronic title management. First, primary screening was performed independently by two reviewers (NWH and JJL) based on the titles and abstracts. Then, the full-text articles were used to assess the eligibility further. Any disagreement was solved by consulting a third reviewer.

Study inclusion and exclusion criteria

The populations were patients diagnosed with halitosis. The intervention was probiotic therapy, representing the experiment group. The control group was done with a placebo treatment. The considered outcomes were halitosis parameters and other indexes before and after treatment. During the first stage of the study selection, studies meeting the following conditions were considered eligible for this review: 1) study types: randomized controlled clinical trials (RCTs) or randomized controlled cross-over studies; 2) participants: systemically healthy patients diagnosed with halitosis via accepted standards (the organoleptic scores and/or the concentrations of VSC); 3) interventions: evaluating the efficacy of probiotics with placebo, regardless of the probiotics species and the consumption method; 4) control interventions: placebo treatment; 5) clinical data: the measurement values, including halitosis parameters and other indexes before and after treatment. At the second stage of the selection, eligible studies acquired in the first stage were identified according to the following exclusion criteria: 1) *in vitro* and animal studies, letter to the editor, review articles, interviews, meta-analysis; 2) unclear halitosis identification; 3) studies with no completed data obtained even by contacting the authors. 4) interventions included other measures (e.g., studies comparing tongue scraping plus chlorhexidine plus probiotics and tongue scraping plus chlorhexidine).[34]

Halitosis assessment

The primary outcomes were evaluated for organoleptic (OLP) scores and the VSC concentration levels. OLP scores reflecting subjective perception were often treated as the gold standard for diagnosing halitosis clinically and in research.[40 41] The OLP scores were estimated by two or three evaluators (with training and experience in calibrating tests). Subjects closed their mouth for 1 min and then exhaled slowly from their mouth into the evaluator's nose at a distance of 10 cm. The score was evaluated according to a six-point '0–5' scale (Rosenberg scale).[42]

The VSC concentrations measurement is an objective method through using the Halimeter or Oral Chroma with no significant difference.[43] Compared with organoleptic evaluation, VSC measurement is a quantitative variable with high sensitivity and reproducibility.[44-46] Subjects had to keep their mouth closed and stop talking for 5 min before measurements. Halimeter: a beverage straw (fixed and attached to the device) was inserted into the subject's mouth, located at the back of the tongue dorsum. Subjects should keep their mouth slightly open and breathe through the nose. Oral Chroma: Subjects were asked to keep their mouths closed for 30 s with an air-tight syringe. Then, 1 mL of mouth air was extracted from the subject and injected into Oral Chroma to measure the VSC concentration.[47] Then the mean of the results given by the evaluators or machines was used.

Risk of bias

The included studies underwent a quality assessment with the Revised Cochrane risk of bias tool for randomized trials (RoB2).[48] This tool assesses the risk of bias in five domain areas, including randomization process, deviations from intended interventions, missing outcome data, measurement of outcome, and selection of the reported result. Each domain assessed bias following several signaling questions. The overall bias was classified as a high risk of bias, some concerns, or a low risk of bias determined by a validated algorithm. After screening the articles, two reviewers (NWH and JJL) conducted the assessment independently to reach an agreement.

Data extraction

Data were extracted with a researcher-designed data form with the following information: 1) basic information of the included studies (first author's name and the year of publication); 2) study type (RCT); 3) diagnostic criteria for halitosis; 4) characteristics of the participants (sample volume, the age range); 5) treatment (probiotic administration, including the type of bacteria, vehicles, doses, and frequencies); 6) clinical parameters (including the primary and secondary outcomes of final participants); 7) significance and follow-up periods.

Of all these variables, the follow-up periods referred to the duration of probiotic use. If probiotic treatment ceased during the observation period, only the data before ceasing treatment were included. Concerning clinical parameters, OLP scores and VSC concentrations were considered the primary outcomes, directly associated with oral malodor. The secondary outcomes in this review included tongue coating scores (TCS) and plaque index (PI) because they are commonly regarded as halitosis causes.

Statistical analysis

The statistical analysis was performed with Review Manager 5.3 and Stata 12.0. All the data were group-analyzed according to the follow-up time. The time ≤4 weeks was considered the short-term period and the time >4 weeks was considered the long-term period. In one study with three observation periods, the values of 4 weeks were analyzed in the short term to keep consistent with other studies.[49] Study heterogeneity was evaluated using Q statistics and the I^2 test. P value <0.10 was treated as the standard test. When I^2 >50% or p value <0.10, there was significant heterogeneity between the studies.[50-52] Then, subgroup analysis and sensitivity analysis were performed to analyze the sources of heterogeneity. The continuous data on the halitosis parameters of the present studies were expressed with the standardized mean difference (SMD) and 95% CI (confidence interval). A random-effect model was used for analysis. Therefore, the mean difference and standard deviation had to be acquired. If the original text did not provide the related data, the mean difference could be calculated, and the standard deviation was obtained with the formula (r_d =sqrt (r_1^2/n_1 + r_2^2/n_2). The excel sheets in the articles were used to convert the values when provided with median and interquartile.[53 54] Publication bias was performed subjectively by funnel plots and objectively by Egger's tests. In Egger's test, p value <0.05 indicates the presence of publication bias.[55] Sensitivity analysis (leave-one-out method) was conducted to evaluate the consistency of outcomes by sequential omission of individual studies.[56]

RESULTS

Study selection

In total, 238 articles were potentially identified by electronic and manual searches. After eliminating the duplicates, 14 articles were included by screening the titles and abstracts. Then, these studies were evaluated by reading the full texts, and seven articles met the final inclusion criteria (Figure 1).[42 49 57-61]

Study characteristics

Table 1 presents the main characteristics of the included studies. In this review, all the studies were randomized control trials. The number of participants in the studies ranged between 23 and 68, with an age range of 19 to 70. Halitosis was diagnosed with OLP scores and/or VSC concentrations. The probiotics and placebo groups were compared, and the follow-up periods varied from two weeks to 12 weeks.

Table 1 Characteristics of the included studies.

Study	Type	Halitosis criterion	Subjects Age	Clinical parameters	Probiotics Administration (Vehicle, strains and frequency)	Follow-up
Mousquer et al. (2020)	RCT Placebo-double masked, parallel	OLP score≥1	29 ≥18	OLP VSC TCS	A gum including 1 billion colony forming units (CFU) Lactobacillus salivarius G60 taken twice per day	Baseline 2 weeks
Lee et al. (2020)	RCT Placebo-double blind parallel	VSC≥1.5ng/10 mL	68 20-39	OLP VSC (H_2S, CH_3S, C_2H_6S)	An 800-mg tablet contained 1.0×10^8 CFU/g Weissella cibaria taken once per day	Baseline 4 weeks 8weeks
He et al. (2020)	RCT Placebo-double blind parallel	OLP score ≥2 VSC ≥150ppb	28 23-44	OLP VSC TCS PI	A tablet containing 1×10^9 CFU Streptococcus salivarius K12 taken twice per day	Baseline 4 weeks
Keller et al. (2012)	RCT Placebo-double blind cross-over	OLP score>1	25 19-25	OLP VSC	A chewing gum containing Lactobacillus reuteri DSM 17938 and Lactobacillus reuteri ATCC PTA 5289 -both with a concentration of 1×10^8 CFU taken twice per day	Baseline 2 weeks
Suzuki et al. (2014)	RCT Double-blind placebo-controlled Cross-over	OLP score≥1.5	23 22-67	OLP VSC (H_2S, CH_3S, C_2H_6S) PI TCS	A tablet containing 6.7×10^8 CFU Lactobacillus salivarius WB21 and 280mg xylitol taken 3 times per day	Baseline 2 weeks
Penala et al. (2016)	RCT Placebo-double blind parallel	OLP score >2	29 25-59	OLP PI	A capsule mixture included Lactobacillus salivarius (2×10^9 CFU) and Lactobacillus reuteri (2×10^9 CFU) dissolved into 10ml distilled water to rinse for 1min, daily twice	Baseline 4 weeks 12 weeks
Kim et al. (2020)	RCT Placebo-double blind parallel	OLP score≥2 VSC≥0.15ng/ ml	58 20-70	VSC (H_2S, CH_3S, C_2H_6S) OLP	A bag of powder mixture included Weissella. cibaria CMU (1.0×10^8 CFU) melted in the mouth once per day	Baseline 2 weeks 4 weeks 8weeks

*RCT: randomized controlled trials; OLP: organoleptic; VSC: volatile sulfur compounds; TCS: tongue coating scores; CFU: colony forming units; H_2S: hydrogen sulfide; CH_3S: methyl mercaptan; C_2H_6S: methanethiol; PI: plaque index

Risk of bias

The bias estimation results showed that one study had a low risk of bias, one had a high risk, and five showed some concerns. The reason for a high risk of bias was the incomplete outcome data of the OLP scores. Five articles were identified as some concerns because there were many uncertain factors in their full texts. Figure 2 presents concrete data on the risk of bias.

Study outcomes

Primary outcomes

Concerning OLP, studies by Keller et al. (2012) and Penala et al. (2016) reported a significant decrease in the probiotic group compared to the placebo group after treatment ($p < 0.05$).[58 59] In the study by Lee et al. (2020) involving different follow-up periods, OLP scores decreased significantly in the test groups at four weeks ($p = 0.002$) but not eight weeks ($p = 0.188$) compared to the baseline.[60] Additionally, the results of the other four studies indicated that the OLP scores did not differ between the two groups.

Concerning VSC, six articles determined VSC concentrations, with three studies detecting the p values of VSC and subgroups (H_2S, CH_3SH, and C_2H_6S).[49 57 60] According to the results, only two studies[57 60] reported a significant improvement in VSC levels in experimental groups versus placebo groups.

Secondary outcomes

Concerning TCS, three studies evaluated the changes between the probiotic and placebo groups at four weeks.[42 57 61] Although a reduced tendency was observed after treatment compared with baseline p values, there was no significant difference between the two groups.

Concerning PI, in the three studies involved,[42 57 59] only one study showed a significant reduction in PI in the experimental group compared with the controlled group at 12 weeks.[59]

Quantitative synthesis

A meta-analysis was performed including studies with similar clinical parameters of OLP, VSC, TCS, and PI, according to the follow-up time. Although the detection methods of VSC were different, both of the devices exhibited similar sensitivity and specificity in the detection of halitosis.[43] Therefore, we analyzed these values together. Considering the limitations of the included studies and follow-up time, the pooled estimation of TCS and PI was only performed in the short term.

In the short term, the OLP scores significantly decreased in the probiotic group compared to the control group [SMD =-0.58; 95% CI (-0.87, -0.30), $p < 0.0001$] (Figure 3). A similar result was observed in VSC [SMD =-0.26; 95% CI (-0.51, -0.01), $p = 0.04$] and H_2S levels [SMD =-0.73; 95% CI (-1.36, -0.10), $p = 0.02$]. Other items (TCS, PI, CH_3S, and C_2H_6S) were not significantly different between the experimental and control groups. The heterogeneity of each outcome was low ($I^2 < 50\%$) except for H_2S levels ($I^2 = 75\%$) (Figures 3 and 4).

In the long term, there was a significant improvement in OLP scores in the experimental group [SMD =-0.45; 95% CI (-0.85, -0.04), $p = 0.03$] (Figure 5). The results failed to show a significant difference in VSC concentrations and their subgroups levels (Figures 5 and 6). The heterogeneity of VSC concentrations was substantial ($I^2 = 58\%$).

Publication bias

In this systematic review and meta-analysis, we found no evidence of publication bias by the result of the funnel plots and Egger's tests ($p > 0.05$) (supplementary file 2-Figures S1-S5).

Sensitivity analysis

Sensitivity analysis (leave-one-out method) revealed no significant change in the pooled estimation when excluding any individual study (supplementary file 2-Figures S6-S9).

DISCUSSION

Summary of the findings

This meta-analysis demonstrated that probiotics significantly reduced the OLP scores compared with the placebo group regardless of the duration of observation, confirming the benefits of probiotics for halitosis treatment. The probiotics group exhibited a significant reduction in VSC concentrations in the short term (≤4 weeks), with no noticeable difference in the long term (>4 weeks). Meta-analyses were also performed in the subgroups of H_2S, CH_3SH, and C_2H_6S to assess the concrete difference in VSC levels. The results showed that only H_2S levels reduced noticeably in the short term when the probiotic treatment was administered. As for TCS and PI, the results showed no significant differences between the experimental and placebo groups in the short term. There was no risk of publication bias. The sensitivity analysis confirmed the consistency of the findings.

Outcomes comparison and possible mechanisms

Concerning the primary outcomes, in the included articles, the pooled estimation of OLP scores and VSC concentrations were in favor of probiotic therapy rather than placebo in the short term.[42 49 57-59 61] The biological mechanisms may be related to the interaction between probiotics and oral microbiota. According to present studies, probiotic therapy reduces odorous compound levels by inhibiting the decomposition of amino acids and proteins by anaerobic bacteria.[7 62] The significantly lower VSC levels under probiotic treatment in the short term might indicate a decrease in anaerobic bacteria activity. In contrast to our findings, a previous study indicated that it could not confirm the effect of probiotics on reducing VSC in the short term.[38] The number of included articles may result in this difference. However, when comes to the results in the long term, only OLP scores showed a significant reduction rather than VSC concentrations. Oral microbiota contains not only VSC-producing bacteria but also other bacteria capable of producing other oral malodor compounds (e.g., indoles, skatole, pyridine, picolines, and polyamines).[63] The underlying mechanisms of the difference may result from the variation and abundance of microbiota community over time, which in turn affects the efficacy of probiotics, especially VSC concentration levels.[35 49 61] Therefore, the no significant effect on VSC concentrations in the long term may be due to probiotics' inhibition effect on those other bacteria. Therefore, the data about microorganisms changing in different periods are significant for the evaluation of probiotic effects. However, from the present studies, insufficient data in the included studies, the differences in detection methods, bacterial species, and heterogeneity of clinical trials limited the microorganism statistical analysis in this review.

Meanwhile, we found that the short-term outcome of H_2S concentration change other than CH_3SH, and C_2H_6S was consistent with the total VSC. This might be related to differences in the function of probiotics and in the number and species of bacteria associated with each VSC reduction.[12 35 64]

Additionally, the regular VSC measurement device was reported to be more sensitive towards H_2S than CH_3SH and C_2H_6S,[46] which may also account for the above result.

Regarding the secondary outcomes, based on the present meta-analysis, there was no significant difference between the experimental and placebo groups on secondary outcomes during the observation time. The possible reason was the short observation time in the included studies, as one study included in the analysis showed a significant improvement in PI at 12 weeks. [59] Tongue coating and periodontitis are often regarded as the leading causes of halitosis. [42 65] However, in an original article, the TCS and PI showed a pronounced decline after using probiotics compared with the baseline, with no decrease in the placebo group.[61] This phenomenon might be related to the type of probiotics, some of which were reported to boost salivary flow by interacting with the oral microbiota.[66]

From the current studies, there are two main types of studies on the effect of probiotics on halitosis, one is to observe the effect during continuous use of probiotics and the other is to observe the effect at follow-up after stopping the use of probiotics. A recently published study indicated that no significance of probiotic effect was found, different from ours. The reason for the difference may be that this study analyzed the collected follow-up data after stopping using probiotics for at least 2 weeks.[67] Therefore, more clinical and systematic studies are needed to explore and verify the probiotic effect on the management of halitosis in future research.

