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Impact of Probiotic Supplementation on Folate, Vitamin B12, and Homocysteine Levels in Humans: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Systematic Review

Keywords: Probiotic, folate, homocysteine, vitamin B12, meta-analysis

Posted Date: May 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9563368/v1

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Additional Declarations: The authors declare no competing interests.

Abstract

Background: Previous pre-clinical studies and human clinical trials have suggested that probiotic suppplementation may affect the homocysteine metabolism. This systematic review and meta-analysis aimed to evaluate the effects of probiotic supplementation on homocysteine level and its closely related cofactors—folate and vitamin B12—in humans.

Methods: This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. PubMed, EMBASE, Web of Science, and the Cochrane Library were searched from inception to September 2025 for randomized controlled trials investigating the effects of probiotics on homocysteine, folate, or vitamin B12 levels. Pooled effect estimates were calculated using random-effects meta-analysis.

Results: Among the 13 included studies, 9 randomized controlled trials involving 395 participants were used for homocysteine analysis. Probiotic supplementation was associated with a significant reduction in homocysteine levels compared with control (mean difference [MD]: −1.61 μmol/L; 95% confidence interval [CI]: −2.61 to −0.62; P = 0.0058). Six studies (n = 300) reported folate outcomes, with no significant effect observed following probiotic intervention (MD: 0.69; 95% CI: −0.22 to 1.62; P = 0.1099). In contrast, meta-analysis of six studies including 330 participants demonstrated a significant increase in vitamin B12 levels in the probiotic group (MD: 39.13; 95% CI: 14.41 to 63.85; P = 0.0096).

Conclusions: Probiotic supplementation was associated with a significant reduction in circulating homocysteine levels and an increase in vitamin B12 concentrations, but not folate levels, in humans. These findings suggest a potential role for probiotics in modulating homocysteine metabolism and micronutrients. Further well-designed trials are warranted to elucidate whether the homocysteine-lowering effect of probiotics is mediated through improvements in vitamin B12.

1. Introduction

Folate and vitamin B12 are essential micronutrients involved in one-carbon metabolism, a fundamental biochemical pathway required for DNA synthesis, methylation reactions, and amino acid metabolism[1]. Adequate status of these B vitamins is necessary to support normal hematopoiesis, neurological function, and cardiovascular health [2]. Inadequate folate or vitamin B12 intake or absorption can lead to hyperhomocysteinemia, a metabolic disturbance that has been consistently associated with increased risks of cardiovascular disease, cognitive decline, adverse pregnancy outcomes, and all-cause mortality[3, 4]. Consequently, strategies aimed at improving folate and vitamin B12 status and reducing circulating homocysteine levels have important clinical and public health implications.

Although dietary intake remains the primary determinant of folate and vitamin B12 status, emerging evidence suggests that the gut microbiota may play a contributory role in host B-vitamin metabolism[5]. Certain commensal bacteria are capable of synthesizing folate and VB12 de novo [6, 7], while others may influence vitamin absorption and utilization[7, 8]. Alterations in gut microbial composition have been linked to impaired micronutrient status, particularly in vulnerable populations such as older adults and individuals with metabolic or gastrointestinal disorders[9, 10]. These observations have stimulated interest in probiotics as a nutritional intervention to support micronutrient homeostasis through modulation of the gut microbiome [11].

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. In addition to their established roles in gastrointestinal health and immune modulation, probiotics have been proposed to influence metabolic pathways related to B-vitamin synthesis and homocysteine regulation. Preclinical studies indicate that specific probiotic strains, including Lactobacillus and Bifidobacterium species, are capable of producing folate or vitamin B12 and may increase intestinal folate production or enhance B-vitamin bioavailability[12–14]. Therefore, from a clinical perspective, probiotic supplementation represents a potentially safe, accessible, and non-pharmacological approach to improve micronutrient status, particularly in populations at risk of folate or VB12 deficiency or elevated homocysteine levels.

In recent years, multiple randomized controlled trials (RCTs) have evaluated the effects of probiotic supplementation on circulating folate, vitamin B12, and homocysteine levels in humans[15]. These trials have been conducted across diverse populations and clinical contexts, including healthy individuals, patients with metabolic syndrome, type 2 diabetes and pregnant women[16]. However, the results have been inconsistent, with reported effects ranging from significant improvements in folate status and reductions in homocysteine to null findings. Variability in probiotic strain composition, dosage, intervention duration, baseline nutritional status, and study design may partly explain these discrepancies.

Therefore, the present study aimed to systematically review and meta-analyze data from randomized controlled trials to evaluate the effects of probiotic supplementation on circulating folate, vitamin B12, and homocysteine levels in humans. By providing a quantitative assessment of available evidence, this work seeks to clarify the clinical relevance of probiotics as an adjunct nutritional strategy for optimizing one-carbon metabolism and reducing disease-related metabolic risk.

2. Methodology

2.1. Search strategy

This meta-analysis evaluated the effects of probiotic supplementation on homocysteine metabolism and related biomarkers, specifically vitamin B12 and folate, in human clinical studies providing quantitative outcome data. The study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[17]. The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO; registration number: CRD420251165113).

The following databases were searched for relevant publications from inception to September 2025: PubMed, EMBASE, Web of Science, and the Cochrane Library. Publications from inception to February 2025 were included. In addition, Google Scholar was spot searched to find relevant studies that may have been published in journals that are not indexed in above databases. The primary search strategy was developed for PubMed and subsequently adapted for use in the other databases as appropriate. The search strategies are provided in **Appendix A**.

