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Effectiveness of Probiotic Supplementation in Enhancing Iron Therapy for Iron Deficiency Anemia in Children: A Systematic Review and Meta Analysis

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EFFECTIVENESS OF PROBIOTIC SUPPLEMENTATION IN ENHANCING IRON THERAPY FOR IRON DEFICIENCY ANEMIA IN CHILDREN: A SYSTEMATIC REVIEW AND META ANALYSIS

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All the data were obtained from publicly available sources. Relevant data supporting the findings of this study are included in the manuscript and supplementary material. Additional details can be shared upon reasonable request to the corresponding author.

AUTHOR'S CONTRIBUTION

Fahad Amin contributed to the conceptualization and topic selection of the study, reviewed the study design, supervised the project, performed the data analysis, and carried out the GRADE assessment; **Mehreen Hameed** served as co-lead author, contributed to project supervision, performed data curation, reviewed the manuscript, and wrote the abstract; **Muhammad Ubaidullah Arshad** performed data extraction, conducted the risk of bias assessment, and wrote the results section; **Meer Hassan Khalid** developed the search strings, conducted the literature search, and wrote the discussion section; **Meryeme Sahraoui** performed data extraction, wrote the introduction, and prepared the baseline characteristics tale; **Dalia Mehmood** contributed to manuscript compilation, formatting,

final **integration; Sofia Intikhab** contributed to study screening and prepared the PROSPERO registration; **Muskan Lohana** developed the data extraction sheet and wrote the methods section; **Aamna Ahmad** contributed to study screening and prepared the PRISMA flow diagram; **Ayesha Ihsan** reviewed the entire manuscript; and **Syed Anas Hussain** also reviewed the manuscript.

ABBREVIATIONS

WHO – World Health Organization
Hb – hemoglobin
ID – iron deficiency
BRINDA – Biomarkers reflecting inflammation and nutritional determinants of anemia
IDA – iron deficiency anemia
CFU – colony-forming units
hs-CRP – high-sensitivity C-reactive protein
AGP – alpha-1-acid glycoprotein
MPO – myeloperoxidase
AAT – alpha-1-antitrypsin
I-FABP-2 – intestinal fatty acid–binding protein 2
AEs – adverse events
MCV – mean corpuscular volume
sTfR – soluble transferrin receptor
CRP – C-reactive protein
PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RoB 2 – Cochrane Risk of Bias 2 tool
MD – Mean difference
RR – Risk ratio
CI – Confidence interval
HK – Hartung–Knapp

ABSTRACT

Background:

Iron deficiency anemia (IDA) remains a leading cause of childhood morbidity worldwide, and standard oral iron therapy is frequently limited by poor gastrointestinal tolerability and suboptimal adherence. Probiotic cosupplementation has been proposed as an adjunctive strategy to increase iron absorption and improve the tolerability of iron therapy. This meta-analysis aimed to evaluate the effectiveness and safety of probiotic supplementation combined with iron therapy versus iron therapy alone in children with IDA.

Methods:

A systematic literature search of PubMed, Embase, Scopus, clinical trials and the Cochrane Library was conducted from inception until 12 July 2026 to identify randomized controlled trials comparing probiotic-supplemented iron therapy with iron therapy alone in children with IDA. Two reviewers independently screened studies, extracted data and assessed outcomes, including changes in hemoglobin, changes in serum ferritin, resolution of iron deficiency, resolution/prevalence of IDA, treatment compliance, and adverse events (overall and gastrointestinal). The mean differences (MDs), risk ratios (RRs), and 95% confidence intervals (CIs) were pooled via random effects models. The risk of bias was assessed via the Cochrane Rob 2 tool.

Results:

Six studies comprising 799 children met the inclusion criteria. Compared with iron therapy alone, probiotic cosupplementation significantly improved serum ferritin (MD 6.08, 95% CI 4.54–7.62, P<0.0001) and reduced adverse events (RR 0.62, 95% CI 0.43–0.91, P=0.0139). No statistically significant differences were observed for change in hemoglobin (MD 0.68, 95% CI −0.55 to 1.90, P=0.2800) or treatment success (RR 1.08, 95% CI 0.99–1.17, P=0.0786). Considerable heterogeneity was observed across several outcomes.

Conclusion: Probiotic supplementation in combination with iron therapy may improve serum ferritin levels and significantly reduce treatment-related adverse events in children with IDA. However, it does not significantly improve hemoglobin or overall treatment success.

INTRODUCTION

Iron deficiency anemia (IDA) remains a major global health problem in children and represents an important manifestation of iron deficiency during a critical period of growth and development. According to the World Health Organization (WHO), approximately 40% of children aged 6–59 months worldwide were affected by anemia in 2019, corresponding to an estimated 269 million children. Although anemia has multiple etiologies, iron deficiency is recognized as one of its most common nutritional causes [1].

Recent evidence indicates that IDA also remains prevalent among school-aged children, with a pooled prevalence of 9.4% (95% CI, 6.5–12.7%) among children aged 5–12 years, with a substantially higher prevalence reported in sub-Saharan Africa and South Asia [2]. In addition to its hematological manifestations, prolonged or severe iron deficiency during early childhood has been associated with adverse neurodevelopmental outcomes, including cognitive, motor, and behavioral impairments [3]. Therefore, effective prevention and treatment of iron deficiency and anemia during childhood remain important therapeutic and public health priorities.

Oral iron is generally recommended as the initial treatment for IDA in children when oral therapy is appropriate. Among oral preparations, ferrous sulfate and other oral iron salts are widely used because of their established efficacy, accessibility, and relatively low cost [4]. However, the therapeutic response to oral iron may be influenced by several factors, including the dose and formulation of iron, intestinal absorption, treatment duration, nutritional status, and patient adherence [4, 5]. Adverse gastrointestinal effects, including abdominal pain, nausea, diarrhea, and constipation, are commonly reported and may negatively affect tolerability and adherence, particularly when prolonged treatment is needed [4, 5]. These limitations have prompted interest in adjunctive strategies that could improve the effectiveness and tolerability of oral iron therapy without compromising safety.

Iron supplementation may also affect the intestinal microbiota and the gut environment. Available evidence suggests that supplemental iron, particularly unabsorbed luminal iron, can alter the composition and diversity of the intestinal microbiota. However, the direction and clinical relevance of these effects appear to vary according to iron dose, age, nutritional status, baseline microbiota, and other host and environmental factors [6, 7]. These observations have generated interest in the intestinal microbiota as a potential modulator of iron availability and host iron metabolism.

The intestinal microbiota may influence the intestinal environment through several mechanisms, including modification of the luminal pH, production of short-chain fatty acids,

and interactions with dietary and supplemental iron, potentially affecting the solubility and bioavailability of nonheme iron [7, 8]. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host [9]. Accordingly, specific probiotic strains have been investigated as potential adjuncts to oral iron therapy. Evidence suggests that certain strains may influence iron availability or absorption; however, these effects appear strain–specific and may not be generalizable to probiotics [8-10]. A systematic review and meta-analysis revealed increased iron absorption with Lactiplantibacillus plantarum 299v, whereas evidence for improvements in broader iron status indices was more limited [10].