Limitation

There were several limitations in the present study throughout the whole review process. First, although both electronic and hand searches were conducted in four primary databases, it was impossible to retrieve all the relevant studies. Second, the number of eligible studies and included subjects was small. Third, all included interventions differed in the species of probiotics, the doses used, frequencies, and administration periods. A subgroup analysis was necessary to evaluate the source of efficacy concerning the probiotic species, but the small size of the included articles prevented further analysis. All these factors would inevitably affect the accuracy of outcomes. Fourth, the detection methods of VSC were different. Although there is no significant difference between them, the combined analysis might still affect the reliability of the results. Fifth, in some included studies, the primary outcomes were presented in different forms, such as percentages or range interquartile. Finally, some important parameters, including the microorganism species and changes, were not presented completely in some articles. The absence of partial original data or the differences caused by data conversion equally impaired the final results though many methods were tried to reduce the bias.

CONCLUSION

The present systematic review and meta-analysis indicated that probiotics (e.g., *Lactobacillus salivarius, Lactobacillus reuters, Streptococcus salivarius, and Weissella cibaria*) may ease halitosis by reducing the VSC concentration levels in the short term, but there is no significant effect on the major cause of halitosis such as plaque and tongue coating. Considering the heterogeneity of clinical trials included and the small sample size, more high-quality random clinical trials are required in the future to verify the results and to evidence the usefulness of probiotics in the management of halitosis.

Contributors

NWH and JJL collected and analyzed data, and drafted the manuscript; NWH and XHQ helped with the literature searching and statistical analysis; YZW and CZW provided help in the literature searching and figure revises; XHQ and YKL critically reviewed the manuscript. LJL designed the experiment and critically reviewed the manuscript. NWH and JJL contributed equally to this paper. All authors agree to be accountable for the study.

Competing interests

None declared.

Ethics approval statement

No applicable.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1: Flow diagram of literature search strategy and inclusion, exclusion criteria.
Figure 2: Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represents a high risk of bias.
Figure 3: Forest plot of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC

concentrations; (C) TCS; (D) PI.

Figure 4: Forest plot of VSC subgroups in short-term (≤4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

Figure 5: Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

Figure 6: Forest plot of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

Figure S1: Funnel plot of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

Figure S2: Funnel plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

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Figure S4: Funnel plot of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

Figure S5: The result of Egger's test in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI; (E) H_2S; (F) CH_3S; (G) C_2H_6S.

Figure S6: Sensitivity analysis of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

Figure S7: Sensitivity analysis of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

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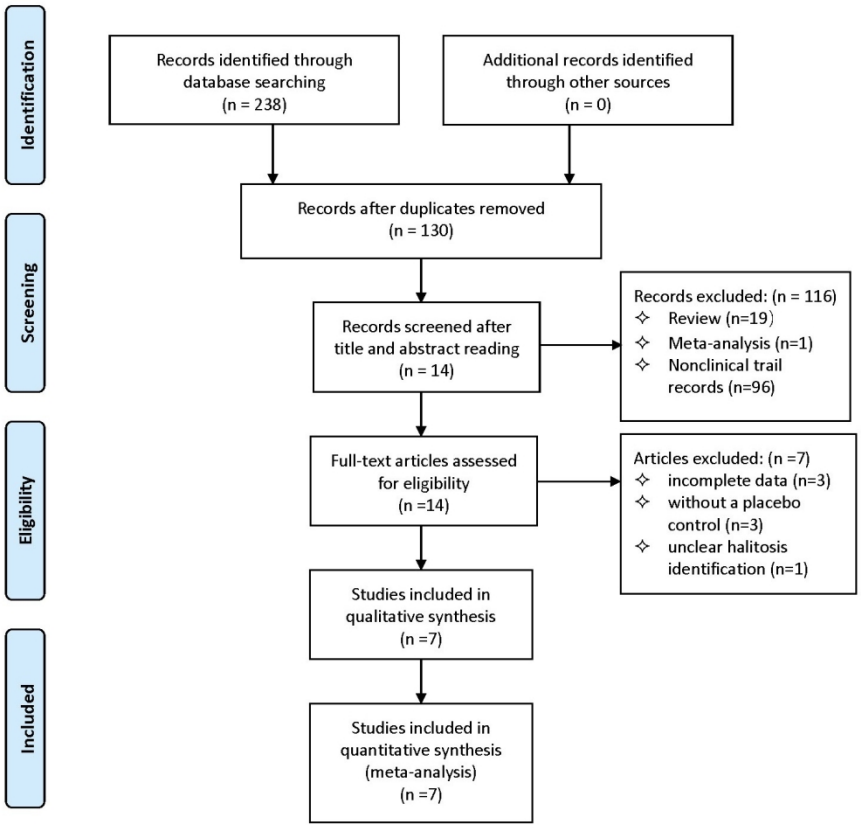

Flow diagram of literature search strategy and inclusion, exclusion criteria.

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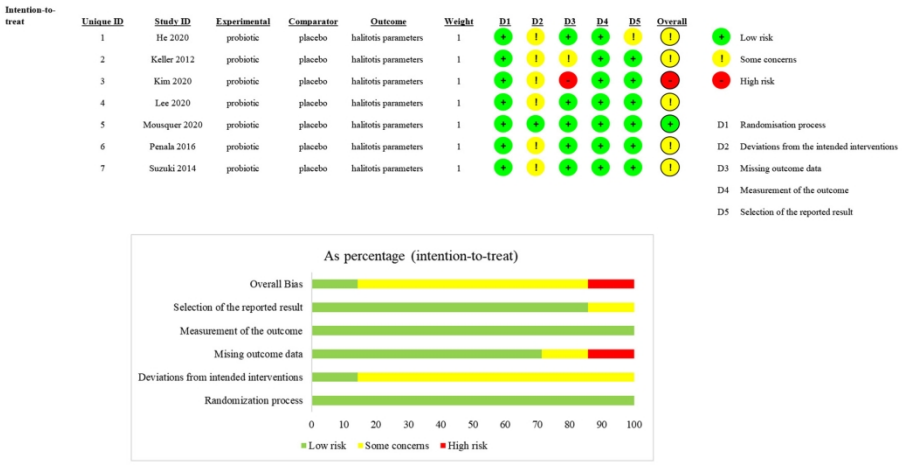

Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represent high risk of bias.

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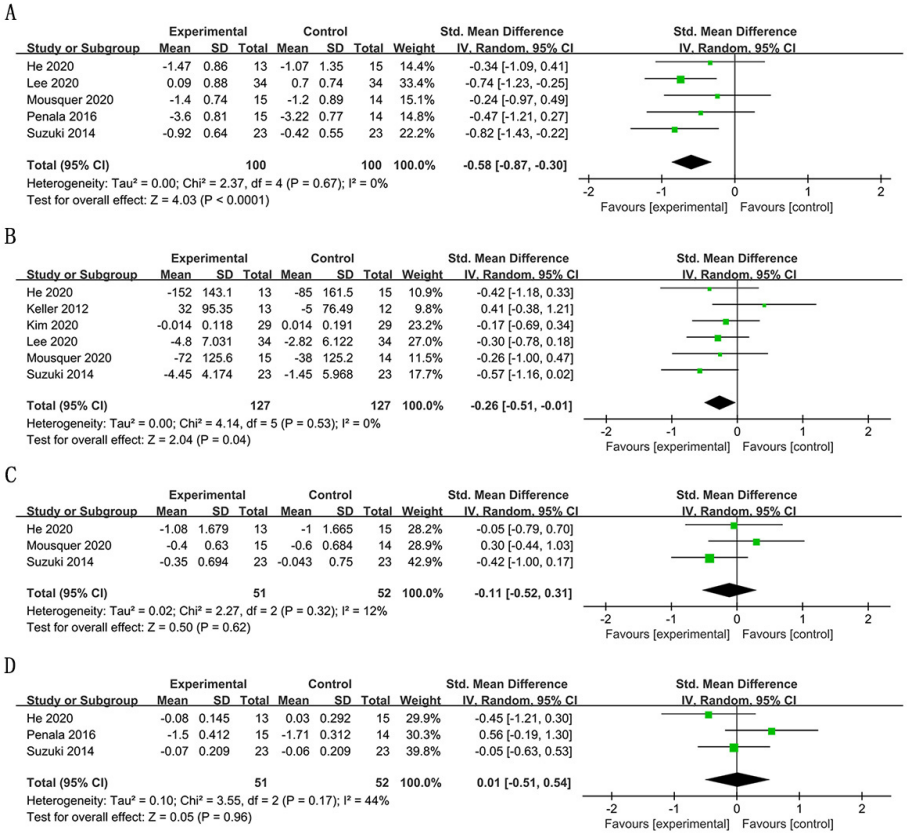

Forest plot of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

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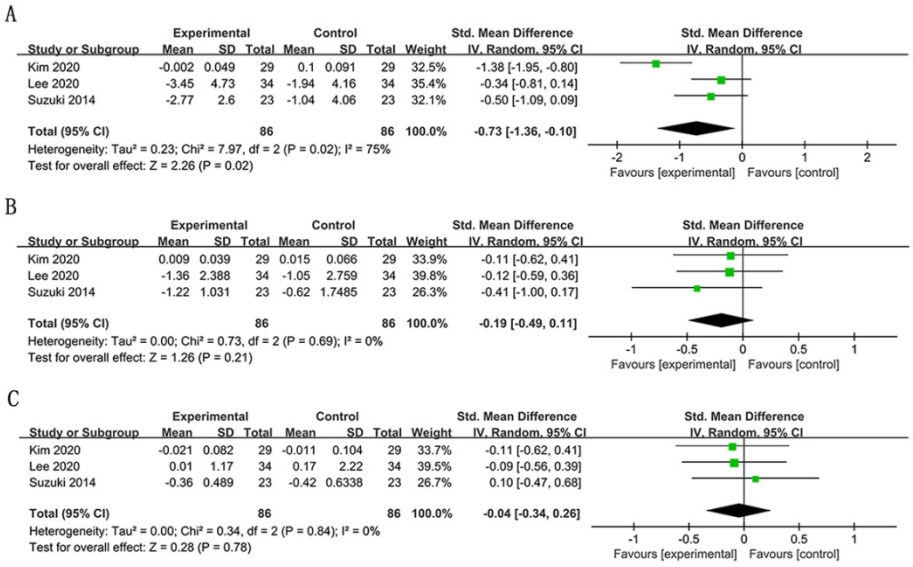

Forest plot of VSC subgroups in short-term (≤4 weeks): (A) H2S; (B) CH3S; (C) C2H6S.

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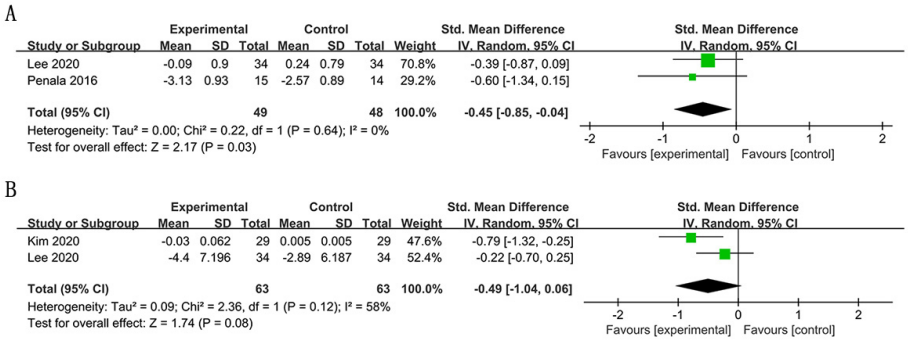
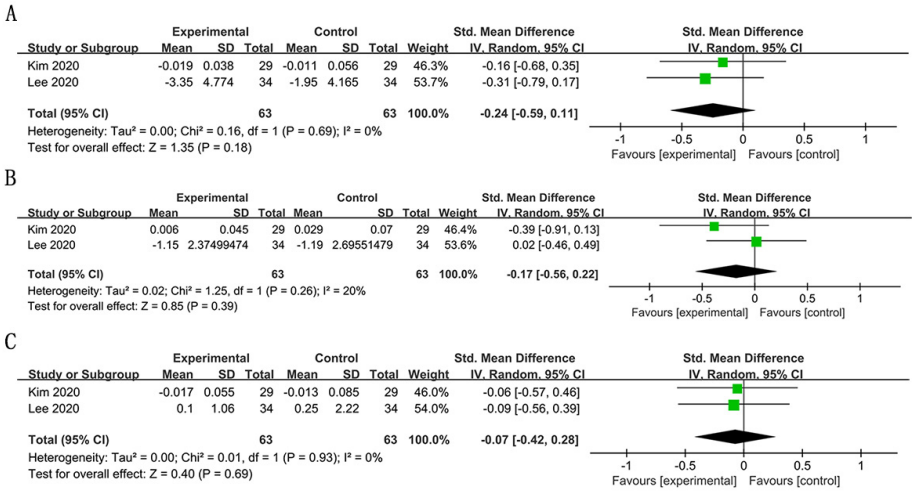


Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

108x45mm (300 x 300 DPI)



Forest plot of VSC subgroups in long-term (>4 weeks): (A) H2S; (B) CH3S; (C) C2H6S.