2.2. Eligibility criteria

Only studies published in English were considered for inclusion. Case reports, book chapters, review papers, editorials and animal model or cell culture studies were excluded.

Studies were selected according to the PICOS design (PICOS: patients, intervention, comparator, outcome and study design): the population include both healthy individuals and those with conditions associated with folate deficiency or hyperhomocysteinemia (e.g., cardiovascular disease, hypertension); intervention included studies that utilized probiotic supplementation with clearly defined doses, bacterial strains (such as Lactobacillus and Bifidobacterium) or substrates containing probiotics (e.g., fermented milk or yogurt); comparison included adequate control or placebo group for comparison; outcomes included studies reporting outcomes related to homocysteine metabolism and associated vitamins (VB12 and folate); study design included randomized controlled trials (RCTs).

Studies were excluded if they met any of the following criteria: (1) Duplicate studies. (2) Studies where the composition of the probiotic intervention was not specified. (3) Studies with insufficient information regarding experimental details or findings. (4) Any narrative review studies, meta-analyses, or any other articles that did not meet the requirement of being an original research study were also excluded.

2.3. Data extraction

Eligible studies were independently reviewed by two authors and the following data were extracted: first author's name, publication year, RCT design, sample size (intervention and control groups), participant characteristics (health status), duration of intervention, delivery format and dose of probiotic intervention, and outcome data for homocysteine, folate, and vitamin B12. Outcome data included means and standard deviations (SDs) at baseline, post-intervention, and/or change from baseline, when available. Two independent reviewers searched for additional articles by reviewing the references of articles selected from the abstract and title searches. Any discrepancies in study selection or data extraction were resolved through discussion and consensus among all authors.Extracted data were compiled and summarized in tabular form (Table 1).

2.4. Risk of bias

We evaluated the methodological quality using the Cochrane Risk of Bias Tool (RoB 2) for the RCTs [18]. Two investigators (Changming Ye, Xinxin Zhang) independently assessed each study, with conflicts resolved by a third reviewer (Mingfang Pan). Risk of bias was categorized as low, some concerns, or high across five domains: (a) randomization process; (b) deviation from the intended interventions; (c) missing outcome data; (d) measurement of the outcome; and (e) selection of the reported results. For each criterion, bias was assessed as a judgment (expressed as “high risk of bias”, “low risk of bias”, or “some concerns”).

2.5 Sensitivity analysis

To evaluate the robustness of our findings amid substantial heterogeneity, a leave-one-out sensitivity analysis was performed [19]. Each study was sequentially omitted, and the meta-analysis was re-run to assess its impact on the pooled estimate and heterogeneity. All sensitivity analyses were conducted in R statistical environment (4.4.1) using the meta package(version 8.1-0).

2.5 Meta-analysis

For the studies examining probiotic effects on homocysteine/folate/VB12 levels, we conducted a meta-analysis using means and standard deviations. For studies reported data using 95% confidence interval (CI) was converted to a standard deviation (SD) using the formula SD = sqrt (n) * (CI UL−CI LL)/t, and standard error of the mean (SEM) was converted to SD using the formula SD = SEM * sqrt (n). A random-effects model was used to calculate the mean difference (MD). Effect size heterogeneity was assessed using Q-statistics for testing the null hypothesis of homogeneity. Heterogeneity was also assessed through I^2. Restricted Maximum Likelihood (REML) method was used to estimate the between-study heterogeneity[20]. The Hartung-Knapp adjustment was applied to random-effects models to account for uncertainty in between-study variance estimations and to enhance the reliability of confidence intervals when the number of included studies was limited[21].

Results were visualized using forest plots. All analyses were conducted using meta packages (version 8.1-0) in R 4.4.1. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Search results

The study selection process is illustrated in Fig. 1. A total of 541 records were identified through database searching, including PubMed, Web of Science, Embase, and the Cochrane Library (n = 539), with an additional 2 records identified from other sources (google scholar search). After removal of duplicates, 75 records remained for screening. During title and abstract screening, 46 records were excluded because they were case reports, reviews, book chapters, meta-analyses, editorials, not relevant to the research question, or not published in English. Full-text articles were retrieved and assessed for eligibility for 19 records, of which 7 were excluded due to insufficient data. Ultimately, 13 studies met the inclusion criteria and were included in the meta-analysis.

3.2 Study characteristics

Table 1 indicates the characteristics of the pooled trials. According to our surveys, all studies were published between the years 2006–2024. Nine clinical trials had a randomized, double-blind, placebo-control design, while the remaining four trials had only randomized control design without double blinding.