Previous systematic reviews and meta-analyses have evaluated probiotics and related microbiota-directed interventions in anemia and iron deficiency [10-12]. However, these reviews have differed substantially in their populations, interventions, comparators, probiotic preparations, iron regimens, and outcome measures. A recent systematic review and meta-analysis specifically evaluated the use of probiotics combined with iron supplementation in children with IDA but was restricted to Chinese populations [13]. This population restriction may limit the generalizability of its findings to other pediatric settings. Therefore, a focused synthesis of randomized controlled trials evaluating probiotic supplementation as an adjunct to oral iron therapy across pediatric populations is warranted.

We conducted this systematic review and meta-analysis to evaluate the efficacy and safety of probiotic supplementation combined with iron therapy compared with iron therapy alone in children with IDA.

METHODS

2.1. Protocol and Registration

The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [14]. The review was prospectively registered on PROSPERO with the following ID (CRD420261483051) [14].

2.2. Search strategy

Two separate reviewers independently conducted a systematic search in PubMed/MEDLINE, Embase, Scopus, the Cochrane Library and ClinicalTrials.gov from inception to July 12, 2026. For the search, three groups of controlled vocabulary terms were used along with free text and Boolean operators, with no restrictions on publication year, to retrieve articles related to iron deficiency anemia, probiotics, iron therapy, and the pediatric population. The PubMed search strategy used was a representative example: (“iron deficiency anemia” OR “iron deficiency anemia” OR IDA) AND (probiotic OR synbiotic OR Lactobacillus OR Bifidobacterium) AND (iron therapy OR iron supplementation OR oral iron) AND (child OR infant OR pediatric OR

adolescent). The strategy was modified for other databases, and manually screened reference lists of relevant studies and reviews were created.

2.3. Eligibility criteria

Studies were selected if they were randomized controlled trials (RCTs) that included probiotic supplementation with iron therapy and/or placebo products and if the studies compared them with conventional iron therapy and/or placebo products in children with IDA (as defined by the study). In studies, at least one of the predefined hematological, biochemical, treatment-response or tolerability outcomes had to be reported numerically.

Studies were removed if they included adults or lacked separate pediatric data, were animal/in vitro studies, lacked an eligible comparator arm, were case reports or case series, review/editorial/letters, or conference abstracts that presented poor-quality results, anemia from other causes, duplicate or overlapping publications, or had inadequate extractable data. Studies had to be in either English or have been fully translated into English.

2.4. Interventions and Outcomes

Probiotic+iron therapy was used as the intervention. When reported, the following information was extracted: probiotic strain, dose, iron formulation and dose, duration of intervention and follow-up. The primary outcomes were changes from baseline to the endpoint in hemoglobin and serum ferritin. The secondary endpoints were treatment success, changes in serum iron, transferrin saturation, other iron indices and adverse events, including gastrointestinal adverse events and tolerability of the treatment.

2.5. Selection of studies

Titles and abstracts were separately evaluated for eligibility by two reviewers, and then the full texts of potentially eligible studies were reviewed. Disagreements were resolved by discussion and consensus, with the advice of a third reviewer when needed. The process for study selection is described in the PRISMA 2020 flow diagram.

2.6. Data extraction and risk of bias assessment.

Study characteristics such as year, country, study design, study setting, sample size, age range, demographic and clinical characteristics, baseline hemoglobin and serum ferritin, strain and dose of the probiotic, dose of the iron formulation, duration of intervention and follow-up, method of randomization, method of blinding, and time points of outcome assessment were independently extracted by 2 reviewers. Means, standard deviations and sample sizes were extracted for continuous outcomes; the number of events and total number of participants were extracted for dichotomous outcomes. Differences were discussed and resolved with consensus or with the help of a third reader.

Bias as a result of the randomization process and deviations from intended interventions, missing outcome data, measurement of the outcome and selection of the reported result were evaluated in the five domains via the Cochrane Risk of Bias 2 (RoB 2) tool [15].

2.7. Data Synthesis and Analysis

Random effects meta-analyses were performed with the metafor package (version 4.5.2) in R, as it was expected that there would be clinical and methodological heterogeneity [17]. For dichotomous outcomes, the risk ratio or odds ratio was calculated, and for continuous outcomes, the mean difference or standardized mean difference was calculated, as appropriate. When appropriate, the Peto odds ratio method was used for rare dichotomous outcomes, as needed. If there were no events in either treatment group, the relative effects could not be calculated.

The conventional 95% confidence interval (CI) was reported, and when appropriate, the Hartung-Knapp (HK)-adjusted confidence intervals were used as a sensitivity approach [18]. Statistical heterogeneity was assessed via I^2 and τ^2, with I^2 values of 25%, 50%, and 75% considered low, moderate, and high heterogeneity, respectively [20]. Leave-one-out sensitivity analysis was carried out to evaluate the influence of single studies. For small-study effects and for fewer than 10 studies, publication bias was not present [19].

2.8. Certainty of Evidence

The GRADE approach was used to assess the certainty of evidence for each outcome: evidence from RCTs was initially classified as high certainty unless there were serious concerns, in which case it was downgraded. The certainty of evidence was independently coded by two reviewers, and disagreements were resolved by discussion with a third reviewer if necessary [16].

RESULTS

Study selection

The study selection process is presented in the PRISMA 2020 flow diagram (Figure 1). A total of 123 records were identified, comprising 112 records from electronic databases (PubMed, n = 12; Cochrane Library, n = 30; Scopus, n = 31; Embase, n = 39), 8 records from the clinical trial registry ClinicalTrials.gov, and 3 additional records identified through manual searching. Thirty-nine duplicate records were removed before screening, leaving 84 unique records for title and abstract screening. Among these, 73 records were excluded. Eleven reports were sought for retrieval, and all 11 were retrieved and assessed for eligibility. Five reports were excluded at the full-text stage. A total of six studies were therefore included in the systematic review and quantitative synthesis. **[Figure 1]**