108x61mm (300 x 300 DPI)

Supplementary file 1

1. PubMed

Search	Query	Items found
#1	((((((Probiotic[Text Word]) OR (Probiotic[MeSH Terms])) OR (Probiotic therapy[Text Word])) OR (Probiotic effect[Text Word])) OR (Probiotic treatment[Text Word])))	27215
#2	(((((((halitosis[Text Word]) OR (halitosis[MeSH Terms])) OR (malodor[Text Word])) OR (oral malodor[Text Word])) OR (malodour[Text Word])) OR (bad breath[Text Word])) OR (fetor oris[Text Word])))	2788
#3	#1 and #2	68

2. Web of science

Search	Query	Items found
#1	(((TS=(Probiotic)) OR TS=(Probiotic therapy)) OR TS=(Probiotic effect)) OR TS=(Probiotic treatment)	28458
#2	(((((TS=(halitosis)) OR TS=(malodor)) OR TS=(oral malodor)) OR TS=(malodour)) OR TS=(bad breath)) OR TS=(fetor oris)	3018
#3	#1 and #2	42

3. Embase ovid search strategy

Search	Query	Items found
#1	((Probiotic or Probiotic or Probiotic therapy or Probiotic effect or Probiotic treatment) and (halitosis or halitosis or malodor or oral malodor or malodour or bad breath or fetor oris)).af.	119

4. Cochrane Central Register of Controlled Trials (CENTRAL) search strategy

Search	Query	Items found
#1	MeSH descriptor: [Halitosis] explode all trees	236
#2	(halitosis):ti,ab,kw (Word variations have been searched)	573
#3	(malodor):ti,ab,kw (Word variations have been searched)	399
#4	(oral malodor):ti,ab,kw (Word variations have been searched)	300
#5	(malodour):ti,ab,kw (Word variations have been searched)	399
#6	(bad breath):ti,ab,kw (Word variations have been searched)	258
#7	(fetor oris):ti,ab,kw (Word variations have been searched)	0
#8	#1 or #2 or #3 or #4 or #5 or #6 or #7	996
#9	MeSH descriptor: [Probiotics] explode all trees	2571

#10	(Probiotic):ti,ab,kw (Word variations have been searched)	8519
#11	(Probiotic therapy):ti,ab,kw (Word variations have been searched)	3834
#12	(Probiotic effect):ti,ab,kw (Word variations have been searched)	6398
#13	(Probiotic treatment):ti,ab,kw (Word variations have been searched)	4579
#14	#9 or #10 or #11 or #12 or #13	8603
#15	#8 and #14	8

5. Gray literature in European and Google Scholar

Search	**Query**	**Items found**
#1	Probiotic OR Probiotic therapy OR Probiotic effect OR Probiotic treatment AND halitosis OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris	1

Supplementary file 2

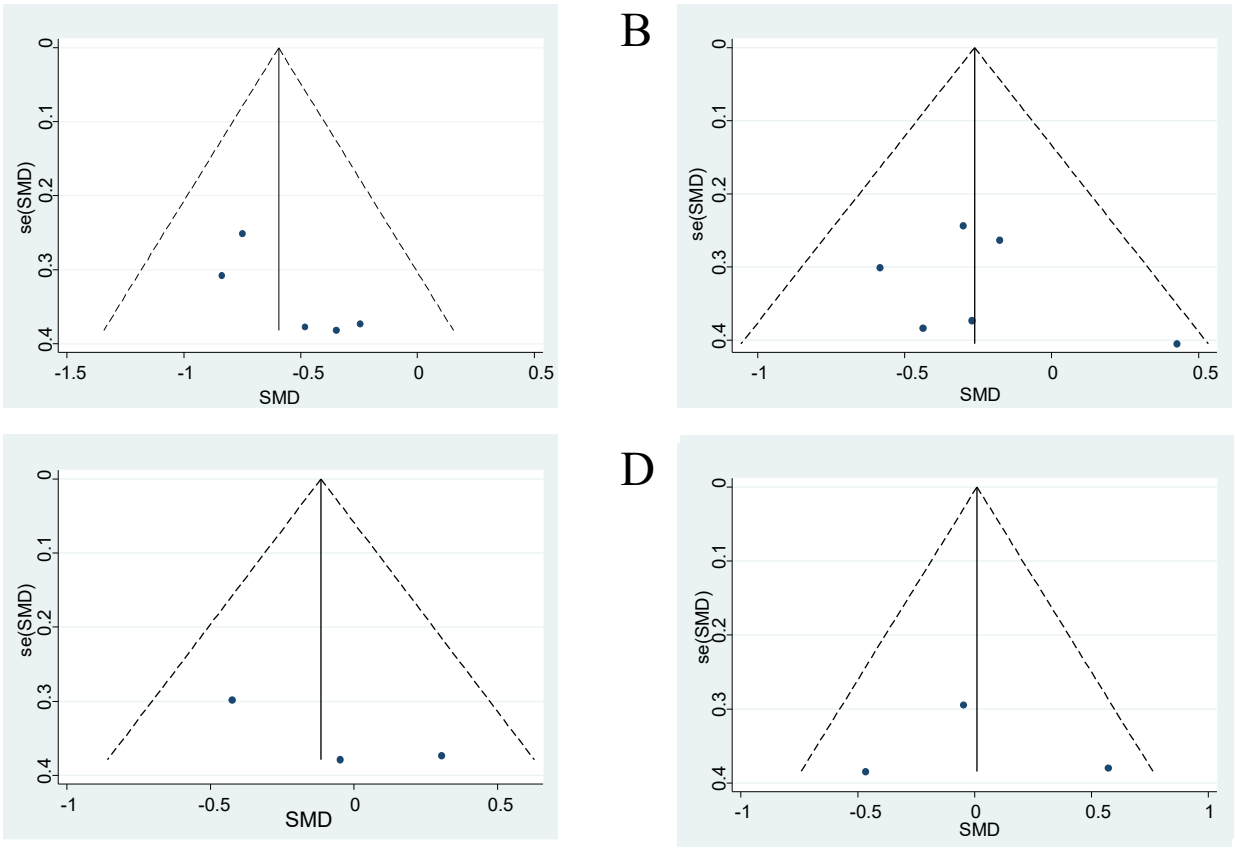


Figure S1: Funnel plot of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

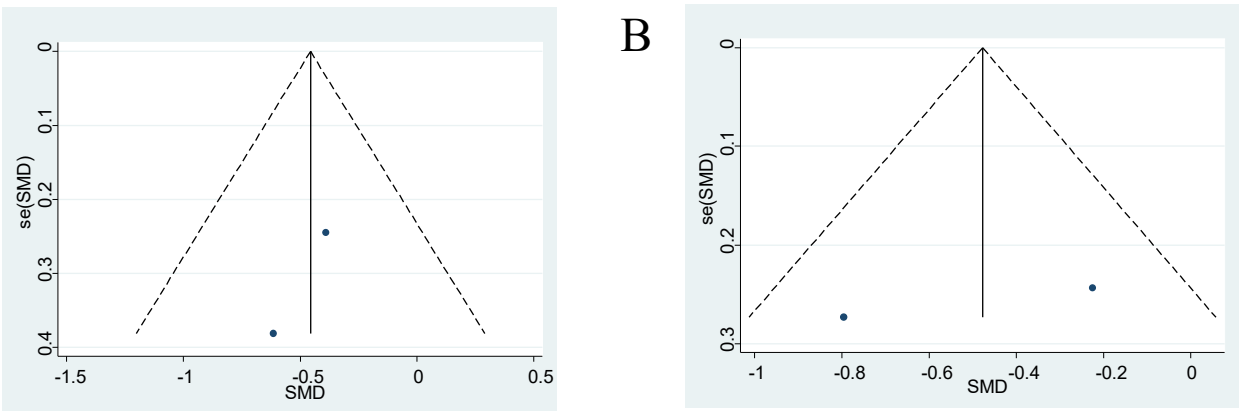


Figure S2: Funnel plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

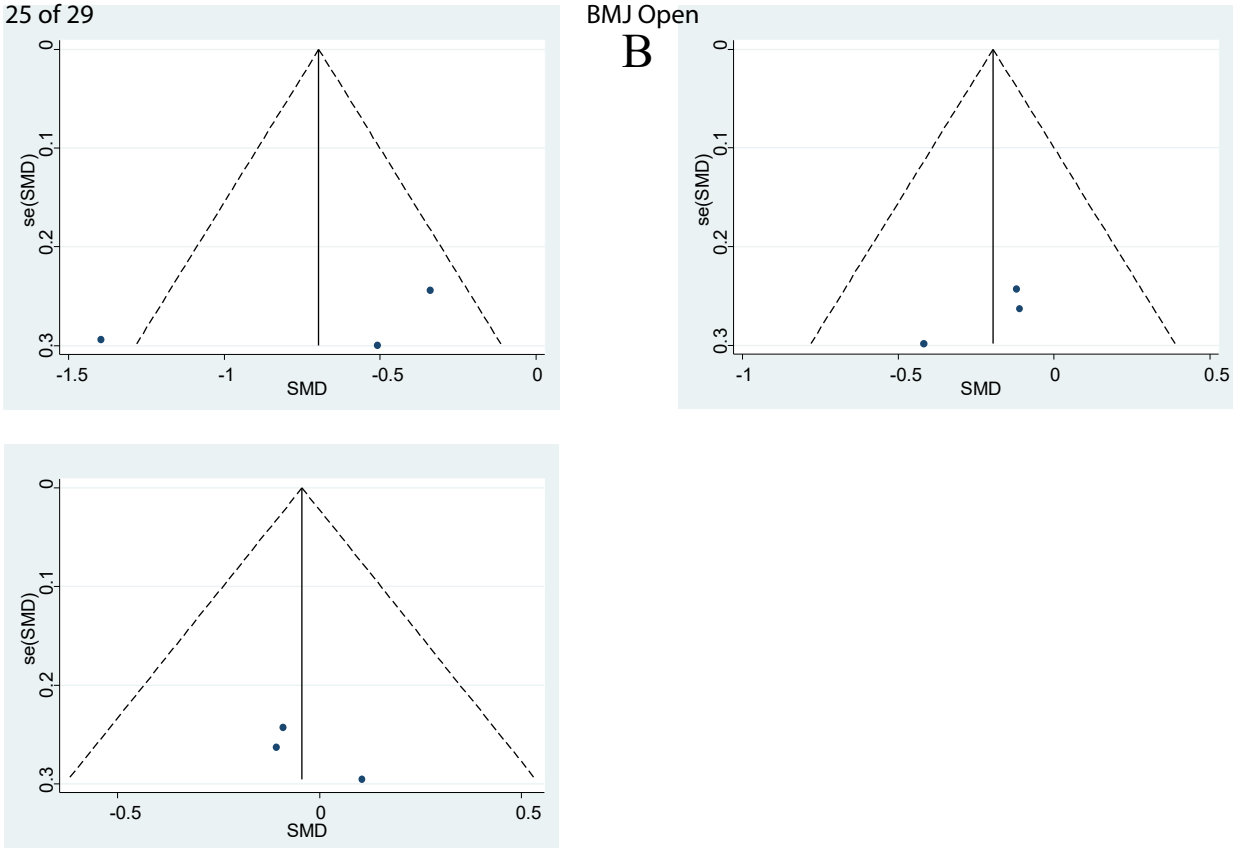


Figure S3: Funnel plot of VSC subgroups in short-term (≤4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

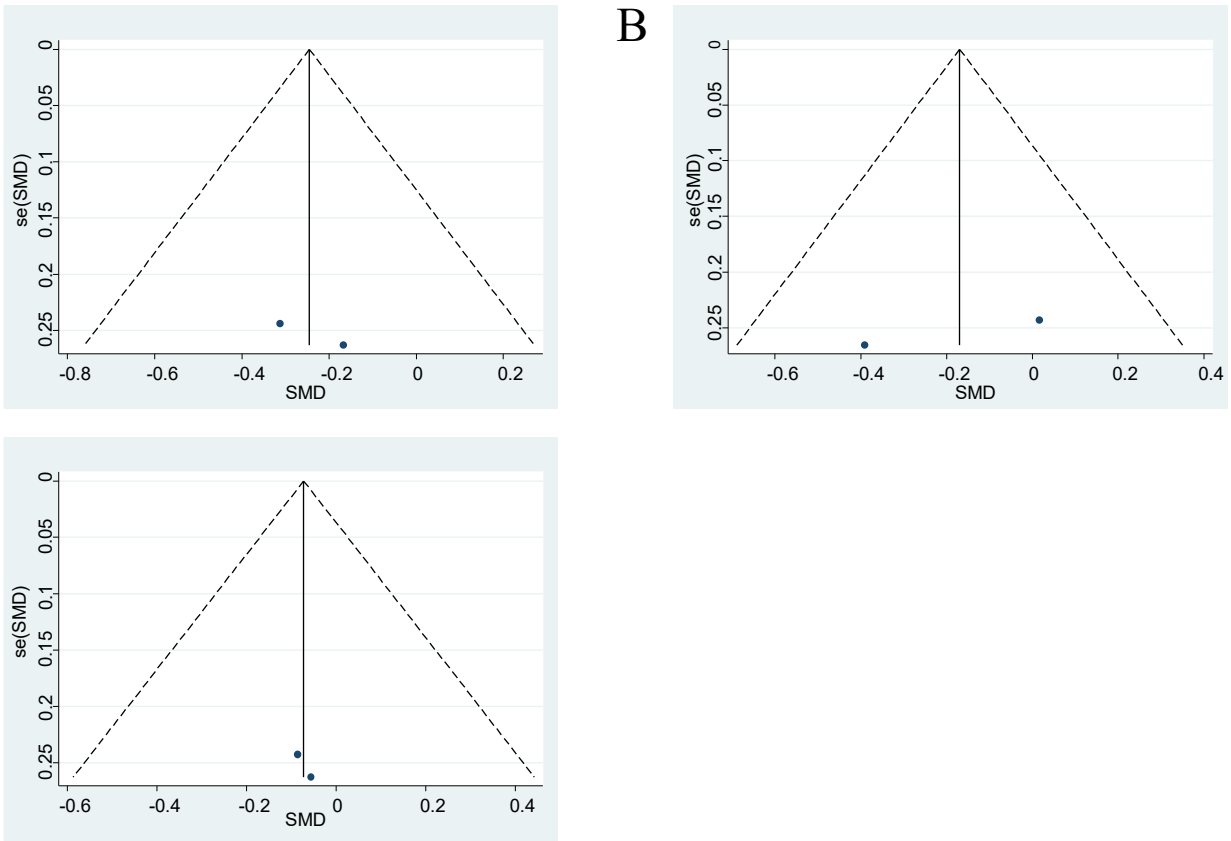


Figure S4: Funnel plot of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

A

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-1.654658	.4354301	-3.80	0.032	-3.04039	-.2689248
bias	3.312431	1.341309	2.47	0.090	-.9562116	7.581074

B

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.6982326	.6453982	-1.08	0.340	-2.490145	1.09368
bias	1.432804	2.079837	0.69	0.529	-4.341748	7.207357

C

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-2.485861	1.282746	-1.94	0.303	-18.7847	13.81297
bias	6.934603	3.728761	1.86	0.314	-40.4438	54.313

D

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.2878511	3.056969	-0.09	0.940	-39.13033	38.55463
bias	.8711259	8.860095	0.10	0.938	-111.7071	113.4493

E

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	2.223943	3.837093	0.58	0.666	-46.53094	50.97883
bias	-10.63929	13.91572	-0.76	0.584	-187.4552	166.1766

F

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	1.252312	.6648476	1.88	0.311	-7.195378	9.700003
bias	-5.480127	2.507946	-2.19	0.273	-37.3466	26.38635

G

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-1.041425	.5483955	-1.90	0.309	-8.00945	5.926601
bias	3.788236	2.074839	1.83	0.319	-22.57509	30.15157

Figure S5: The result of Egger's test in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI; (E) H_2S; (F) CH_3S; (G) C_2H_6S.