The population of participants in the studies included healthy individuals, obese patients undergoing OAGB/RYGB, hyperlipidemia patients, type 2 diabetes patients and patients with metabolic syndrome. The study participants were from Egypt, Turkey, Italy, France, Germany, Poland, Iran, and Brazil, with ages ranging from 11 to 85 years. Sample sizes for all 11 studies ranged from 11 to 38. The total sample size for the nine studies included in the meta-analysis for homocysteine was 395 participants. The total sample size for the six studies included in the meta-analysis for folate and VB12 was 300 and 330 participants, respectively. The duration of probiotic intervention for the trials was between 42 and ~ 180 days. There was wide variation in delivery of the probiotic, ranging from capsules, tablets, powder, yogurt or fermented milk. Probiotic composition included *L. casei*, *L. rhamnosus*, *L. bulgaricus*, *L. acidophilus*, *L. salivarius*, *L. paracasei*, *L. plantarum*, *B. breve*, *B. longum*, *B. animalis subsp. lactis*, *B. bifidum*, *B. lactis*, *B. bacterium infantis* and *S. thermophiles*. Nine studies determined the change of homocysteine, six studies measured folate values and six measured levels of VB12 (Table 1).

Table 1
Study characteristics

Study	Study Design	Duration	Population /group	Country	Age (years)	Intervention /probiotic strains	Delivery Format	Dose (daily)	Hcy/FA/VB12
Mahmoud 2006[22]	RCT	42 days	Healthy children: Probiotic=12 Placebo = 12	Egypt	11	Probiotic: yogurt with *L. acidophilus* (La1), *L. acidophilus* (La1), $5*10^9$ CFU Control: yogurt without probiotic	Yogurt	2 cups	Hcy↓ FA↑ VB12↑
Valentini 2015[23]	RCT	56 days	Healthy persons: Probiotic=31 Placebo = 31	France Germany Italy	65–85	Probiotic: probiotic blend (*B. bacterium infantis* DSM 24737, *B. longum* DSM 24736, *B. breve* DSM 24732, *L. acidophilus* DSM 24735, *L. delbrückii* ssp. bulgaricus DSM 24734, *L. paracasei* DSM 24733, *L. plantarum* DSM 24730 and *S. thermophilus* DSM 2473),$1.12*10^{11}$CFU Control: without addition of probiotic	Capsule	2 capsules	Hcy↓ FA↑
Karbaschian 2018[24]	RDBPC	112 days	Obese patients undergoing OAGB-MGB surgery: Placebo=23 Probiotic = 23	Iran	18–60	Probiotic: 7 probiotic bacteria (*L. casei*, *L. rhamnosus*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B. longum*, and *L. bulgaricus* and 38.5 mg fructo-oligosaccharide Control: same amount of maltodextrin	Tablet	1 tablet	Hcy↓ FA↑ VB12↑
Majewska 2020[25]	RDBPC	84 days	Obese women: Placebo=25 Probiotic = 25	Poland	45–70	Probiotic: *B. bifidum* W23, *B. lactis* W51, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *L. lactis* W19 and *L. lactis* W58, 2.5×10^9 CFU/g Control: no probiotic	Powder	2 sachets (4 g)	Hcy↓
Okburan (a) 2024 [26]	RDBPC	56 days	Patients with hyperlipidemia: Placebo=16 Probiotic = 18	Turkey	30–64	Probiotic: $1*10^6$ CFU *L. rhamnosus* GG, Control: emptied capsule	Capsule	1 capsule	Hcy↓
Okburan (b) 2024 [26]	RDBPC	56 days	Patients with hyperlipidemia: Placebo=16 Probiotic = 17	Turkey	30–64	Probiotic group: $1*10^9$ CFU *L. acidophilus* and $1*10^9$ CFU *B. animalis* subsp.lactis Control: emptied capsule	Capsule	1 capsule	Hcy↓
Bellikci-Koyu 2022[27]	RCT	84 days	Patients with metabolic syndrome: Probiotic=31 Placebo = 31	Turkey	18–65	Probiotic: fermented milk (Lactobacillus spp., Leuconostoc spp., and Lactococcus spp, 10^6 CFU/g)	Fermented milk	180 ml	Hcy↓

Notes: study design: RDBPC, randomized, double-blind placebo control; RCT, randomized control trial. RYGB: Roux-en-Y gastric bypass; OAGB-MGB: One Anastomosis Gastric Bypass-Mini Gastric Bypass; ↑ statistically significant increase, ↓statistically significant decrease; ← no statistically significant change. L. = Lactobacillus, B. = Bifidobacterium, S. = Streptococcus. Hcy=homocysteine