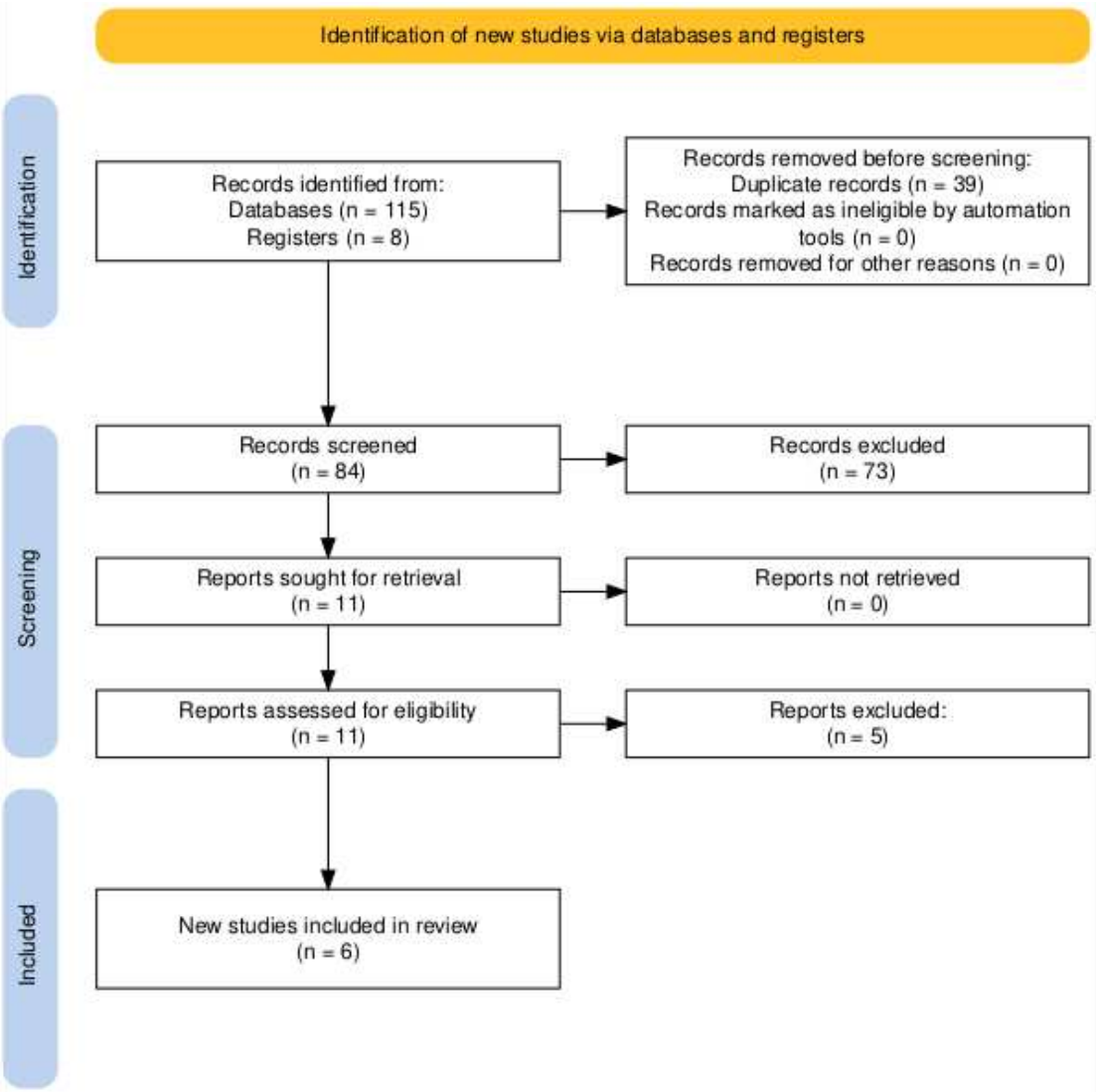


Figure 1. PRISMA 2020 flow diagram of the study selection process.

Included studies and participants

Across the included studies, 403 participants received probiotics with iron therapy (Biotics+) and 396 received iron therapy alone (Biotics−), totaling 799 participants. Four outcomes were quantitatively pooled: change in serum ferritin, change in serum hemoglobin, treatment success, and adverse events. The number of studies and participants differed by outcome because not all studies reported every endpoint. Random−effects models were used for all pooled analyses. **[Table 1]**

Table 1. Baseline characteristics of the included studies

First author	Year	Study design	Country	Sample size, n (intervention/control)	Age, years	Male (%)	Baseline Hb, g/dL	Baseline ferritin, ng/mL	IDA diagnostic criteria	Intervention (iron regimen + Probiotic strain and dose)	Control	Treatment duration, weeks	Outcomes reported
Palika	2026	Randomized, single-blind, placebo-controlled trial	India	248 (124/124)	3.55 ± 0.85	56.4	9.65 ± 1.05	4.23 ± 4.52	WHO Hb thresholds (anemia: Hb 7–10.9 g/dL for enrollment: severe <7, moderate 7.0–9.9, mild 10.0–10.9, normal ≥11 g/dL). Iron deficiency (ID) = inflammation-adjusted (BRINDA method) plasma ferritin <12 µg/L. IDA = ID + anemia present concurrently	Iron-folic acid (IFA) syrup: 3 mg/kg/day (ferrous sulphate, 20 mg/mL elemental iron + 100 µg/mL folic acid) + *Lactobacillus plantarum* UBLP40 (MTCC 5380): 10^{10} CFU/sachet/day	Iron-folic acid (IFA) syrup: 3 mg/kg/day (ferrous sulphate, 20 mg/mL elemental iron + 100 µg/mL folic acid) + placebo sachet	12.8	Hb ferritin sTfR inflammatory markers (hs-CRP, AGP) inflammation markers (fecal MPO, AAT, occult blood, plasma IFABP-2) prevalence of anemia/ID/IDA AEs (vomiting, diarrhea, fever, abdominal pain)
Chethan	2025	Prospective, parallel-group, randomized controlled trial	India	100 (50/50)	7.95 ± 3.35	54	8.25 ± 0.65	9.20 ± 3.35	Hemoglobin <11 g/dL, serum ferritin <15 ng/mL, and normal CRP	Oral ferrous sulfate: 3 mg/kg/day + Lactobacillus rhamnosus GG: 10^9 CFU/day	Oral ferrous sulfate alone: 3 mg/kg/day	8	Hb ferritin MCV reticulocyte count AEs (nausea, abdominal pain, constipation, diarrhea)
Suryawan	2025	Randomized, double-blind, placebo-controlled trial	Indonesia	100 (50/50)	-	45.7	12.78 ± 1.65	16.76 ± 7.83	-	Standard iron: 3 mg/kg/day + Saccharomyces boulardii 2.5x10^9 CFU/capsule (1 capsule/day <3y; 2 capsules/day for 3-18y)	Standard iron: 3 mg/kg/day + placebo (250 mg lactose powder capsules)	4.3	Ferritin Prevalence of ID
Rosen	2019	Randomized, double-blind, placebo-controlled trial	USA	52 (2725)	10.2 ± 4.2	-	-	23.7	iron deficiency defined as ferritin <50 ng/mL + CRP <0.3	Oral ferrous sulfate: 3 mg/kg/day + vitamin c: 250 mg for age >5 yrs, 125 mg for <5 yrs + *Lactobacillus plantarum* 299v (LP299v): 10^{10} CFU/day	Oral ferrous sulfate: 1.8 ± 0.8 mg/kg/day + vitamin c: 250 mg for age >5 yrs, 125 mg for <5 yrs + placebo	6-8	ferritin CRP AEs (abdominal pain, constipation, vomiting, diarrhea)
Yang	2015	Randomized controlled trial	China	152 (76/76)	0.5-5	-	-	-	Nutritional iron-deficiency anemia	Iron dextran + Biostime®	Iron dextran alone	8	Treatment efficacy/effective