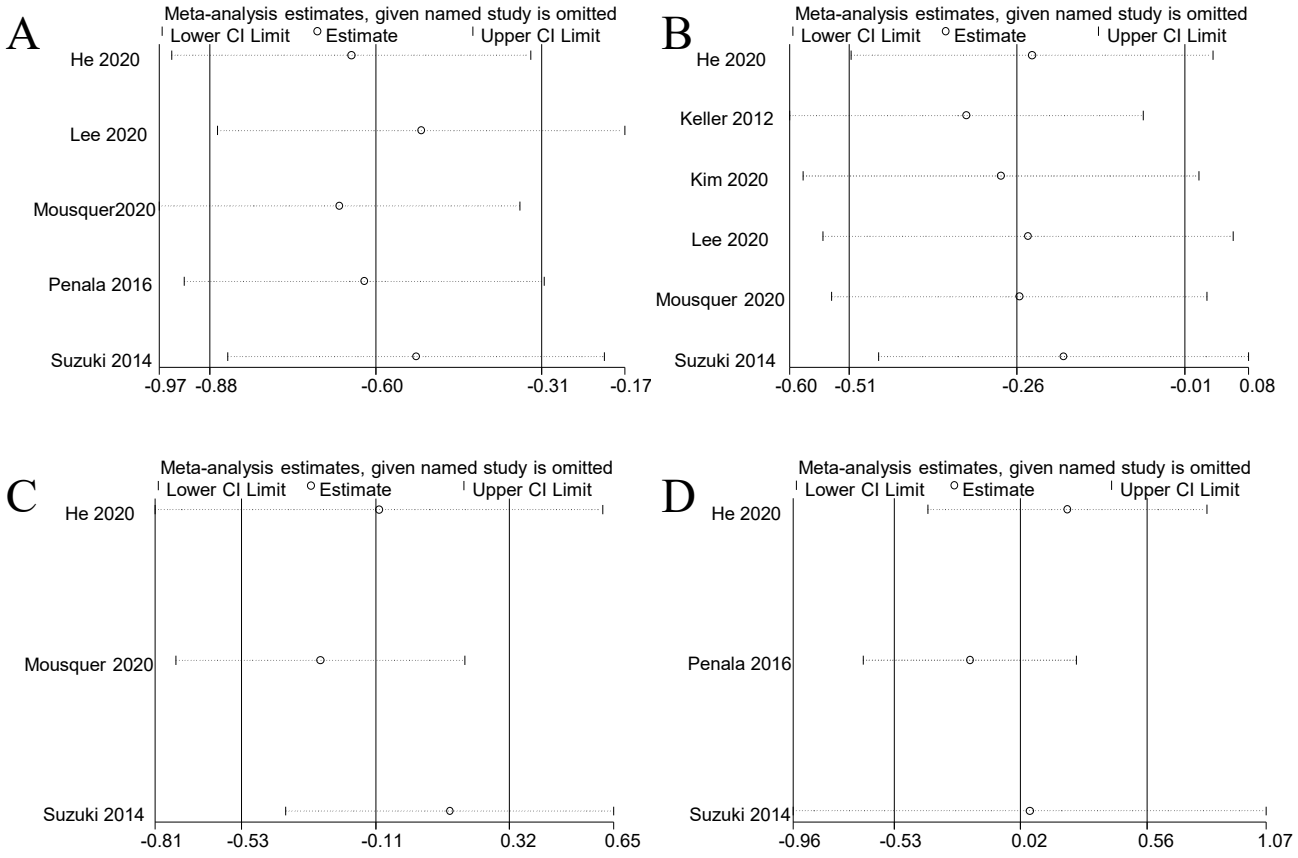


Figure S6: Sensitivity analysis of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

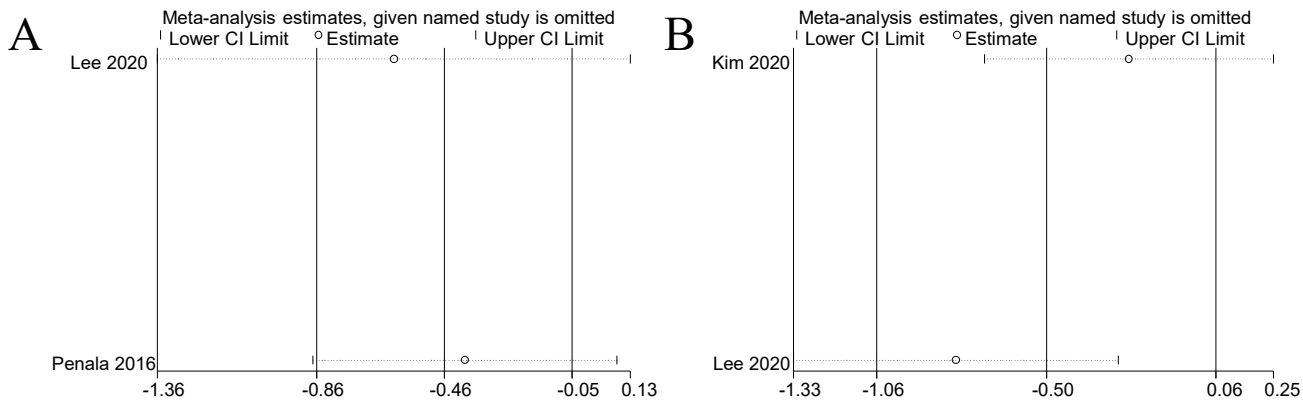


Figure S7: Sensitivity analysis of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

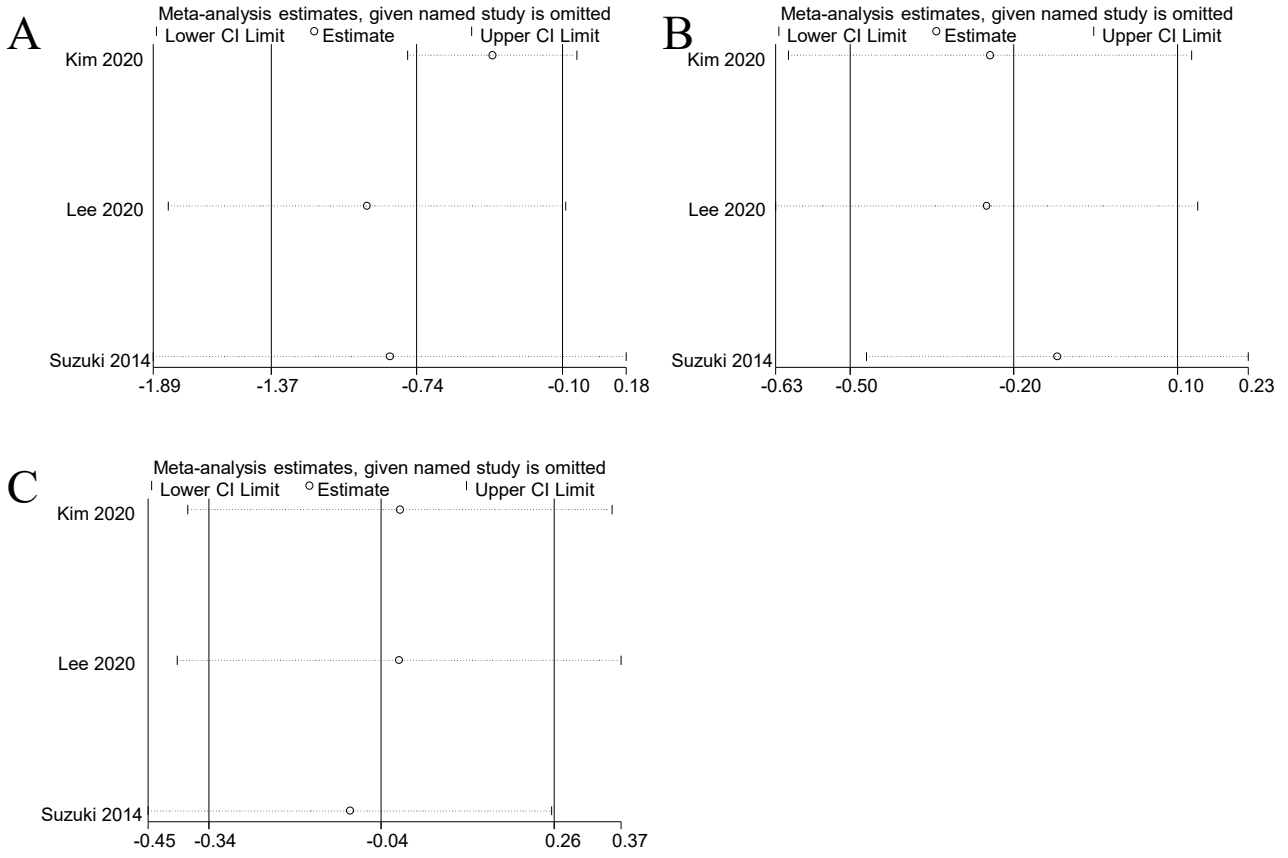


Figure S8: Sensitivity analysis of VSC subgroups in short-term (≤4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

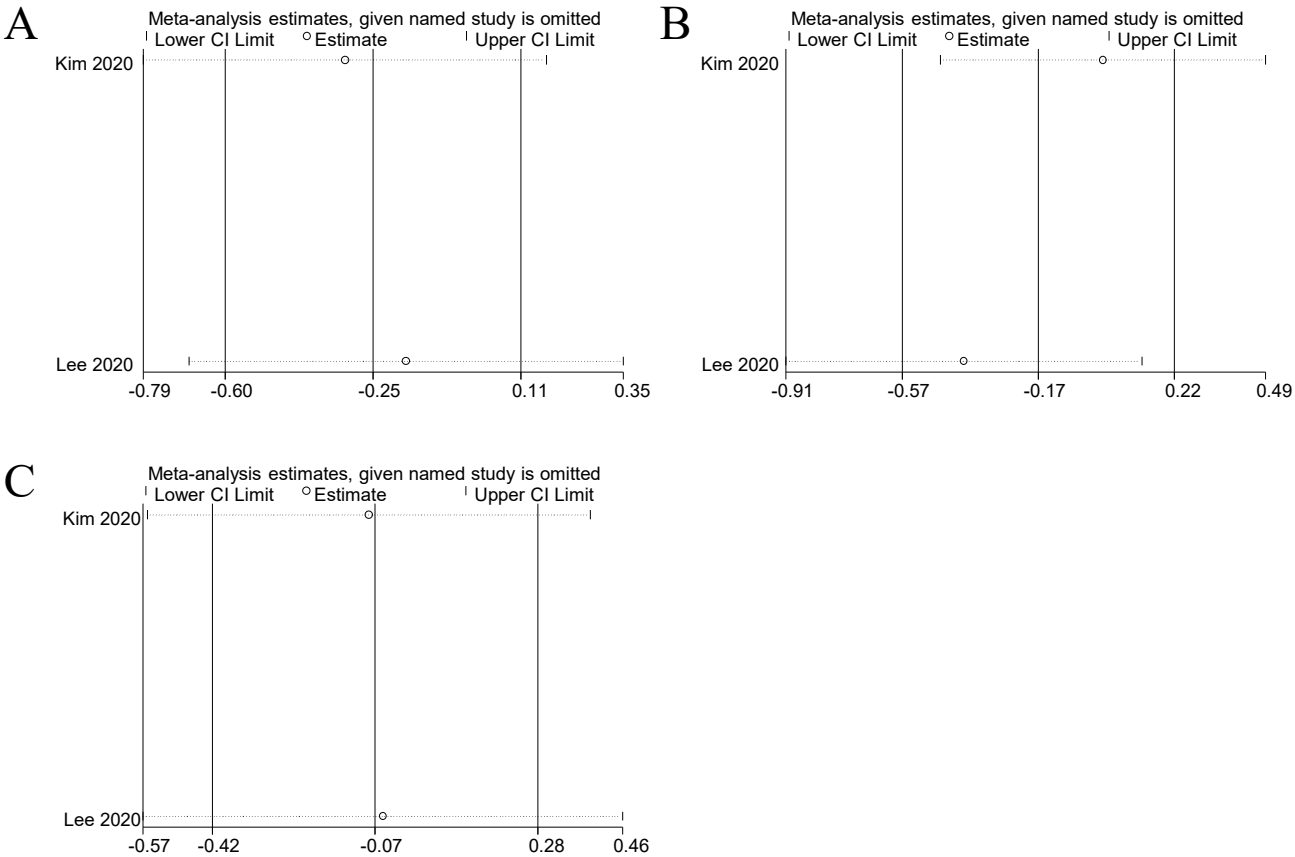


Figure S9: Sensitivity analysis of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2,3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	4
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	4
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	4
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	4
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	5

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	5
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	5
Study characteristics	17	Cite each included study and present its characteristics.	5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	6
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	6
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	6
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7
	23b	Discuss any limitations of the evidence included in the review.	7,8
	23c	Discuss any limitations of the review processes used.	7,8
	23d	Discuss implications of the results for practice, policy, and future research.	8
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	3
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	3
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	3
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	2
Competing interests	26	Declare any competing interests of review authors.	2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	4

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

BMJ Open

The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

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Primary Subject Heading:	Dentistry and oral medicine
Secondary Subject Heading:	Dentistry and oral medicine
Keywords:	Microbiology < PATHOLOGY, Infectious diseases & infestations < DERMATOLOGY, Public health < INFECTIOUS DISEASES

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Title page

Title: The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

Nengwen Huang[1*] Jinjin Li[1*] Xianghe Qiao[1] Yongzhi Wu[1] Yunkun Liu[1] Chenzhou Wu[1] Longjiang Li[1]

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[*] Nengwen Huang and Jinjin Li contributed equally to this paper.

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Running title: Probiotic treatment of halitosis

ABSTRACT

Background Halitosis is defined as a foul odor emitted from the oral cavity. Many interventions have been used to control halitosis from mouthwashes to chewing gums. Probiotics have been reported as an alternative method to alleviate halitosis.

Objective The present study aimed to investigate the effect of probiotics on halitosis from a time perspective.

Design and methods This is a meta-analysis study performed in indexed databases up to February 2021. Randomized controlled trials were included that compared probiotics and placebo concerning primary outcomes [organoleptic (OLP) scores and volatile sulfur compounds (VSC) levels)] and secondary outcomes [tongue coating scores (TCS) and plaque index (PI)]. Data extraction and quality assessment were conducted independently by two reviewers. Publication bias and leave-one-out analyses were performed.

Results Standardized mean difference (SMD) and 95% confidence interval (CI) were calculated to synthesize data. The data was sub-grouped and analyzed in the short term (≤4 weeks) and long term (>4 weeks) based on the follow-up time. Seven articles were included in this meta-analysis. Primary outcomes, both OLP scores [SMD =-0.58; 95%CI (-0.87, -0.30), p <0.0001] and VSC levels [SMD =-0.26; 95%CI (-0.51, -0.01), p =0.04], significantly decreased in the probiotics group compared with the placebo group in the short term. However, a significant reduction was observed only in OLP scores [SMD =-0.45; 95%CI (-0.85, -0.04), p =0.03] in the long term. No significant differences were observed in secondary outcomes. There was no evidence of publication bias. The leave-one-out analysis confirmed that the pooled estimate was stable.

Conclusions According to the results of this work, it seems that probiotics (e.g., *Lactobacillus salivarius, Lactobacillus reuteri, Streptococcus salivarius, and Weissella cibaria*) may relieve halitosis in the short term (≤4 weeks). The results of the biased assessment, limited data, and heterogeneity of clinical trials included might reduce the reliability of the conclusions.

Strengths and limitations of this study

- This study included larger RCTs involved in halitosis and probiotics.
- The results were rationally analyzed from the follow-up time perspective.
- Subgroup analysis was done to identify the sources of heterogeneity based on the component of VSC.
- The included studies had limited patients.
- Some studies reported the outcomes with different forms, increasing the heterogeneity of the results.

INTRODUCTION

Halitosis, also known as "oral malodor," is typically defined as an unpleasant odor emanating from the oral cavity.[1] As a cause of patient's referral to the dentist, halitosis is the third most common disease, only ranking behind dental caries and periodontal disease.[2] According to an epidemiological study, the prevalence of halitosis is approximately 27.5% in the Chinese population.[3] People have a higher demand for social interactions and attach more importance to their personal image in today's society. Halitosis has a significant impact on both patients' daily work and social activities and may even result in frequent psychological problems such as anxiety, depression, and social isolation.[4] Clinically, halitosis is categorized into genuine halitosis, pseudo-halitosis, and halitophobia.[5] The latter two types are related to psychological conditions. Only genuine halitosis is caused by pathological and physiological factors. It includes intraoral halitosis (IOH) and extraoral halitosis, with the former accounting for 80-90% of the cases.[6]

The main etiologic factor of genuine halitosis is the volatile sulfur compounds (VSC) produced by oral bacteria via complex microbe–substrate and microbe-microbe interactions and putrefaction of organic substrates in the oral cavity, associated with poor oral hygiene, tongue coating, and periodontal disease.[7-10] In particular, hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulphide (C_2H_6S) are considered significant parameters and markers of halitosis.[11] Some microorganisms, such as *Fusobacterium. nucleatum*, *Porphyromonas. gingivalis*, *Prevotella. intermedia*, *Prevotella. nigrescens*, and *Treponema. Denticola*, not only do participate in periodontal diseases, but they also may facilitate the production of VSC metabolism.[12] Some studies using 16S rRNA amplicon sequencing and GC-MS-based metabolite profiling found that the bacterial composition, diversity, and metabolites of the halitosis group were different from those of the control group.[13 14] Therefore, the anaerobic oral condition might play an important role in the development of halitosis. Consequently, regulating the balance of the oral microbiota to reduce VSC levels is an important method to manage oral malodor.