Study	Study Design	Duration	Population /group	Country	Age (years)	Intervention /probiotic strains	Delivery Format	Dose (daily)	Hcy/FA/VB12
						Control: non-fermented milk			
Barreto 2014[28]	RCT	90 days	Patients with metabolic: Probiotic=12 Placebo = 12	Brazil	58–76	Probiotic: fermented milk (*L. plantarum*, $1.25*10^7$ UFC/g) Control: non-fermented milk	Fermented milk	80 mL	Hcy↓
Alihosseini 2017[29]	RDBPC	56 days	Patients with type 2 diabetes: Probiotic1 = 30 Probiotic2=30	Iran	35–65	Probiotic: probiotic (*S. thermophiles*, *L. bulgaricus, L. casei*, *L.acidophilus* and *B. lactis*) fermented milk Control: conventional fermented milk contained *S. thermophiles* and *L. bulgaricus*	Fermented milk	600 ml	Hcy↓
Ramos 2021[30]	RDBPC	90 days	Obese patients undergoing RYGB: Placebo=33 Probiotic = 38	Brazil	26–54	Probiotic: $5*10^8$CFU *L. acidophilus* NCFM® Strain, $5*10^8$CFU *B. lactis* Bi-07® Control: manipulated tablet consisting of starch and lactose.	Tablet	2 tablets	FA← VB12↑
Bardosono 2018[31]	RDPC	~ 180 days	Pregnant women placebo=22 probiotic = 55	Indonesia	18–35	Probiotic: milk powder with *B. animalis* subsp. lactis DR10TM Control: milk powder	Milk	200 mL	FA← VB12↑
Mokhtari 2019[32]	RDBPC	120 days	Obese patients undergoing OAGB: Placebo=25 Probiotic = 25	Iran	18–60	Probiotic: *L. casei* (3.5×10^9 CFU/g), *L. rhamnosus* (7.5×10^8 CFU/g), *S. thermophiles* (1×10^8 CFU/g), *B. breve* (1×10^{10} CFU/g), *L. acidophilus* (1×10^9 CFU/g), *B. longum* (3.5×10^9 CFU/g), and *L. bulgaricus* (1×10^8 CFU/g) and 38.5-mg fructooligosaccharide. Control: maltodextrin	Capsule	1 capsule	VB12↑
Ballini 2020[33]	RDBPC	56 days	Pregnant women: placebo=10 probiotic = 10	Albania		Probiotic: B. infantis, L. rhamnosus, L. plantarum, L. fermentum, L. reuteri, and L. acidophilus, $5*10^8$CFU, and actinidin powder Control: actinidin powder	Tablet	1 tablet	FA↑

Notes: study design: RDBPC, randomized, double-blind placebo control; RCT, randomized control trial. RYGB: Roux-en-Y gastric bypass; OAGB-MGB: One Anastomosis Gastric Bypass-Mini Gastric Bypass; ↑ statistically significant increase, ↓statistically significant decrease; ← no statistically significant change. L. = Lactobacillus, B. = Bifidobacterium, S. = Streptococcus. Hcy=homocysteine

3.3 Risk of bias and publication bias

According to the RoB 2.0 assessment, six of the 13 included studies were judged as having some concerns or high risk of bias in the domain bias due to deviations from intended interventions, primarily because blinding of participants and personnel was not reported (Fig. 2). Among them, two studies were judged as having some concerns in the domain bias arising from the randomization process due to insufficient reporting of random sequence generation. The remaining randomized controlled trials were judged as having a low overall risk of bias.

Egger's test and funnel plot analysis were conducted to evaluate potential publication bias. The funnel plot demonstrated an approximately symmetrical distribution (Supplementary Figure S1), and Egger's test revealed no statistically significant evidence of small-study effects or publication bias (p = 0.7211)

3.4 Probiotic supplementation on homocysteine

Nine studies with a total of 395 subjects were included in the meta-analysis of homocysteine concentrations. Of these subjects, 199 were in the probiotic group, and 196 were in the placebo group. All the included 9 studies reported a statistically significant reduction in homocysteine levels following probiotic supplementation (Table 1). Random-effects meta-analysis showed that probiotic supplementation significantly reduced the homocysteine levels (MD: -1.61, 95% confidence interval: -2.61 to -0.62, P = 0.0058), with substantial heterogeneity across studies (I^2 = 71.5%).

To investigate potential sources of heterogeneity, a leave-one-out sensitivity analysis was performed by sequentially excluding each study. Exclusion of the study by Mahmoud et al.[22], resulted in a marked reduction in heterogeneity, with the I^2 statistic decreasing from 71.5% to 30.5%, indicating that this study was a major contributor to the observed heterogeneity. Importantly, the pooled effect estimate remained statistically significant (P = 0.0022) regardless of whether the Mahmoud et al. study was excluded, thereby confirming the robustness of the findings. (Fig. 4)

3.5 Probiotic supplementation on folate and vitamin B12

Six studies (300 subjects) evaluated serum folate levels following probiotic intervention. Of these, four reported a statistically significant increase in folate concentrations in probiotic group, whereas two studies observed no significant change (Table 1). Random-effects meta-analysis showed no significant difference between the two groups (MD: 0.69, 95% confidence interval: -0.22 to 1.62, P = 0.1099) (Fig. 5A).

Six studies (330 subjects) provided VB12 data, and the random meta-analysis showed a significant improvement of VB12 in probiotic group by 39.13 pg/ml (95% confidence interval: 14.41 to 63.85, P = 0.0096), accompanied by substantial between-study heterogeneity (I^2 = 67.5%) (Fig. 5B). Similarly, leave-one-out sensitivity analysis showed that exclusion of the study by Mahmoud et al. reduced the I^2 statistic to 0%, suggesting that this study was the primary source of the observed heterogeneity (Fig. 6).

4. Discussion and conclusion

This meta-analysis represents the first systematic evaluation of the effects of probiotic supplementation on homocysteine metabolism, with particular focus on folate and vitamin B12 (VB12) status. Overall, the pooled evidence from randomized controlled trials indicate that probiotic supplementation significantly reduces circulating homocysteine levels in humans. The observed mean reduction in homocysteine was − 1.61 μmol/L (95% confidence interval: −2.61 to − 0.62; P = 0.0058), suggesting a moderate but potentially clinically meaningful effect. This result is consistent with previous evidences from animal studies demonstrating a homocysteine-lowering effect of probiotics [34, 35]. Given the well-established role of elevated homocysteine as a risk factor for cardiovascular and metabolic disorders, our results extend prior preclinical observations by providing quantitative clinical evidence that probiotic supplementation can modulate homocysteine metabolism in humans.