First author	Year	Study design	Country	Sample size, n (intervention/control)	Age, years	Male (%)	Baseline Hb, g/dL	Baseline ferritin, ng/mL	IDA diagnostic criteria	Intervention (iron regimen + Probiotic strain and dose)	Control	Treatment duration, weeks	Outcomes reported
										probiotics 1 sachet BID			rate AEs
Wu	2014	Randomized controlled trial	China	147 (76/71)	0.5-5	-	-	-	Nutritional iron-deficiency anemia	Iron dextran + Biostime® probiotic 1 sachet QD	Iron dextran alone	8	Treatment efficacy/effective rate AEs

WHO, World Health Organization; Hb, hemoglobin; ID, iron deficiency; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; IDA, iron deficiency anemia; CFU, colony-forming units; hs-CRP, high-sensitivity C-reactive protein; AGP, alpha-1-acid glycoprotein; MPO, myeloperoxidase; AAT, alpha-1-antitrypsin; I-FABP-2, intestinal fatty acid–binding protein 2; AEs, adverse events; MCV, mean corpuscular volume; sTfR, soluble transferrin receptor; CRP, C-reactive protein

Summary of Findings

A summary of the findings of the meta-analysis is given in **Figure 2**. There was a statistically significant change in the serum ferritin level in the probiotic group but no significant improvement in the change in the serum hemoglobin level. Moreover, there was no difference between the two groups in terms of the success rate, but the probiotic group was associated with a significantly lower risk of adverse events than the nonprobiotic group was.

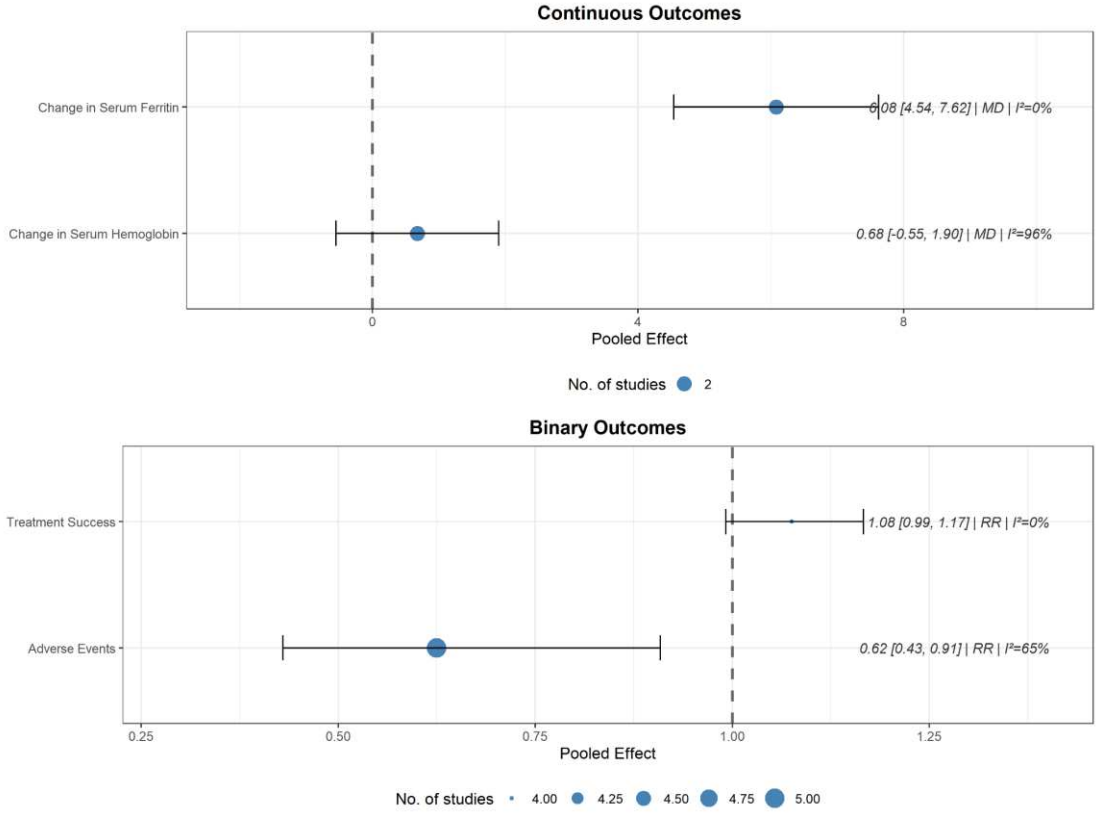


Figure 2. Overall summary forest plot.

Risk of bias in the included studies

The risk of bias was assessed across five RoB 2 domains: bias arising from the randomization process (D1), bias due to deviations from intended interventions (D2), bias due to missing outcome data (D3), bias in measurement of the outcome (D4), and bias in selection of the reported result (D5). Overall, five of the six included studies were judged to have some concerns, whereas one study (Yang et al., 2015) was judged to have a high overall risk of bias. No study was judged to have an overall low risk of bias. **[Figure 3] [Figure 4]**

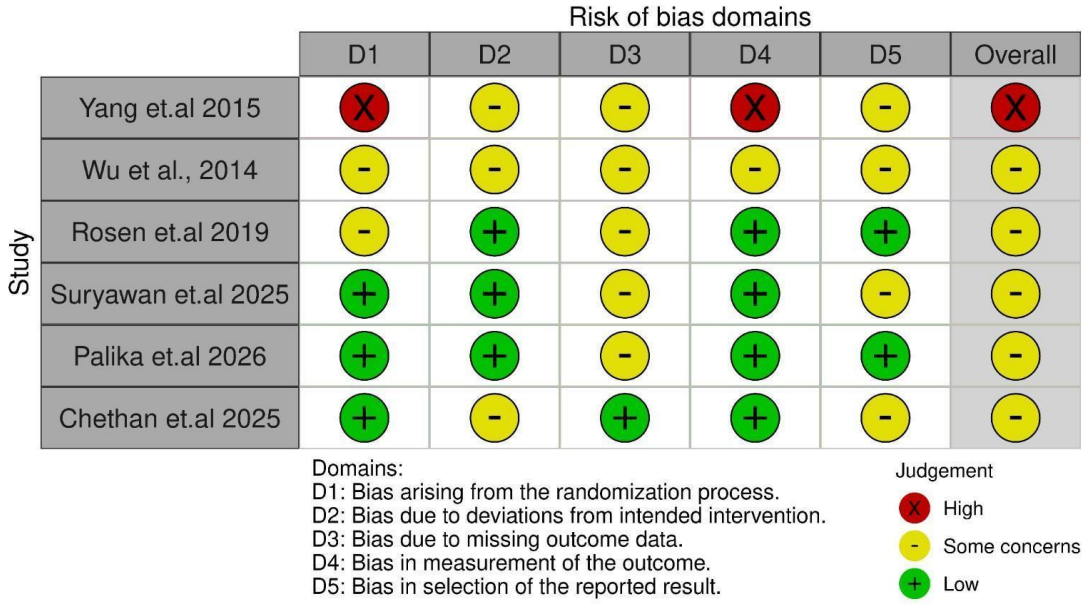


Figure 3. Risk-of-bias judgments across the included studies.