The current treatments for halitosis include mechanical cleaning (scaling and tongue scraping) and chemical therapy (antibiotics, mouthwashes, and other agents).[15 16] However, mechanical therapy is often uncomfortable, even if carried out by the dentist. In addition, although chemical therapy is generally effective for a short time, it is always associated with various side effects, including the emergence of dysbacteriosis and staining of the tongue and tooth.[17-20] Consequently, new methods with fewer side effects are constantly suggested to inhibit oral malodor.

As live microorganisms, probiotics confer benefits to the host when administered in appropriate amounts.[21] Their beneficial effects are primarily related to regulating the local microenvironment through the prevention of adhesion of pathogens and inhibition of growth of pathogens through the production of bacteriocins.[22 23] Recently, probiotics like *Lactobacillus reuteri* and *Bifidobacteria* have been widely used in the oral field.[24] There is a growing body of evidence that the administration of probiotics might affect the composition of oral biofilms. They have also been investigated in the treatment of

periodontal[25 26] and peri-implant diseases,[27 28] caries,[29] oral candidiasis[30 31], and oral mucositis induced by chemo-radiotherapy.[32] Meanwhile, probiotics have also been reported as an alternative strategy to relieve oral malodor.[33-37] However, a previous systematic review showed that probiotic therapy for oral malodor is associated with insufficient evidence for its recommendation.[38] Thus, it is necessary to carry out a focused analysis of the therapeutic effects of probiotics in the treatment of halitosis.

Therefore, this systematic review and meta-analysis was undertaken to investigate the effect of probiotics in managing halitosis from a time perspective to provide some evidence for the administration of probiotics in this field.

METHODS

Patient and public involvement

No patient was involved in the study.

Study design

This systematic review was based on the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered in the PROSPERO (CRD42021227504).[39] According to the PICOS principle, the following focused question was structured: What is the clinical efficacy of probiotics in patients with halitosis when compared with placebo treatment? To answer our research question, we selected clinical trials according to the following study inclusion and exclusion criteria.

Search strategy

A critical electronic search was conducted in the bibliographic databases, mainly including PubMed, EMBASE, Web of Science, and Cochrane Central Register of Controlled Trials up to and including February 2021 to select the published literature. Additionally, gray literature was searched in the database System for Information on Gray literature in European and Google Scholar. The reference lists of the included articles and some related Chinese journals (the Chinese Journal of Stomatology, West China Journal of Stomatology, Journal of Oral Science Research, Journal of Practical Stomatology) were also searched manually. There was no language restriction.

An initial search strategy was conducted in PubMed with the combination of Medical Subject Headings (Mesh) terms identified by an asterisk symbol (*) and free text words as follows: Probiotic OR Probiotic* OR Probiotic therapy OR Probiotic effect OR Probiotic treatment AND halitosis OR halitosis * OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris. The detailed search strategy for each database was mentioned in supplemental file 1. Endnote X7 was used for electronic title management. First, primary screening was performed independently by two reviewers (NWH and JJL) based on the titles and abstracts. Then, the full-text articles were used to assess the eligibility further. Any disagreement was solved by consulting a third reviewer.

Study inclusion and exclusion criteria

The populations were patients diagnosed with halitosis. The intervention was probiotic therapy, representing the experiment group. The control group was done with a placebo treatment. The considered outcomes were halitosis parameters and other indexes before and after treatment. During the first stage of the study selection, studies meeting the following conditions were considered eligible for this review: 1) study types: randomized controlled clinical trials (RCTs) or randomized controlled cross-over studies; 2) participants: systemically healthy patients diagnosed with halitosis via accepted standards (the organoleptic (OLP) scores and/or the concentrations of VSC); 3) interventions: evaluating the efficacy of probiotics with placebo, regardless of the probiotics species and the consumption method; 4) control interventions: placebo treatment; 5) clinical data: the measurement values, including halitosis parameters and other indexes before and after treatment. At the second stage of the selection, eligible studies acquired in the first stage were identified according to the following exclusion criteria: 1) *in vitro* and animal studies, letter to the editor, review articles, interviews, and meta-analyses; 2) unclear halitosis identification; 3) studies with no completed data obtained even by contacting the authors. 4) interventions included other measures (e.g., studies comparing tongue scraping plus chlorhexidine plus probiotics and tongue scraping plus chlorhexidine).[34]

Halitosis assessment

The primary outcomes were evaluated for OLP scores and the VSC concentration levels. OLP scores reflecting subjective perception were often treated as the gold standard for diagnosing halitosis clinically and in research.[40 41] The OLP scores were estimated by two or three evaluators (with training and experience in calibrating tests). Subjects closed their mouth for 1 min and then exhaled slowly from their mouth into the evaluator's nose at a distance of 10 cm. The score was evaluated according to a six-point '0–5' scale (Rosenberg scale).[42]

The VSC concentrations measurement is an objective method through using the Halimeter or Oral Chroma with no significant difference.[43] Compared with organoleptic evaluation, VSC concentrations measurement is a quantitative variable with high sensitivity and reproducibility.[44-46] Subjects had to keep their mouth closed and stop talking for 5 min before measurements. Halimeter: a beverage straw (fixed and attached to the device) was inserted into the subject's mouth, located at the back of the tongue dorsum. Subjects should keep their mouth slightly open and breathe through the nose. Oral Chroma: subjects were asked to keep their mouths closed for 30 s with an air-tight syringe. Then, 1 mL of mouth air was extracted from the subject and injected into Oral Chroma to measure the VSC concentration.[47] Then the mean of the results given by the evaluators or machines was used.

Risk of bias

The included studies underwent a quality assessment with the Revised Cochrane risk of bias tool for randomized trials (RoB2).[48] This tool assesses the risk of bias in five domain areas, including randomization process, deviations from intended interventions, missing outcome data, measurement of outcome, and selection of the reported result. Each domain assessed bias following several signaling questions. The overall bias was classified as a high risk of bias, some concerns, or a low risk of bias determined by a validated algorithm. After screening the articles, two reviewers (NWH and JJL) conducted the assessment independently to reach an agreement.

Data extraction

Data was extracted with a researcher-designed data form with the following information: 1) basic information of the included studies (first author's name and the year of publication); 2) study type (RCT); 3) diagnostic criteria for halitosis; 4) characteristics of the participants (sample volume, the age range); 5) treatment (probiotic administration, including the type of bacteria, vehicles, doses, and frequencies); 6) clinical parameters (including the primary and secondary outcomes of final participants); 7) significance and follow-up periods.

Of all these variables, the follow-up periods referred to the duration of probiotic use. If probiotic treatment ceased during the observation period, only the data before ceasing treatment was included. Concerning clinical parameters, OLP scores and VSC concentrations were considered the primary outcomes, directly associated with oral malodor. The secondary outcomes in this review included tongue coating scores (TCS) and plaque index (PI) because they are commonly regarded as halitosis causes.

Statistical analysis

The statistical analysis was performed with Review Manager 5.3 and Stata 12.0. All the data was group-analyzed according to the follow-up time. The time ≤4 weeks was considered the short-term period and the time >4 weeks was considered the long-term period. In one study with three observation periods, the values of 4 weeks were analyzed in the short term to keep consistent with other studies.[49] Study heterogeneity was evaluated using Q statistics and the I^2 test. P value <0.10 was treated as the standard test. When I^2 >50% or p value <0.10, there was significant heterogeneity between the studies.[50-52] Then, subgroup analysis and sensitivity analysis were performed to analyze the sources of heterogeneity. The continuous data on the halitosis parameters of the present studies were expressed with the standardized mean difference (SMD) and 95% CI (confidence interval). A random-effect model was used for analysis. Therefore, the mean difference and standard deviation had to be acquired. If the original text did not provide the related data, the mean difference could be calculated, and the standard deviation was obtained with the formula (r_d =sqrt ($r_1^2/n_1 + r_2^2/n_2$). The excel sheets in the articles were used to convert the values when provided with median and interquartile.[53] [54] Publication bias was performed subjectively by funnel plots and objectively by Egger's tests. In Egger's test, p value <0.05 indicates the presence of publication bias.[55] Sensitivity analysis (leave-one-out method) was conducted to assess the alteration by sequential omission of individual studies.[56]

RESULTS

Study selection

In total, 238 articles were potentially identified by electronic and manual searches. After eliminating the duplicates, 14 articles were included by screening the titles and abstracts. Then, these studies were evaluated by reading the full texts, and seven articles met the final inclusion criteria (Figure 1).[42 49 57-61]

Study characteristics

Table 1 presents the main characteristics of the included studies. In this review, all the studies were randomized control trials. The number of participants in the studies ranged between 23 and 68, with an age range of 19 to 70. Halitosis was diagnosed with OLP scores and/or VSC concentrations. The probiotics and placebo groups were compared, and the follow-up periods varied from two weeks to 12 weeks.

Table 1 Characteristics of the included studies.

Study	Type	Halitosis criterion	Subjects Age	Clinical parameters	Probiotics Administration (Vehicle, strains and frequency)	Follow-up
Mousquer et al. (2020)	RCT Placebo-double masked, parallel	OLP score≥1	29 ≥18	OLP VSC TCS	A gum including 1 billion colony forming units (CFU) Lactobacillus salivarius G60 taken twice per day	Baseline 2 weeks
Lee et al. (2020)	RCT Placebo-double blind parallel	VSC≥1.5ng/10 mL	68 20-39	OLP VSC (H_2S, CH_3S, C_2H_6S)	An 800-mg tablet contained 1.0×10^8 CFU/g Weissella cibaria taken once per day	Baseline 4 weeks 8weeks
He et al. (2020)	RCT Placebo-double blind parallel	OLP score ≥2 VSC ≥150ppb	28 23-44	OLP VSC TCS PI	A tablet containing 1×10^9 CFU Streptococcus salivarius K12 taken twice per day	Baseline 4 weeks
Keller et al. (2012)	RCT Placebo-double blind cross-over	OLP score>1	25 19-25	OLP VSC	A chewing gum containing Lactobacillus reuteri DSM 17938 and Lactobacillus reuteri ATCC PTA 5289 -both with a concentration of 1×10^8 CFU taken twice per day	Baseline 2 weeks
Suzuki et al. (2014)	RCT Double-blind placebo-controlled Cross-over	OLP score≥1.5	23 22-67	OLP VSC (H_2S, CH_3S, C_2H_6S) PI TCS	A tablet containing 6.7×10^8 CFU Lactobacillus salivarius WB21 and 280mg xylitol taken 3 times per day	Baseline 2 weeks
Penala et al. (2016)	RCT Placebo-double blind parallel	OLP score >2	29 25-59	OLP PI	A capsule mixture included Lactobacillus salivarius (2×10^9 CFU) and Lactobacillus reuteri (2×10^9 CFU) dissolved into 10ml distilled water to rinse for 1min, daily twice	Baseline 4 weeks 12 weeks
Kim et al. (2020)	RCT Placebo-double blind parallel	OLP score≥2 VSC≥0.15ng/ml	58 20-70	VSC (H_2S, CH_3S, C_2H_6S) OLP	A bag of powder mixture included Weissella. cibaria CMU (1.0×10^8 CFU) melted in the mouth once per day	Baseline 2 weeks 4 weeks 8weeks

*RCT: randomized controlled trials; OLP: organoleptic; VSC: volatile sulfur compounds; TCS: tongue coating scores; CFU: colony forming units; H_2S: hydrogen sulfide; CH_3S: methyl mercaptan; C_2H_6S: methanethiol; PI: plaque index

Risk of bias

The bias estimation results showed that one study had a low risk of bias, one had a high risk, and five showed some concerns. The reason for a high risk of bias was the incomplete outcome data of the OLP scores. Five articles were identified as some concerns because there were many uncertain factors in their full texts. Figure 2 presents concrete data on the risk of bias.

Study outcomes

Primary outcomes

Concerning OLP, studies by Keller et al. (2012) and Penala et al. (2016) reported a significant decrease in the probiotic group compared to the placebo group after treatment ($p < 0.05$).[58 59] In the study by Lee et al. (2020) involving different follow-up periods, OLP scores decreased significantly in the test groups at four weeks ($p = 0.002$) but not eight weeks ($p = 0.188$) compared to the baseline.[60] Additionally, the results of the other four studies indicated that the OLP scores did not differ between the two groups.

Concerning VSC, six articles determined VSC concentrations, with three studies detecting the p values of VSC and subgroups (H_2S, CH_3SH, and C_2H_6S).[49 57 60] According to the results, only two studies[57 60] reported a significant improvement in VSC levels in experimental groups versus placebo groups.

Secondary outcomes

Concerning TCS, three studies evaluated the changes between the probiotic and placebo groups at four weeks.[42 57 61] Although a reduced tendency was observed after treatment compared with baseline p values, there was no significant difference between the two groups.

Concerning PI, in the three studies involved,[42 57 59] only one study showed a significant reduction in PI in the experimental group compared with the controlled group at 12 weeks.[59]

Quantitative synthesis

A meta-analysis was performed including studies with similar clinical parameters of OLP, VSC, TCS, and PI, according to the follow-up time. Although the detection methods of VSC were different, both of the devices exhibited similar sensitivity and specificity in the detection of halitosis.[43] Therefore, we analyzed these values together. Considering the limitations of the included studies and follow-up time, the pooled estimations of TCS and PI were only performed in the short term.

In the short term, the OLP scores significantly decreased in the probiotic group compared to the control group [SMD =-0.58; 95% CI (-0.87, -0.30), $p < 0.0001$] (Figure 3). A similar result was observed in VSC [SMD =-0.26; 95% CI (-0.51, -0.01), $p = 0.04$] and H_2S levels [SMD =-0.73; 95% CI (-1.36, -0.10), $p = 0.02$]. Other items (TCS, PI, CH_3S, and C_2H_6S) were not significantly different between the experimental and control groups. The heterogeneity of each outcome was low ($I^2 < 50\%$) except for H_2S levels ($I^2 = 75\%$) (Figures 3 and 4).

In the long term, there was a significant improvement in OLP scores in the experimental group [SMD =-0.45; 95% CI (-0.85, -0.04), $p = 0.03$] (Figure 5). The results failed to show a significant difference in VSC concentrations and their subgroups levels (Figures 5 and 6). The heterogeneity of VSC concentrations was substantial ($I^2 = 58\%$).

Publication bias

In this systematic review and meta-analysis, we found no evidence of publication bias by the result of the funnel plots and Egger's tests ($p > 0.05$) (supplementary file 2-Figures S1-S5).

Sensitivity analysis

Sensitivity analysis (leave-one-out method) revealed no significant change in the pooled estimation when excluding any individual study (supplementary file 2-Figures S6-S9).