Folate and VB12 play essential roles in homocysteine metabolism, and deficiencies in either vitamin are well-established contributors to elevated homocysteine levels [36]. Extensive evidence demonstrates that supplementation with folate and VB12 effectively lowers homocysteine concentrations in humans [37]. Therefore, we speculated that the homocysteine-lowering effect of probiotic supplementation observed in this meta-analysis may be mediated, at least in part, through improvements in folate and/or VB12 status.

Certain probiotic strains have the capacity to synthesize B vitamins, including folate and VB12 [12]. Animal studies have shown that administration of folate-producing probiotics can enhance host folate levels [34, 35]. Among the 13 randomized controlled trials included in this review, six reported the change of folate data after probiotic supplementation. However, no significant increases in folate levels was observed after random meta-analysis. In contrast, meta-analysis of six trials showed a significant increases in VB12 levels following probiotic innervation (MD: 39.13 39.13 pg/mL; 95% confidence interval: 14.41 to 63.85, P = 0.0096). These findings suggest that probiotic supplementation may preferentially improve VB12 status and thereby contribute to enhanced homocysteine metabolism.

Beyond direct vitamin synthesis, probiotics may influence VB12 status through modulation of the gut microbiota. In silico analyses suggest that the human gut microbiome has the potential to produce a substantial proportion of the recommended daily intake of folate and VB12, thereby contributing to host B-vitamin homeostasis[7, 14]. Gut microbiota dysbiosis resulting from antibiotic exposure, pharmacological treatments, or chronic disease has been associated with micronutrient deficiencies, including B vitamins [15]. Additionally, pilot clinical studies indicate that probiotic supplementation may enhance vitamin bioavailability, improvements in gut barrier function and intestinal absorption [30]. Despite these plausible mechanisms, further basic and translational studies are required to clarify how probiotics regulate host VB12 levels and to delineate their direct contribution to homocysteine metabolism.

A relatively high degree of heterogeneity was observed in the meta-analyses of both homocysteine (I^2 = 71.5%) and VB12 (I^2 = 67.5%). Sensitivity analyses, conducted by sequential exclusion of individual trials, showed the heterogeneity appears to be largely driven by a single outlier study conducted by Mahmoud et al. [22]. Exclusion of this study reduced heterogeneity from 71.5% to 30.5% in the homocysteine analysis and from 67.5% to 0% in the VB12 analysis, while preserving statistically significant overall effects. In that trial, probiotic supplementation resulted in a markedly greater

improvement of VB12 (86.2 ± 26.42 pg/ml) and a more pronounced reduction in homocysteine concentrations (− 4.8 ± 1.28 μmol/L) compared with the control group (− 0.89 ± 1.12 μmol/L).

The magnitude of effect observed in this study may be explained by characteristics of the study population. The trial was conducted in Egyptian children (mean age: 11 years) who exhibited substantially higher baseline homocysteine levels (16.89 ± 1.12 μmol/L) than participants in other included studies (< 13.76 ± 8.6 μmol/L). Individuals with elevated baseline homocysteine may experience greater absolute reductions following intervention.

Limitations of this meta-analysis should be acknowledged. First, because of the limitation of resources, only studies published in English language were included in this systematic review. The bias of the included studies may have affected the results

of the meta-analysis. For example, four studies did not use blinding and all of the studies were lacking in justification of sample size. Moreover, the studies included in this review were widely heterogeneous in terms of participant age, probiotic species, dosage, intervention duration, and form of administration. These factors may results in high heterogeneity and have affected the overall results of the meta-analysis. More rigorously designed, randomized, blinded, and adequately powered trials using standardized probiotic formulations are needed to confirm these findings. In additon, only two clinical trials concurrently measured homocysteine alongside folate and/or VB12 levels. limiting the ability to draw firm causal inferences. Addressing these gaps will require large-scale, well-designed prospective trials that simultaneously evaluate probiotic-induced changes in B-vitamin status and homocysteine metabolism.

Despite these limitations, this meta-analysis provides novel evidence that probiotic supplementation may represent a promising strategy for lowering homocysteine levels and improving VB12 status. These findings have potential clinical implications for the prevention and management of homocysteine-related conditions and warrant further investigation in rigorously designed human studies.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consented to the publication of the manuscript.

Availability of data and material

All data and material reported in this review and meta-analysis were from peer-reviewed publications. The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

No potential conflicts of interest were disclosed.

Authors' contributions

MFP and CMY conceived and designed the study and developed the search strategy. MFP, CMY, and XXZ independently performed the abstract and full-text screening, assessed methodological quality and risk of bias, and completed data extraction for all included studies. MFP, LSL, and QQH conducted the meta-analyses, sensitivity analyses, and publication bias assessments, and prepared the figures and tables. MFP and QQH drafted the initial manuscript and revised and finalized the manuscript. XHQ, YS, and RMW contributed to the critical interpretation of the results and provided substantial intellectual input to the manuscript. PC and MQT participated in editing and revising the final version of the manuscript. All authors read and approved the final manuscript and agreed with the authors' theoretical statements.

Acknowlegements

This work was supported by the Science, Technology and Innovation Committee of Shenzhen, Major Special Program (Grant No. KJZD20230923114408018): R& D of Gut Microbiota-Based Probiotic for Stroke Prevention. The funding agency had no involvement in study design, data interpretation, or manuscript preparation.