At the domain level, the randomization process (D1) was judged as low risk in three studies, some concerns in two studies, and high risk in one study. For deviations from intended interventions (D2), three studies were judged as low risk, and three had some concerns. For missing outcome data (D3), one study was judged as low risk, and five had some concerns. The measurement of the outcome (D4) was judged as low risk in four studies, some concerns in one study, and high risk in one study. For selection of the reported result (D5), two studies were judged as low risk, and four had some concerns. These judgments indicate that concerns were particularly concentrated in missing outcome data and selection of the reported result, whereas the main high-risk judgments occurred in the randomization and outcome measurements.

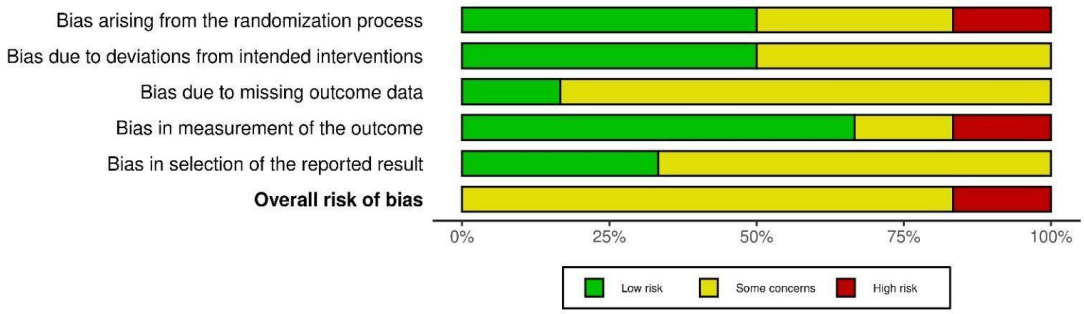


Figure 4. Distribution of risk-of-bias judgments across RoB 2 domains.

Meta-Analysis

Changes in serum ferritin

Two studies involving 200 participants (100 in each group) reported change in serum ferritin. The pooled mean difference was 6.08 (95% CI 4.54 to 7.62; p<0.0001). No statistical heterogeneity was detected (I^2 = 0%). The 95% prediction interval was −3.91 to 16.07. **[Figure 5]**

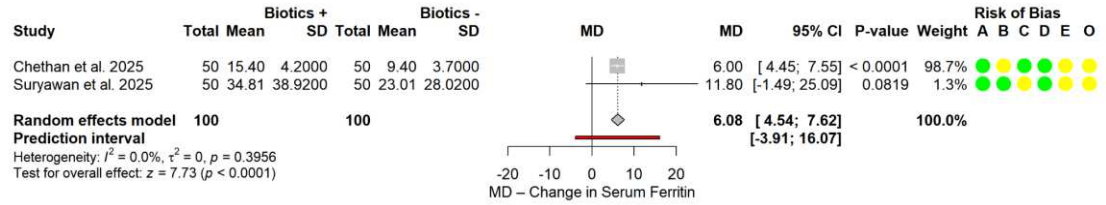


Figure 5. Forest plot for changes in the serum ferritin level.

Leave−one−out analysis showed that the pooled estimate remained positive after exclusion of either study. After omission of Chethan et al. (2025), the pooled MD was 11.80 (95% CI −1.49 to 25.09), whereas after omission of Suryawan et al. (2025), it was 6.00 (95% CI 4.45 to 7.55). **[Figure 6]**

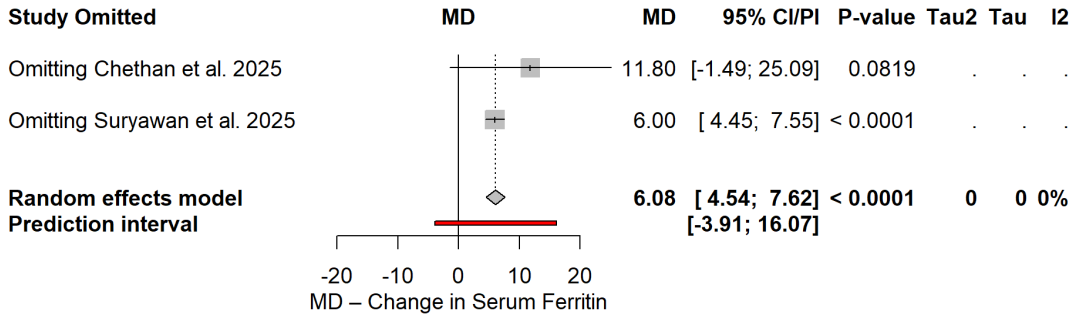


Figure 6. Leave-one-out sensitivity analysis for serum ferritin.

Changes in serum hemoglobin

Two studies including 348 participants (174 in each group) assessed change in serum hemoglobin. The pooled analysis showed no statistically significant difference between groups (MD 0.68, 95% CI −0.55 to 1.90; p = 0.2800). Considerable heterogeneity was present (I^2

= 96.2%, p<0.0001). The 95% prediction interval ranged from −12.91 to 14.26. The Hartung–Knapp adjusted 95% CI was −7.27 to 8.62. **[Figure 7]** **[Figure 8]**

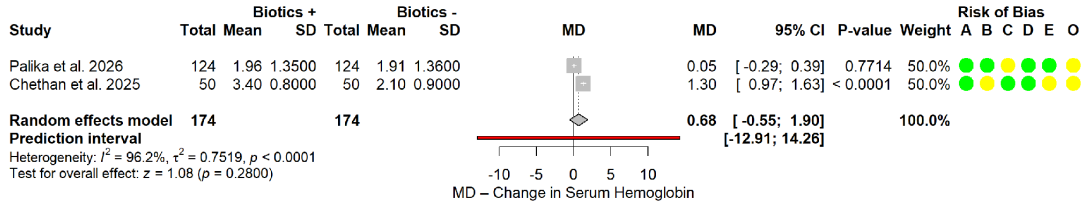


Figure 7. Forest plot for changes in serum hemoglobin.

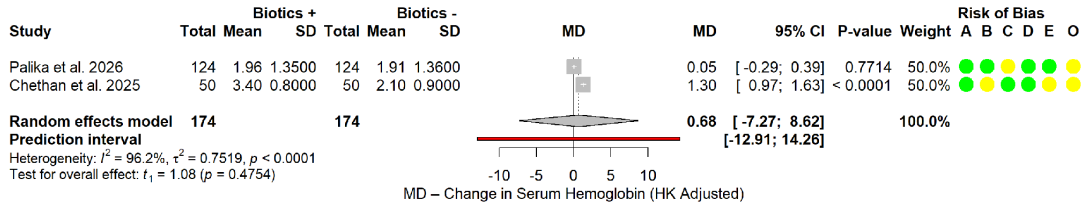


Figure 8. Hartung–Knapp adjusted forest plot for serum hemoglobin.