DISCUSSION

Summary of the findings

This meta-analysis demonstrated that probiotics significantly reduced the OLP scores compared with the placebo group regardless of the duration of observation, confirming the benefits of probiotics for halitosis treatment. The probiotics group exhibited a significant reduction in VSC concentrations in the short term (≤4 weeks), with no noticeable difference in the long term (>4 weeks). Meta-analyses were also performed in the subgroups of H_2S, CH_3SH, and C_2H_6S to assess the concrete difference in VSC levels. The results showed that only H_2S levels reduced noticeably in the short term when the probiotic treatment was administered. As for TCS and PI, the results showed no significant differences between the experimental and placebo groups in the short term. There was no evidence of publication bias. The sensitivity analysis confirmed that the pooled estimate was stable.

Outcomes comparison and possible mechanisms

Concerning the primary outcomes, in the included articles, the pooled estimation of OLP scores and VSC concentrations were in favor of probiotic therapy rather than placebo in the short term.[42 49 57-59 61] The biological mechanisms may be related to the interaction between probiotics and oral microbiota. According to present studies, probiotic therapy reduces odorous compound levels by inhibiting the decomposition of amino acids and proteins by anaerobic bacteria.[7 62] The significantly lower VSC levels under probiotic treatment in the short term might indicate a decrease in anaerobic bacteria activity. In contrast to our findings, a previous study indicated that it could not confirm the effect of probiotics on reducing VSC in the short term.[38] The number of included articles may result in this difference. However, when comes to the results in the long term, only OLP scores showed a significant reduction rather than VSC concentrations. Oral microbiota contains not only VSC-producing bacteria but also other bacteria capable of producing other oral malodor compounds (e.g., indoles, skatole, pyridine, picolines, and polyamines).[63] The underlying mechanisms of the difference may result from the variation and abundance of microbiota community over time, which in turn affects the efficacy of probiotics, especially VSC concentration levels.[35 49 61] Therefore, no significant effect on VSC concentrations in the long term may be due to probiotics' inhibition effect on those other bacteria. Therefore, the data about microorganisms changing in different periods are significant for the evaluation of probiotic effects. However, from the present studies, insufficient data in the included studies, the differences in detection methods, bacterial species, and heterogeneity of clinical trials limited the microorganism statistical analysis in this review.

Meanwhile, we found that the short-term outcome of H_2S concentration change other than CH_3SH, and C_2H_6S was consistent with the total VSC levels. This might be related to differences in the function of probiotics and in the number and species of bacteria associated with each VSC reduction.[12 35 64]

Additionally, the regular VSC measurement device was reported to be more sensitive towards H_2S than CH_3SH and C_2H_6S,[46] which may also account for the above result.

Regarding the secondary outcomes, based on the present meta-analysis, there was no significant difference between the experimental and placebo groups on secondary outcomes during the observation time. The possible reason was the short observation time in the included studies, as one study included in the analysis showed a significant improvement in PI at 12 weeks. [59] Tongue coating and periodontitis are often regarded as the leading causes of halitosis. [42 65] However, in an original article, the TCS and PI showed a pronounced decline after using probiotics compared with the baseline, with no decrease in the placebo group.[61] This phenomenon might be related to the type of probiotics, some of which were reported to boost salivary flow by interacting with the oral microbiota.[66]

From the current studies, there are two main types of studies on the effect of probiotics on halitosis, one is to observe the effect during continuous use of probiotics and the other is to observe the effect at follow-up after stopping the use of probiotics. A recently published study indicated that no significance of probiotic effect was found, different from ours. The reason for the difference may be that this study analyzed the collected follow-up data after stopping using probiotics for at least 2 weeks.[67] In addition, OLP, as the gold standard, demonstrated the efficacy of probiotics in managing halitosis. However, the results of VSC concentration and subgroup analysis in the long term undermined this effect. These results with various different outcomes showed the inconsistency in this study. According to Bradford-Hill criteria, there would be less persuasive evidence for causation between the management of halitosis and probiotics[68]. Therefore, more clinical and systematic studies are needed to explore and verify the probiotic effect on the management of halitosis in future research.

Limitations

There were several limitations in the present study throughout the whole review process. First, although both electronic and hand searches were conducted in four primary databases, it was impossible to retrieve all the relevant studies. Second, this study lacked persuasive evidence for causation between the management of halitosis and probiotics due to the inconsistency of the pooled results. Third, all included interventions differed in the species of probiotics, the doses and frequencies used, and administration periods. A subgroup analysis was necessary to evaluate the source of efficacy concerning the probiotic species, but the small size of the included articles prevented further analysis. All these factors would inevitably affect the accuracy of outcomes. Fourth, the detection methods of VSC were different. Although there is no significant difference between them, the combined analysis might still affect the reliability of the results. Fifth, in some included studies, the primary outcomes were presented in different forms, such as percentages or range interquartile. Finally, some important parameters, including the microorganism species and changes, were not presented completely in some articles. The absence of partial original data or the differences caused by data conversion equally impaired the final results though many methods were tried to reduce the bias.

CONCLUSION

The present systematic review and meta-analysis indicated that probiotics (e.g., *Lactobacillus salivarius, Lactobacillus reuters, Streptococcus salivarius, and Weissella cibaria*) may ease halitosis by reducing the VSC concentration levels in the short term, but there is no significant effect on the major cause of halitosis such as plaque and tongue coating. Considering the heterogeneity of clinical trials included

and the small sample size, more high-quality random clinical trials are required in the future to verify the results and to evidence the efficacy of probiotics in the management of halitosis.

Contributors

NWH and JJL collected and analyzed data, and drafted the manuscript; NWH and XHQ helped with the literature searching and statistical analysis; YZW and CZW provided help in the literature searching and figure revises; XHQ and YKL critically reviewed the manuscript. LJL designed the experiment and critically reviewed the manuscript. NWH and JJL contributed equally to this paper. All authors agree to be accountable for the study.

Competing interests

None declared.

Ethics approval statement

No applicable.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1: Flow diagram of literature search strategy and inclusion, exclusion criteria.

Figure 2: Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represents a high risk of bias.

Figure 3: Forest plot of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

Figure 4: Forest plot of VSC subgroups in short-term (≤4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

Figure 5: Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

Figure 6: Forest plot of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

Figure S1: Funnel plot of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

Figure S2: Funnel plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

Figure S3: Funnel plot of VSC subgroups in short-term (≤4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

Figure S4: Funnel plot of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

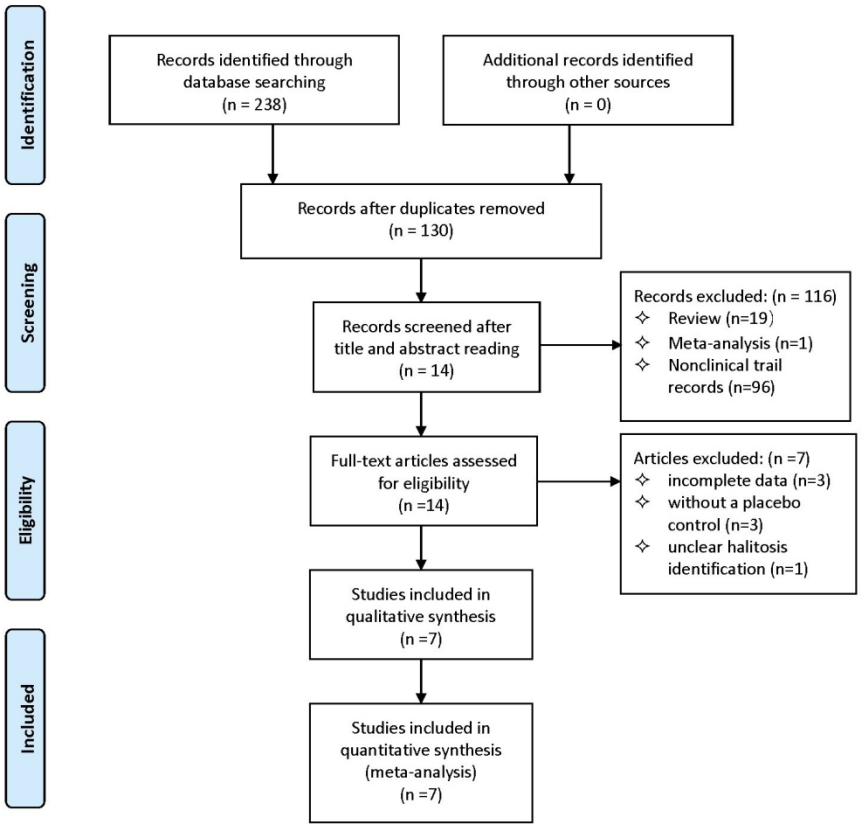
Figure S5: The result of Egger's test in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI; (E) H_2S; (F) CH_3S; (G) C_2H_6S.

Figure S6: Sensitivity analysis of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

Figure S7: Sensitivity analysis of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

Figure S8: Sensitivity analysis of VSC subgroups in short-term (≤4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

Figure S9: Sensitivity analysis of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.



Flow diagram of literature search strategy and inclusion, exclusion criteria.

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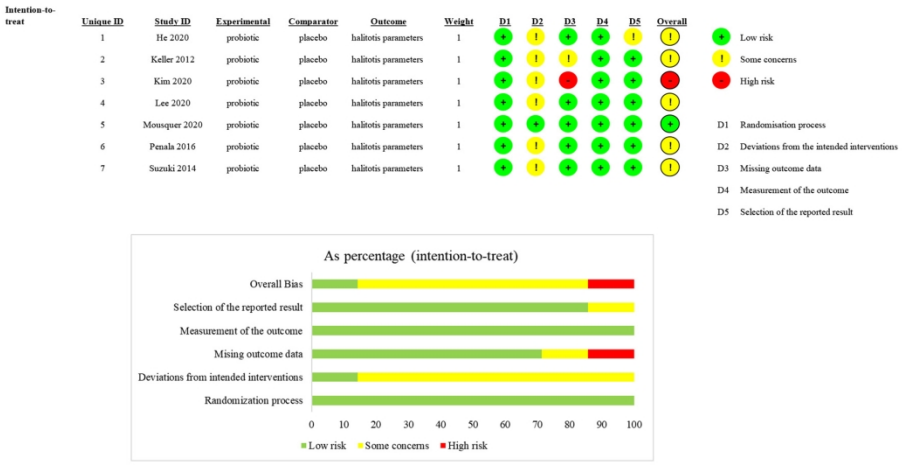

Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represent high risk of bias.

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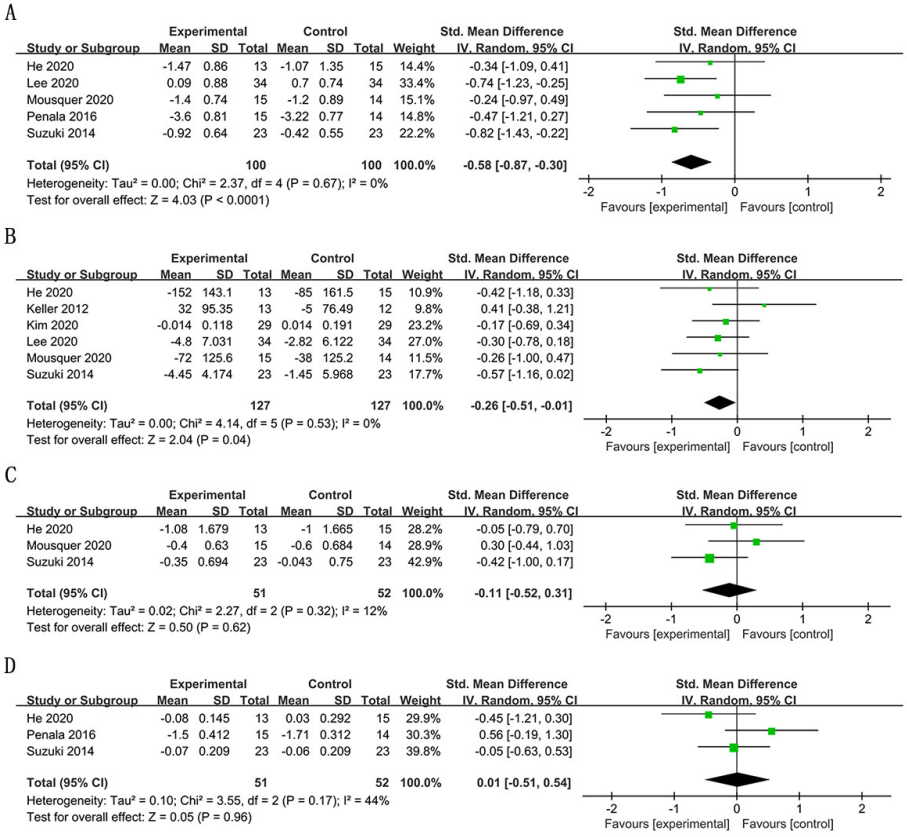

Forest plot of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

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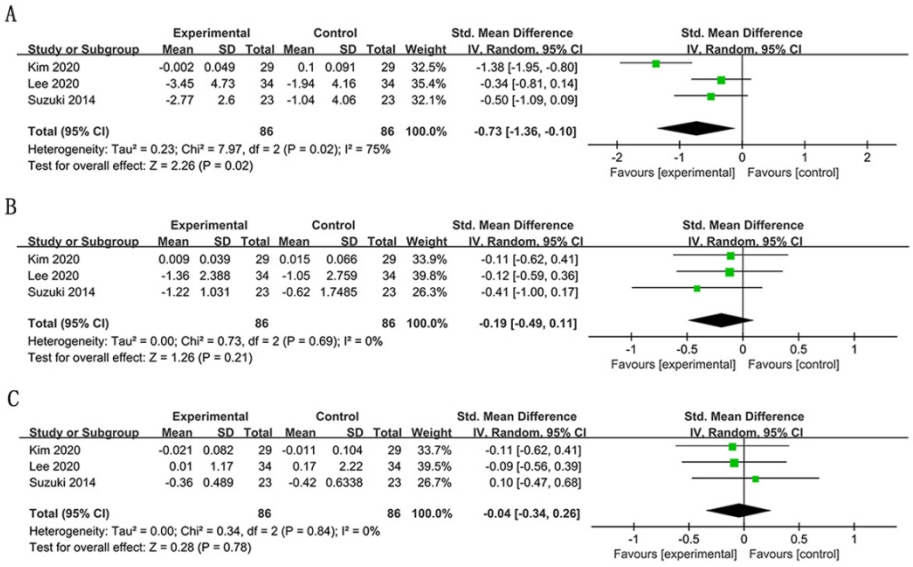

Forest plot of VSC subgroups in short-term (≤4 weeks): (A) H2S; (B) CH3S; (C) C2H6S.