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Figures

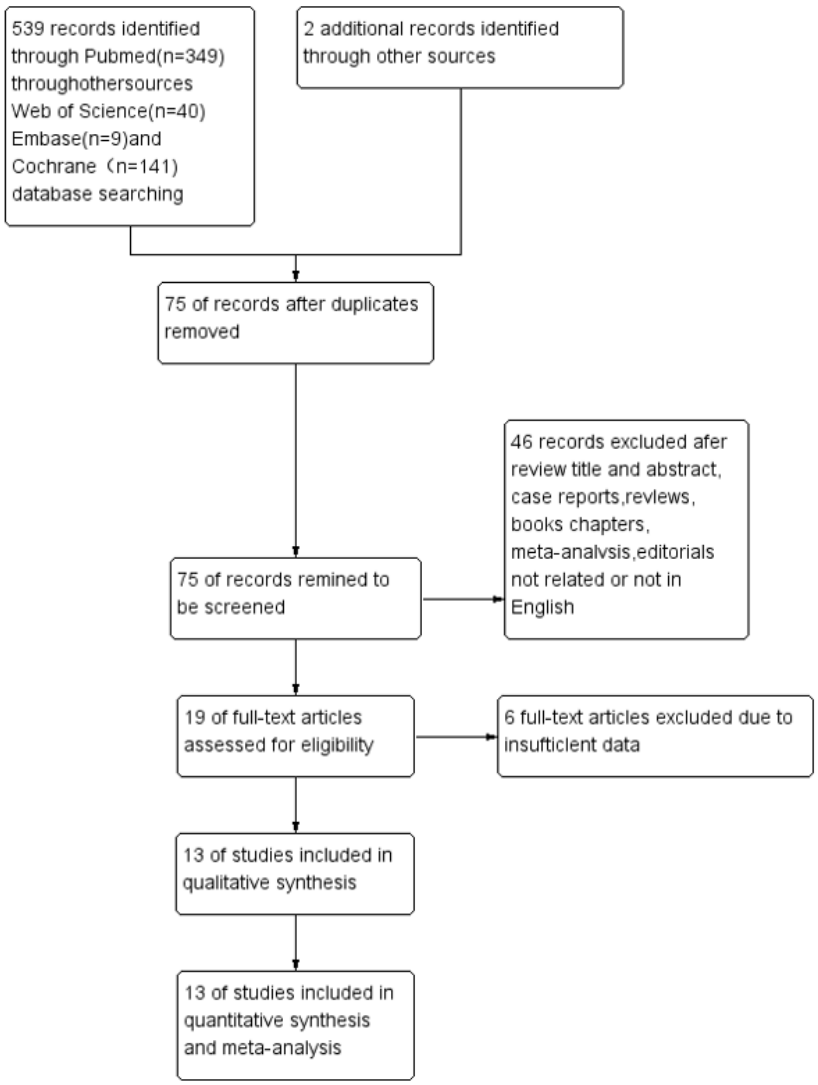


Figure 1

PRISMA flow diagram of the systematic review.

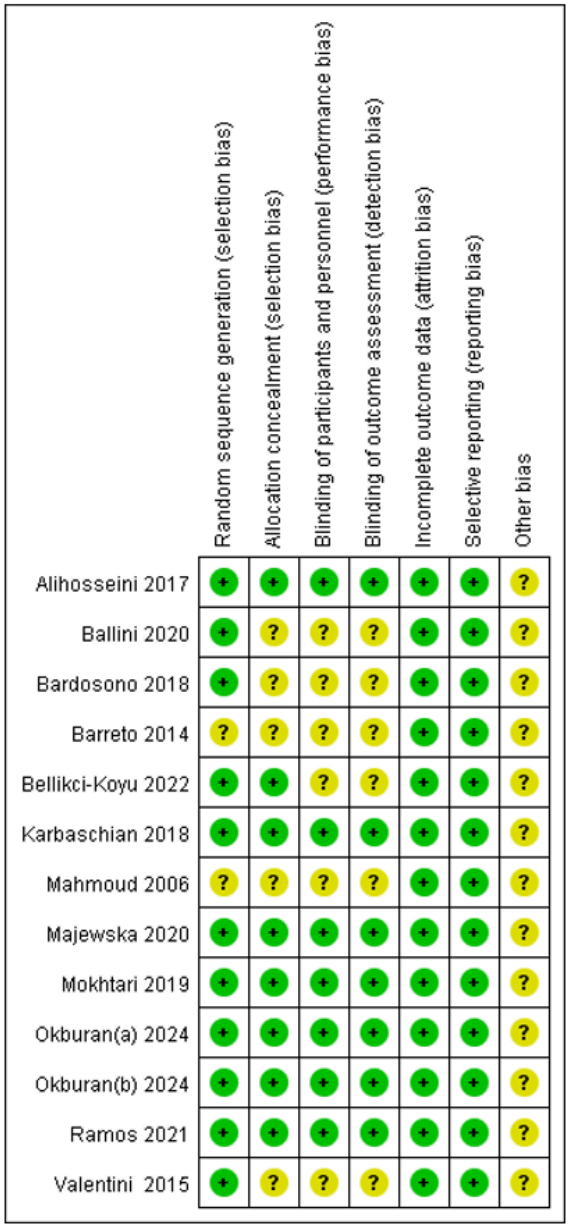


Figure 2

Quality assessment for RCT studies. Green circle with plus sign = low risk of bias; yellow circle with question mark = unclear risk of bias; red circle with minus sign = high risk of bias. (a) (b) Reports two studies in one article.