Leave-one-out analysis showed substantial sensitivity to individual studies. After omission of Palika et al. (2026), the pooled MD was 1.30 (95% CI 0.97 to 1.63), whereas after omission of Chethan et al. (2025), the pooled MD was 0.05 (95% CI −0.29 to 0.39). **[Figure 9]**

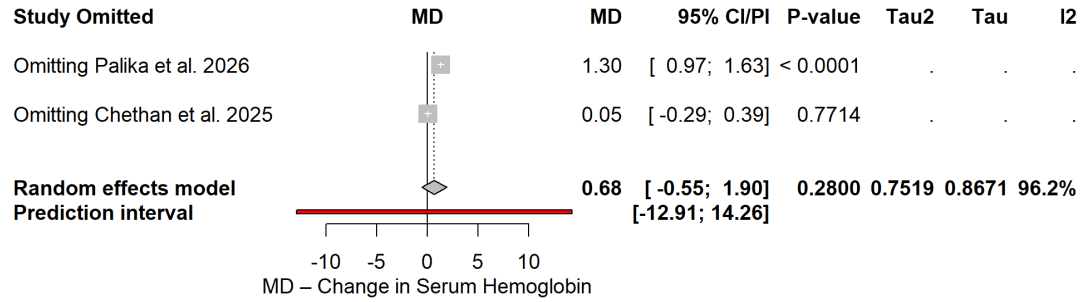


Figure 9. Leave-one-out sensitivity analysis for serum hemoglobin.

Treatment success

Four studies involving 647 participants (326 in Biotics+ and 321 in Biotics−) reported treatment success. Treatment success occurred in 223/326 (68.4%) and 208/321 (64.8%), respectively. The pooled risk ratio was 1.08 (95% CI 0.99 to 1.17; p = 0.0786), with no statistically significant difference between groups. No heterogeneity was detected (I^2 = 0%). **[Figure 10]**

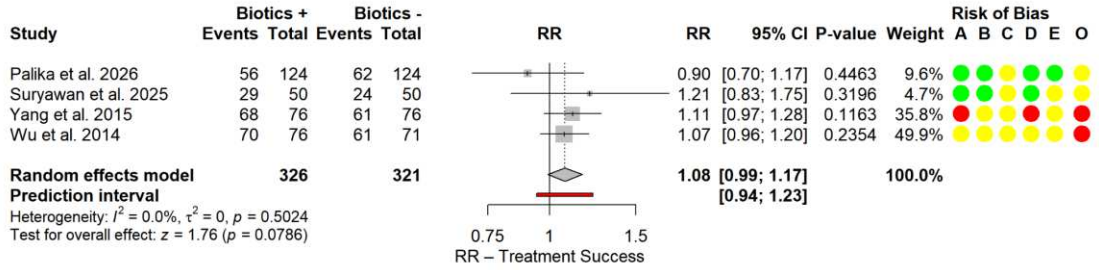


Figure 10. Forest plot for treatment success.

Leave-one-out analyses revealed that heterogeneity decreased to zero after the exclusion of Palika et al., and the effect of probiotics became statistically significant. **[Figure 11]**

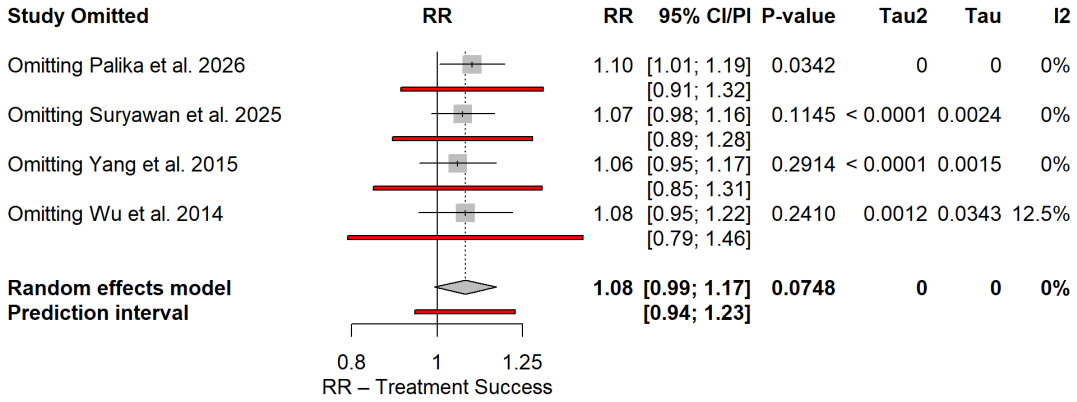


Figure 11. Leave-one-out sensitivity analysis for treatment success.

Adverse events

Five studies involving 699 participants (353 in Biotics+ and 346 in Biotics−) reported adverse events. Adverse events occurred in 87/353 (24.6%) versus 133/346 (38.4%). The pooled RR was 0.62 (95% CI 0.43 to 0.91; p = 0.0139), corresponding to an approximately 38% relative reduction in adverse-event risk. Moderate heterogeneity was observed (I^2 = 65.3%, p = 0.0212). The 95% prediction interval was 0.22 to 1.81 and the Hartung–Knapp adjusted 95% CI was 0.38 to 1.02. **[Figure 12] [Figure 13]**

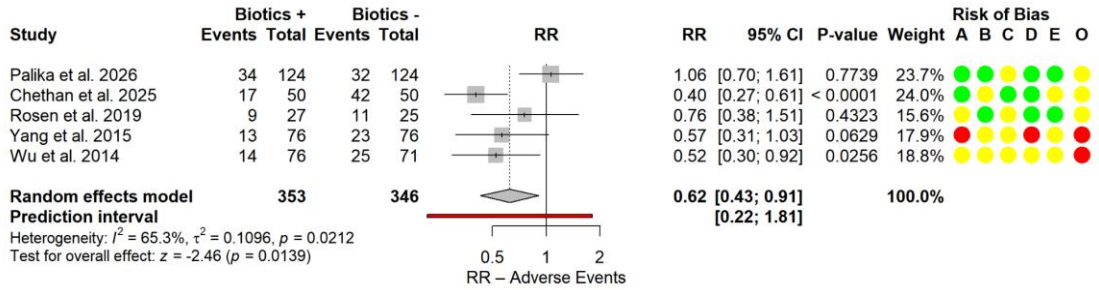


Figure 12. Forest plot for adverse events.

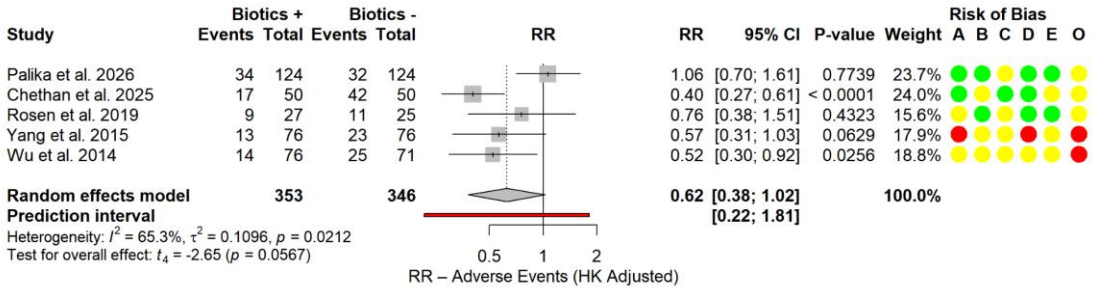


Figure 13. Hartung–Knapp adjusted forest plot for adverse events.