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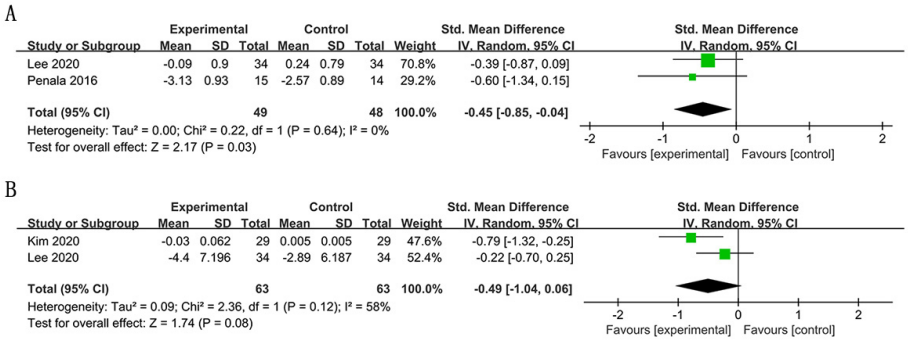

Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

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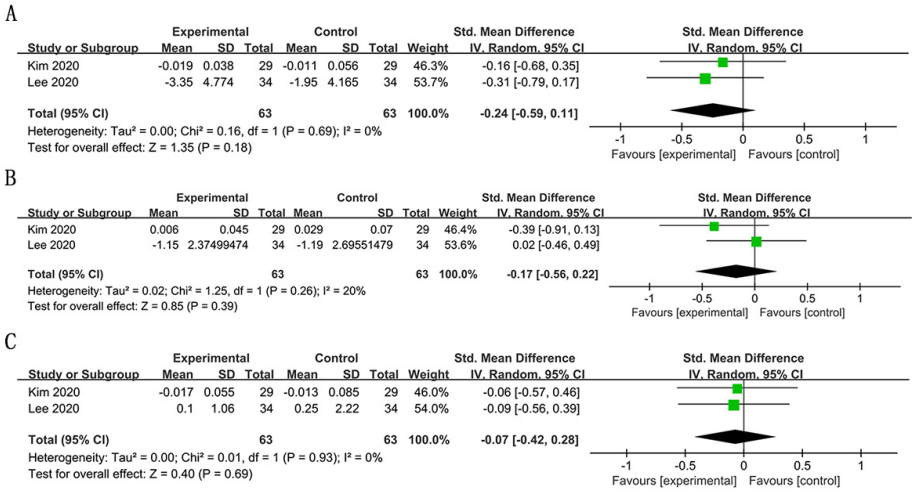

Forest plot of VSC subgroups in long-term (>4 weeks): (A) H2S; (B) CH3S; (C) C2H6S.

108x61mm (300 x 300 DPI)

Supplementary file 1

1. PubMed

Search	Query	Items found
#1	((((((Probiotic[Text Word]) OR (Probiotic[MeSH Terms])) OR (Probiotic therapy[Text Word])) OR (Probiotic effect[Text Word])) OR (Probiotic treatment[Text Word])))	27215
#2	(((((((halitosis[Text Word]) OR (halitosis[MeSH Terms])) OR (malodor[Text Word])) OR (oral malodor[Text Word])) OR (malodour[Text Word])) OR (bad breath[Text Word])) OR (fetor oris[Text Word])))	2788
#3	#1 and #2	68

2. Web of science

Search	Query	Items found
#1	(((TS=(Probiotic)) OR TS=(Probiotic therapy)) OR TS=(Probiotic effect)) OR TS=(Probiotic treatment)	28458
#2	(((((TS=(halitosis)) OR TS=(malodor)) OR TS=(oral malodor)) OR TS=(malodour)) OR TS=(bad breath)) OR TS=(fetor oris)	3018
#3	#1 and #2	42

3. Embase ovid search strategy

Search	Query	Items found
#1	((Probiotic or Probiotic or Probiotic therapy or Probiotic effect or Probiotic treatment) and (halitosis or halitosis or malodor or oral malodor or malodour or bad breath or fetor oris)).af.	119

4. Cochrane Central Register of Controlled Trials (CENTRAL) search strategy

Search	Query	Items found
#1	MeSH descriptor: [Halitosis] explode all trees	236
#2	(halitosis):ti,ab,kw (Word variations have been searched)	573
#3	(malodor):ti,ab,kw (Word variations have been searched)	399
#4	(oral malodor):ti,ab,kw (Word variations have been searched)	300
#5	(malodour):ti,ab,kw (Word variations have been searched)	399
#6	(bad breath):ti,ab,kw (Word variations have been searched)	258
#7	(fetor oris):ti,ab,kw (Word variations have been searched)	0
#8	#1 or #2 or #3 or #4 or #5 or #6 or #7	996
#9	MeSH descriptor: [Probiotics] explode all trees	2571

#10	(Probiotic):ti,ab,kw (Word variations have been searched)	8519
#11	(Probiotic therapy):ti,ab,kw (Word variations have been searched)	3834
#12	(Probiotic effect):ti,ab,kw (Word variations have been searched)	6398
#13	(Probiotic treatment):ti,ab,kw (Word variations have been searched)	4579
#14	#9 or #10 or #11 or #12 or #13	8603
#15	#8 and #14	8

5. Gray literature in European and Google Scholar

Search	**Query**	**Items found**
#1	Probiotic OR Probiotic therapy OR Probiotic effect OR Probiotic treatment AND halitosis OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris	1

Supplementary file 2

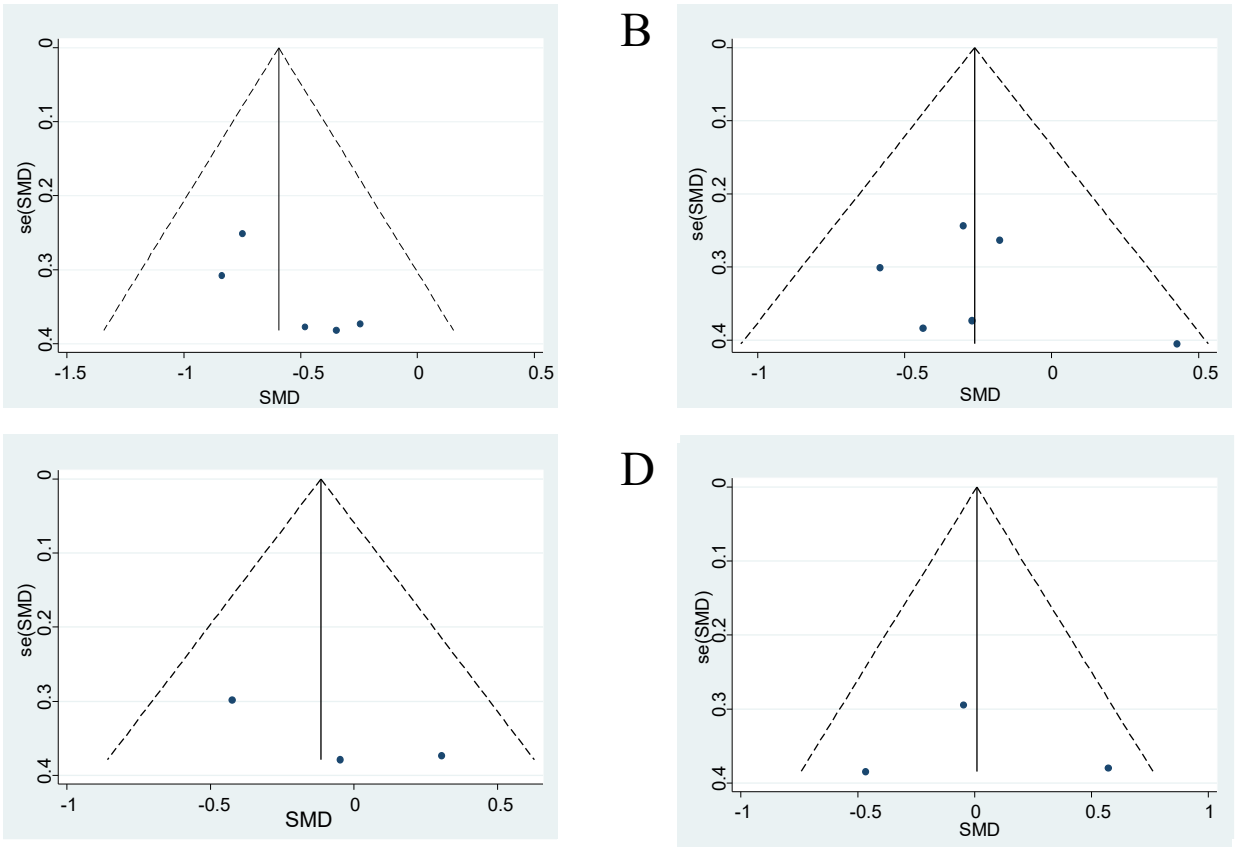

Figure S1: Funnel plot of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

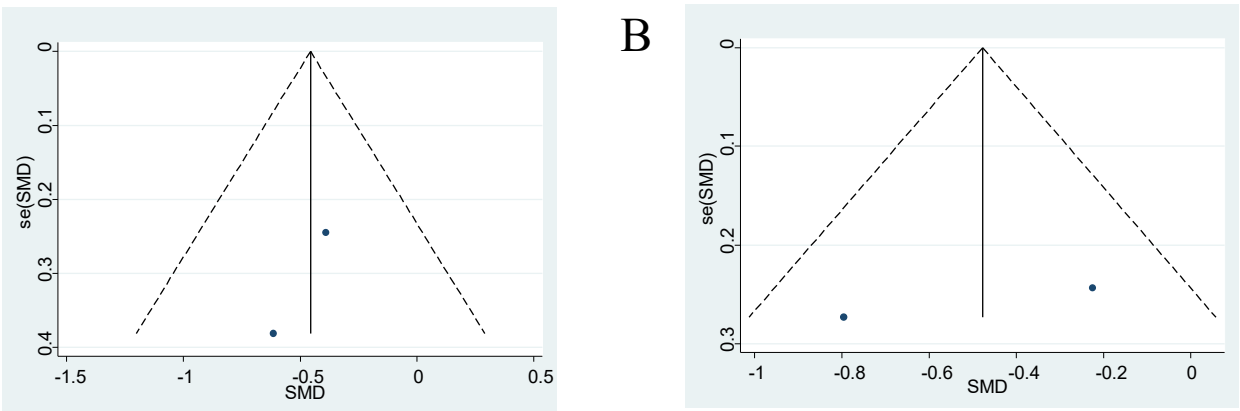

Figure S2: Funnel plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

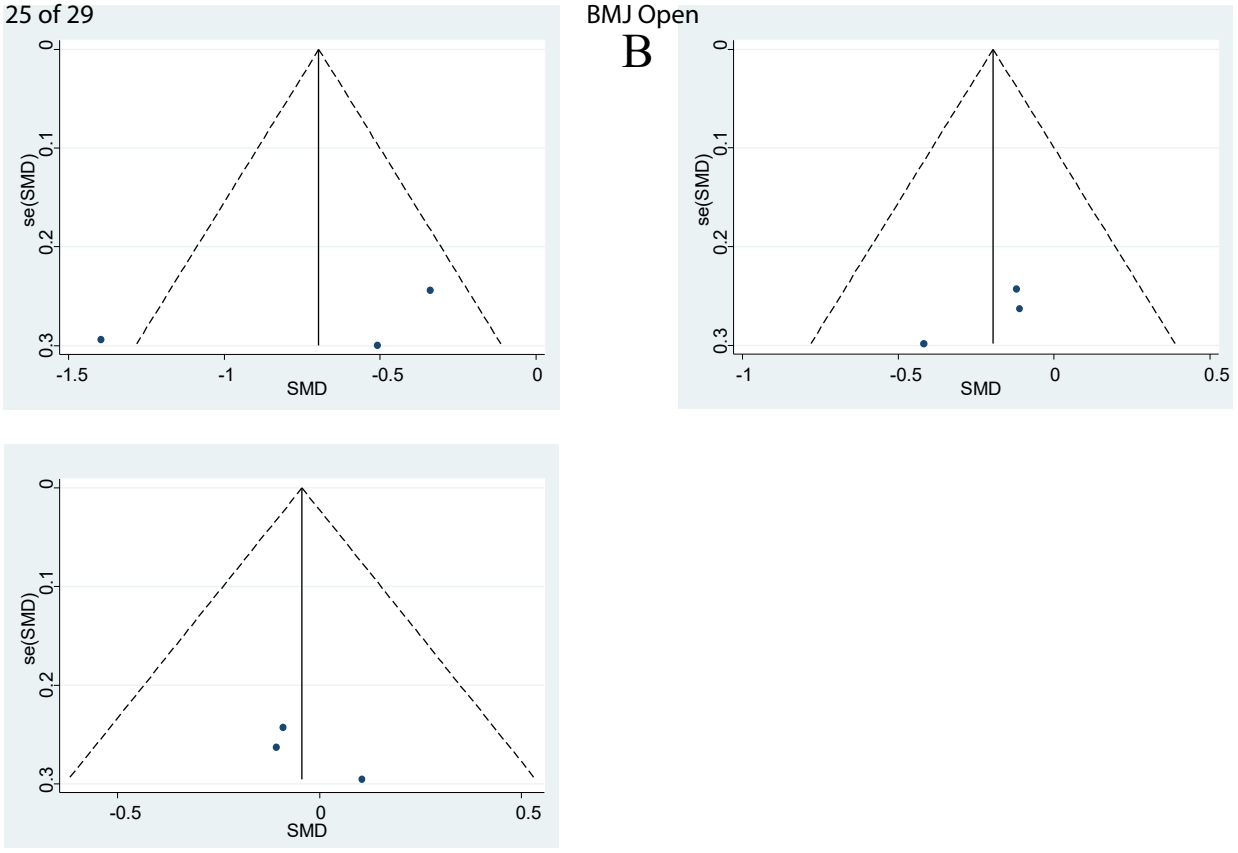

Figure S3: Funnel plot of VSC subgroups in short-term (≤4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

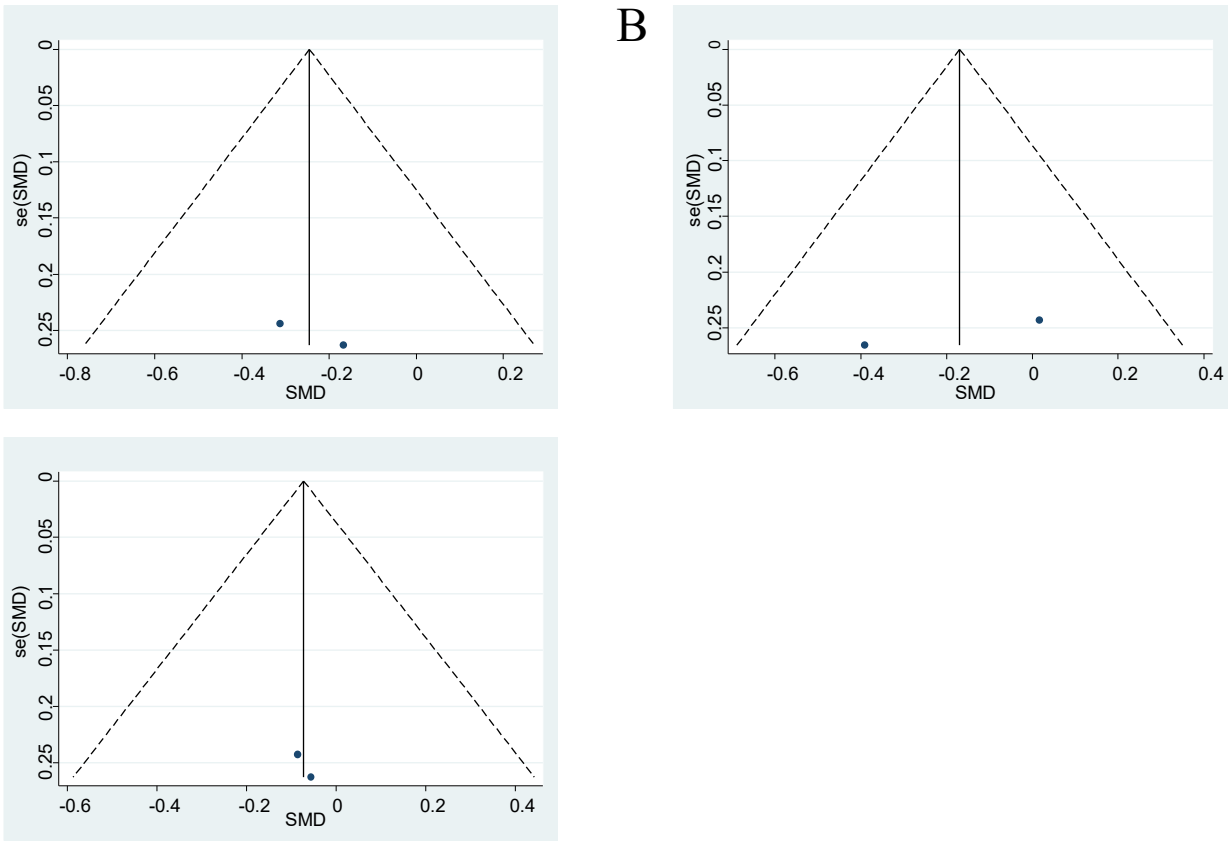

Figure S4: Funnel plot of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

A

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-1.654658	.4354301	-3.80	0.032	-3.04039	-.2689248
bias	3.312431	1.341309	2.47	0.090	-.9562116	7.581074

B

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.6982326	.6453982	-1.08	0.340	-2.490145	1.09368
bias	1.432804	2.079837	0.69	0.529	-4.341748	7.207357

C

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-2.485861	1.282746	-1.94	0.303	-18.7847	13.81297
bias	6.934603	3.728761	1.86	0.314	-40.4438	54.313

D

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.2878511	3.056969	-0.09	0.940	-39.13033	38.55463
bias	.8711259	8.860095	0.10	0.938	-111.7071	113.4493

E

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	2.223943	3.837093	0.58	0.666	-46.53094	50.97883
bias	-10.63929	13.91572	-0.76	0.584	-187.4552	166.1766

F

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	1.252312	.6648476	1.88	0.311	-7.195378	9.700003
bias	-5.480127	2.507946	-2.19	0.273	-37.3466	26.38635

G

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-1.041425	.5483955	-1.90	0.309	-8.00945	5.926601
bias	3.788236	2.074839	1.83	0.319	-22.57509	30.15157

Figure S5: The result of Egger's test in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI; (E) H_2S; (F) CH_3S; (G) C_2H_6S.