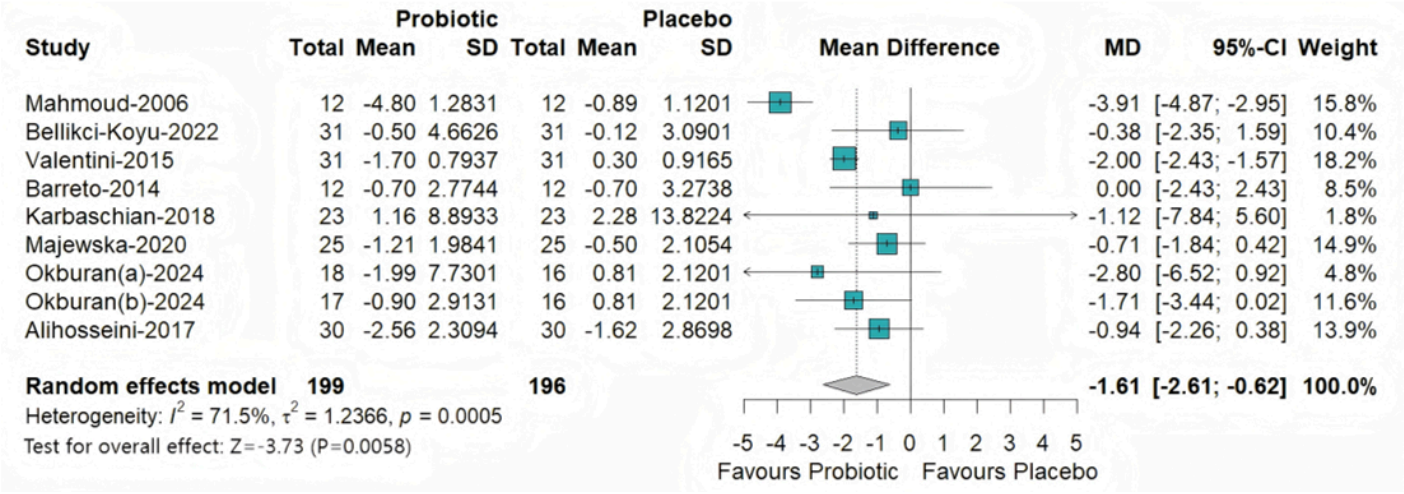


Figure 3

Forest plot of randomized controlled trials investigating the effects of probiotic supplementation on homocysteine. MD: mean difference. CI: confidence interval; I-squared: variation in MD attributable to heterogeneity, I^2 calculated based on Q statistic; p value was associated with Q statistic; the Hartung-Knapp method was used for adjustment of the random effects model.

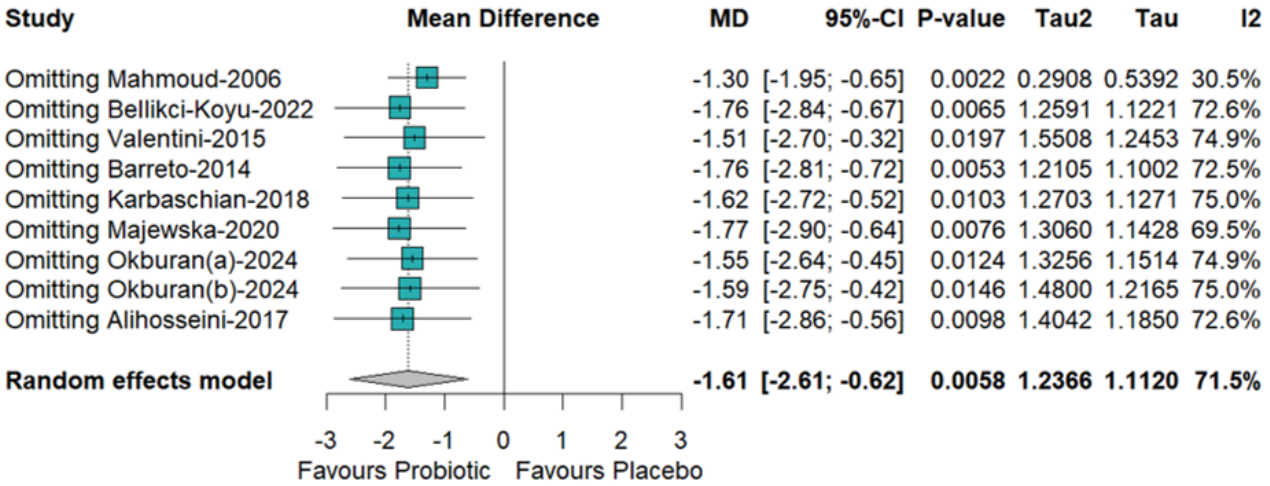


Figure 4

Sensitivity analysis of the mean difference (MD) for homocysteine changes.MD: mean difference. CI: confidence interval; I-squared: variation in MD attributable to heterogeneity, I^2 calculated based on Q statistic; p value was associated with Q statistic; the Hartung-Knapp method was used for adjustment of the random effects model.