Leave-one-out analyses revealed that heterogeneity decreased to zero after the exclusion of Palika et al., and the effect of probiotics became statistically significant. **[Figure 14]**

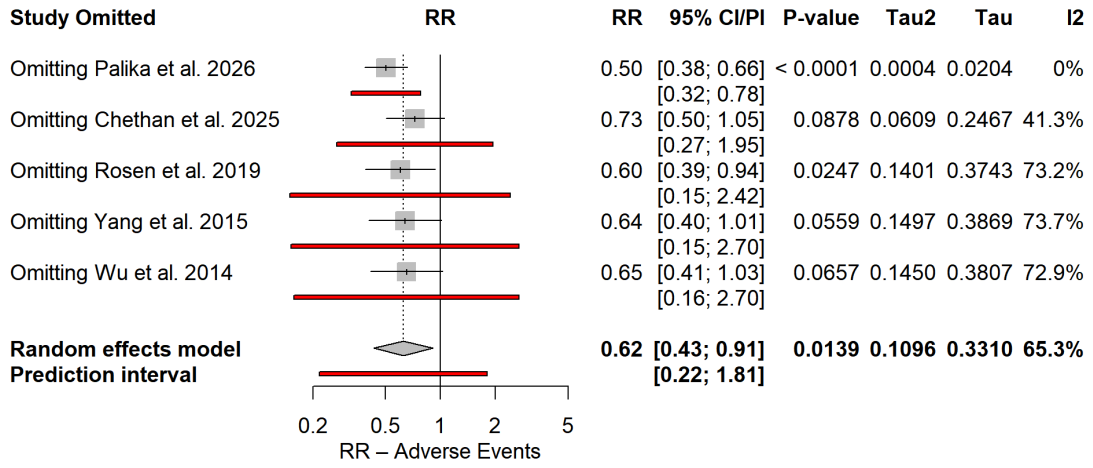


Figure 14. Leave-one-out sensitivity analysis for adverse events.

Certainty of evidence (GRADE)

The certainty of evidence was moderate for changes in serum ferritin, very low for changes in serum hemoglobin, low for treatment success, and very low for adverse events. Downgrading was related to the risk of bias and, where applicable, inconsistency and imprecision. **[Table 2]**

Table 2. GRADE assessment

Outcome	No. Studies	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other	Biotics + (N/evts)	Biotics -	Relative Effect	Absolute Effect	GRADE Certainty
Change in Serum Ferritin	2	serious^a	not serious	not serious	not serious	none	100 patients	100 patients	N/A (Continuous)	MD 6.08 (4.54–7.62)	⊕⊕⊕◯Moderate
Change in Serum Hemoglobin	2	serious^a	very serious^b	not serious	serious^c	none	174 patients	174 patients	N/A (Continuous)	MD 0.68 (-0.55–1.90)	⊕◯◯◯Very Low
Treatment Success	4	serious^a	not serious	not serious	serious^d	none	223/326 (68.4%)	208/321 (64.8%)	RR 1.08 (0.99–1.17)	16 more/1000 (2 fewer to 34 more)	⊕⊕◯◯Low
Adverse Events	5	serious^a	serious^e	not serious	serious^d	none	87/353 (24.6%)	133/346 (38.4%)	RR 0.62 (0.43–0.91)	104 fewer/1000 (173 fewer to 22 fewer)	⊕◯◯◯Very Low

a: Downgraded 1 level; >50% studies moderate/some concerns/serious RoB.
b: Downgraded 2 levels; severe heterogeneity (I^2>75%).
c: Downgraded 1 level; OIS not met (N<400) and CI crosses 0.
d: Downgraded 1 level; OIS not met or CI crosses 1.
e: Downgraded 1 level; moderate heterogeneity ($I^2 \geq 40\%$).

DISCUSSION

We included six RCTs in this systematic review and meta-analysis. All of these included 799 children, and the probiotics administered as adjunctive therapies showed inconsistent results for the different outcomes. Probiotic supplementation was statistically significantly associated with increasing the level of serum ferritin (MD 6.08, 95% CI 4.54–7.62) and the reduction of treatment-related adverse events (RR 0.62, 95% CI 0.43–0.90) but not with increasing hemoglobin (MD 0.68, 95% CI −0.55–1.90) and treatment success (RR 1.08, 95% CI 0.99–1.17). This discrepancy is also similar to the general literature for probiotics as a therapy for iron therapy, which has been steadily increasing [8, 21] and is now of interest to the microbiota of the gut as an iron bioavailability modulator. However, there has been varying randomized evidence regarding the probiotic strain, dosing, and population [11]. Importantly, the certainty of the evidence in our current meta-analysis was moderate for ferritin and very low for adverse events and hemoglobin. This suggests that there is concern about risk of bias and/or imprecision and inconsistency within the interventions combined (no study was judged to be at overall low risk of bias). These results, therefore, must be interpreted with caution and will be discussed with each of the outcomes coming up in the following.

The pooled results for the change in serum ferritin in favor of probiotic supplementation was the most statistically homogenous in this analysis, and there was no evidence of heterogeneity between the two studies selected ($I^2 = 0\%$). In spite of all the above, there are some caveats to the above finding. First, the estimate was based on only 2 trials (N = 200), and although the I^2 value was reassuring [22], it was not high enough to conclude that there was a true consistency among the results of the studies [20]. This small number of studies increased the width of the confidence interval to 95% CI (−2.41 to 14.56) with the Hartung-Knapp adjustment, suggesting that the

apparent precision of the standard random−effects model was likely to have underestimated the true uncertainty [22]. A second sensitivity analysis, dropping one trial each time, revealed that the pooled effect was influenced strongly by one trial, namely, the exclusion of Chethan et al. [23], which shifted the mean difference by 5.72 points, from excluding to including zero, for the 95% CI. This was one of the largest effect sizes reported in this database, and this single center study has the typical limitations of smaller studies and should therefore be interpreted with caution and not as proof of a genuine effect of a probiotic on iron stores.

Unlike the ferritin result, the pooled effect of probiotics' effect on hemoglobin increase did not provide any significant statistical result and had a significant and unexplained heterogeneity (I^2 = 96.2%, $p < 0.0001$). This is a high level of uncertainty, as both trials tested different effects, likely because of differing clinical and methodologic features, and the combined point estimate is not very meaningful [20]. This separation was clearly reflected when we excluded Palika et al. [24], one of the well−designed, placebo−controlled studies in pediatric probiotic−IDA that did not show a beneficial effect of probiotics in addition to iron−folic acid, from the pooled mean difference, which was then 1.30 (95% CI 0.97 to 1.63); and when we excluded Chethan et al. [23], one of the largest hemoglobin increase reports in the pediatric probiotic−IDA literature, the pooled mean difference was close to null (MD 0.05, 95% CI −0.29 to 0.39). This could suggest that the overall null result is not just because of lack of power but because there's some disagreement between the larger trial (with low risk of bias) that did not find an effect and the smaller trial (with high risk of bias) that did. This aligns with other children's populations, which have found inconsistent results in systematic reviews of probiotics or prebiotics for any reproducible and measurable benefit in children's

hemoglobin levels compared to adults or children aged 12 or older [11].