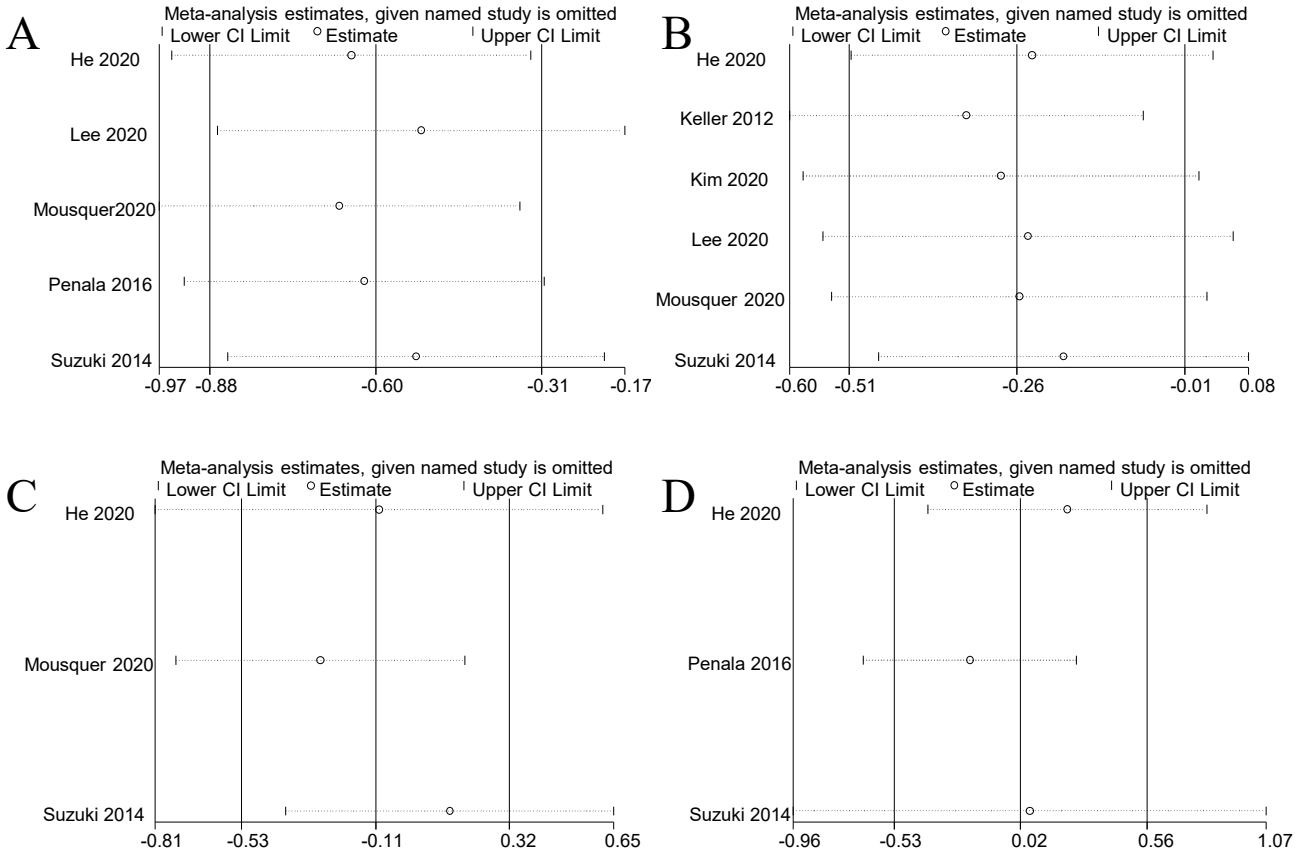

Figure S6: Sensitivity analysis of halitosis parameters in short-term (≤4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

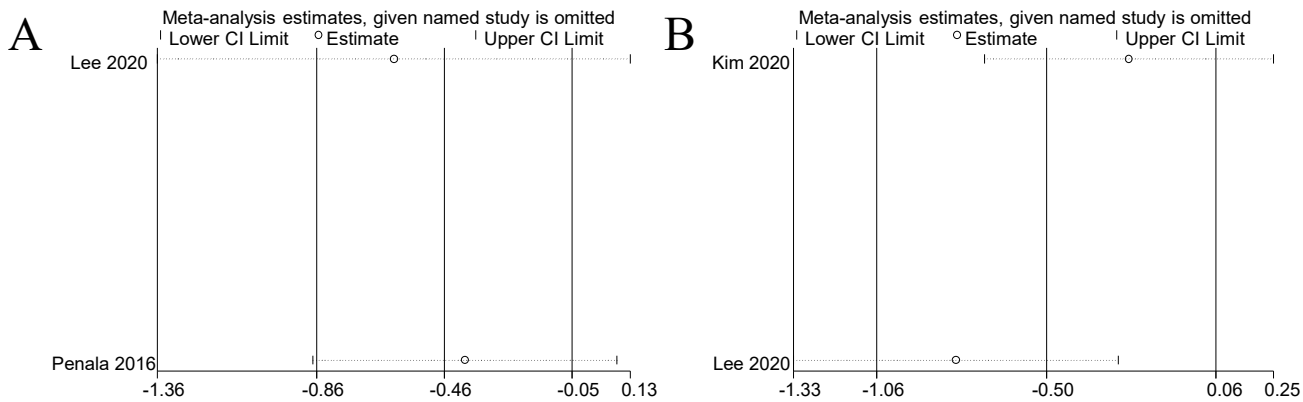

Figure S7: Sensitivity analysis of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

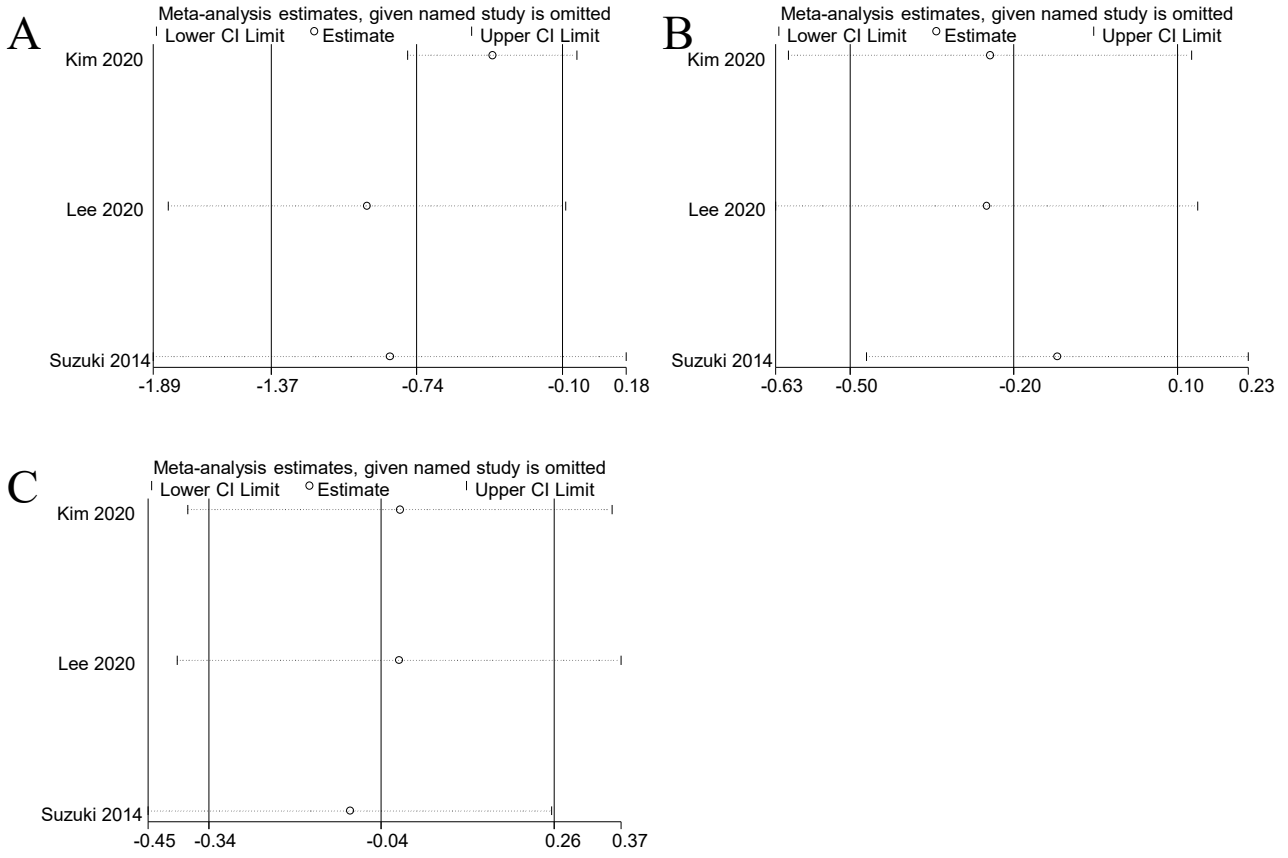

Figure S8: Sensitivity analysis of VSC subgroups in short-term (≤4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

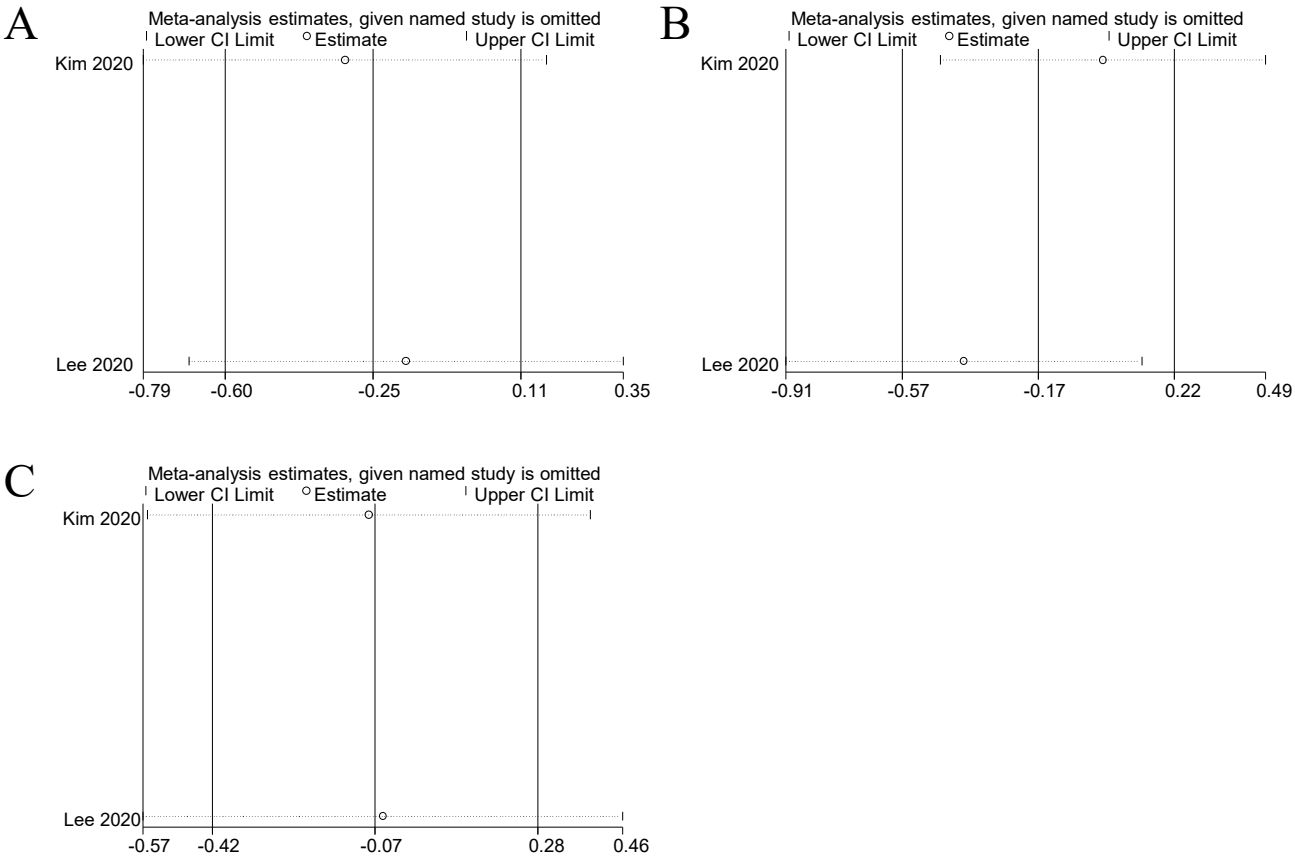

Figure S9: Sensitivity analysis of VSC subgroups in long-term (>4 weeks): (A) H_2S; (B) CH_3S; (C) C_2H_6S.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2,3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	4
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	4
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	4
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	4
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	5

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	5
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	5
Study characteristics	17	Cite each included study and present its characteristics.	5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	6
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	6
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	6
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7
	23b	Discuss any limitations of the evidence included in the review.	7,8
	23c	Discuss any limitations of the review processes used.	7,8
	23d	Discuss implications of the results for practice, policy, and future research.	8
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	3
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	3
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	3
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	2
Competing interests	26	Declare any competing interests of review authors.	2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	4

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71