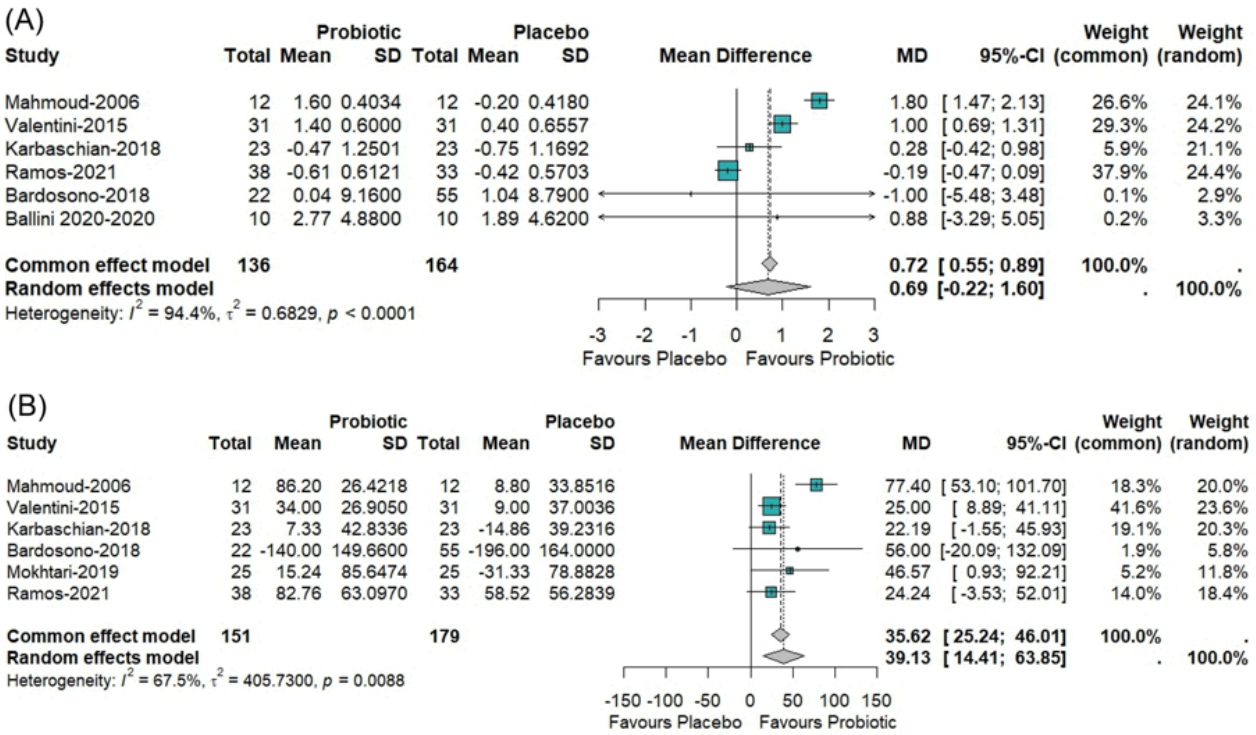


Figure 5

Forest plot of randomized controlled trials investigating the effects of probiotic supplementation on folate(A) and VB12(B). MD: mean difference. CI: confidence interval; I-squared: variation in MD attributable to heterogeneity, I^2 calculated based on Q statistic; p value was associated with Q statistic; the Hartung-Knapp method was used for adjustment of the random effects model.

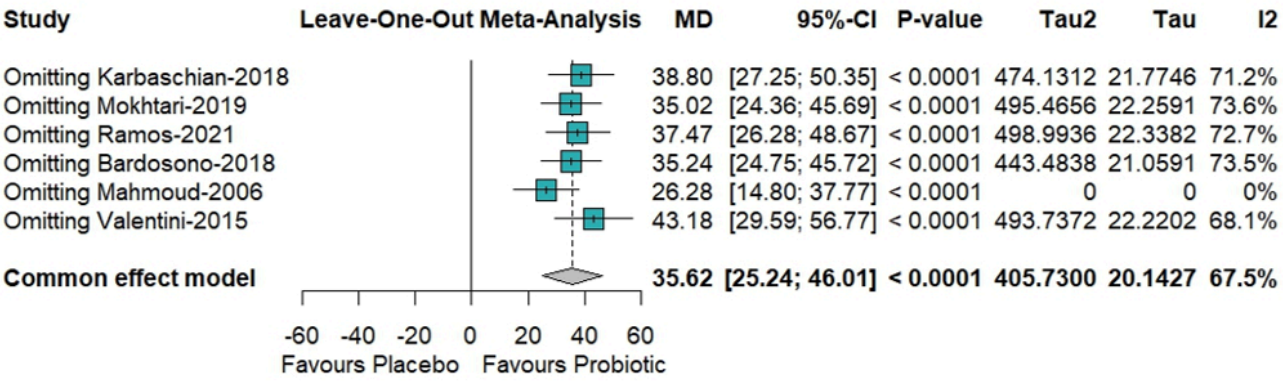


Figure 6

Sensitivity analysis of the mean difference (MD) for VB12 changes.MD: mean difference. CI: confidence interval; I-squared: variation in MD attributable to heterogeneity, I^2 calculated based on Q statistic; p value was associated with Q statistic; the Hartung-Knapp method was used for adjustment of the random effects model.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AppendixA.docx