Heterogeneity ($I^2 = 0\%$) and the highest number of patients (628) in the four trials considered for pooled treatment success (defined slightly differently across the trials as clinically meaningful hematologic or symptomatic improvement) did not lead to different treatment success rates between the probiotic and control arms (RR 1.08, 95% CI 0.99–1.17; p = 0.0748). This narrow confidence interval and a p value just above the conventional level of significance suggest that this is not a true trend toward probiotic supplementation but rather a reflection of the variability of the point estimates, as evidenced by leave–one-out analysis of the data, which revealed point estimates between 1.06 and 1.10 when any single trial was removed. The probiotic group had a much lower rate of adverse events (0.62 (95% CI 0.43--0.90, p = 0.0119); that is, approximately 38% RRR); each of the leave-one-out analyses also yielded a signal in the same direction, as shown in Table 2. This finding corroborates previous studies in children reporting a similar 47% reduction in iron-related side effects when probiotics are administered together [25] and mechanistic studies that indicate that probiotics are protective of the gut mucosa without directly impacting iron absorption [26]. The adverse events estimate was slightly heterogeneous ($I^2 = 62.5\%$) and had very low GRADE certainty, and the tolerability benefit result of this analysis also did not reach the level of certainty yet, as the interval would still contain unity (0.38–1.01).

The various effects of probiotic supplementation observed throughout these trials and outcomes may be due to a large number of biologically possible factors. Strain specificity is one of the most important methods, as the strains used in the included trials all work through different mechanisms: Lactobacillus rhamnosus GG [23]; *Lactobacillus plantarum* strains UBLP40 and 299v [24, 27]; and synbiotic Biostime formulations [28, 29], with the latter two mechanisms being the production of short-chain fatty acids, which lower the gut pH and increase mucin production at the enterocyte surface. Across such diverse interventions, pooling the interventions for a broad efficacy question is justified but may be a source of clinical heterogeneity, especially in hemoglobin. A second explanation is the role of hepcidin in the regulation of iron absorption; probiotics are thought to increase the availability of iron, in part through their ability to decrease inflammation in the gut, which causes a reduction in hepcidin-mediated blockade of iron export from enterocytes [30]. This pathway could have little impact in trial populations where anemia is mostly nutritional and where iron supplementation is already sufficient to treat iron deficiency, which is explicitly hypothesized by both Palika et al. [24] and Suryawan et al. [31] as an explanation for their null results. Finally, the length of intervention in several trials (30 days to 12 weeks) may not be enough to produce meaningful

and lasting changes in the gut microbial composition that can impact iron metabolism in the body, as highlighted in several of the key primary trials in this area [24, 31].

The trend seen in this meta-analysis, with a likely tolerability benefit and no hemoglobin benefit, mirrors the overall trend in the literature on probiotics and iron, with an emerging focus on population-specific benefits. Apte et al. [11] also conducted a 2025 systematic review and meta-analysis on probiotic and prebiotic supplementation for iron absorption and hemoglobin in both women of reproductive age and children and were similarly able to observe measurable improvements in iron absorption but not hemoglobin in pediatric populations, whereas our findings for ferritin and hemoglobin in adult women were different. When the results of all probiotic trials in women of reproductive age, children and pregnant women, and adult males were combined, a previous systematic review and meta-analysis by Vonderheid et al. [10] concluded that probiotics had a modest positive effect on iron absorption but had no significant effect on serum ferritin or hemoglobin levels. In concert with the present findings, these findings suggest the presence of children with iron deficiency anemia, who may be a population in which the effect of enhancing iron absorption by probiotics, if it exists, is too small and/or too short-lived to yield a detectable hemoglobin response, especially with the iron absorption response to background therapy. On the other hand, the tolerability signal described here has been reinforced by the literature: Tremblay et al. [25] reported a similar decrease in Fe-related side effects with adjunctive probiotics, and a more recent randomized controlled trial by Koker et al. [33] highlighted improved GIT tolerance as the main demonstrated effect of coadministration of *Lactobacillus plantarum* 299v, even if there was no strong independent positive effect on Fe status. The p values appeared to be stronger for the ferritin and treatment-success outcomes than for the adverse event findings in the present analysis, but this convergence across independent bodies of evidence would be useful to increase confidence in the adverse event findings relative to the other outcomes.

Limitations

There are several limitations to consider for this meta-analysis. Only two to five trials reported each pooled outcome, limiting the power of the analyses and making heterogeneity and small-study-effect analyses mainly exploratory [22, 32]. No included study was judged to be at overall low risk of bias, and the clinical trials differed in the type of probiotic strain, dose, treatment duration, background iron regimen, and characteristics of the study population, which is likely to explain the high level of heterogeneity found with regard to hemoglobin. The follow-up duration in all trials included was short, typically 30 days to 12 weeks, and evaluation of the observed ferritin and tolerability benefits for participants was not conducted to determine if such benefits are durable. Finally, some trials that were published in Chinese-language journals with limited reporting were conducted in a single center, meaning that the trials may have been

subject to unmeasured methodological or reporting bias, which was not assessed by typical risk-of-bias tools.

Conclusion

At this time, there is no evidence that probiotics provide a meaningful benefit to complement hemoglobin therapy for children who have IDA. A statistically significant improvement in ferritin was observed, but the estimate was unreliable and was dominated by one trial; thus, this finding should be interpreted with caution. Reduced treatment-related adverse events were the most consistent treatment benefit reported, indicating the potential for using probiotics as a means of improving tolerability and, thus, adherence to iron therapy without providing any specific evidence of an effect on iron absorption itself. However, further adequately powered and longer follow-up studies with larger, strain-standardized trials are needed prior to the routine use of coadministration of probiotics with iron therapy in this population.

REFERENCES

1. World Health Organization. Anemia. Geneva: World Health Organization; 2025 Feb 10.
2. Sukwuttichai P, Tidwong N, Chaipichit N, Dhippayom N, Dilokthornsakul W, Dilokthornsakul P. Global prevalence of iron deficiency anemia among children aged 5-12 years: a systematic review and meta-analysis. J Glob Health. 2026;16:04027. doi:10.7189/jogh.16.04027.
3. Theola J, Andriastuti M. Neurodevelopmental impairments as long-term effects of iron deficiency in early childhood: a systematic review. Balkan Med J. 2025;42(2):108-120. doi:10.4274/balkanmedj.galenos.2025.2024-11-24.
4. Iolascon A, Andolfo I, Russo R, Sanchez M, Busti F, Swinkels D, et al. Recommendations for diagnosis, treatment, and prevention of iron deficiency and iron deficiency anemia. HemaSphere. 2024;8(7). doi:10.1002/hem3.108.
5. Andersen CT, Marsden DM, Duggan CP, Liu E, Mozaffarian D, Fawzi WW. Oral iron supplementation and anemia in children according to schedule, duration, dose and cosupplementation: a systematic review and meta-analysis of 129 randomized trials. BMJ Glob Health. 2023;8(2). doi:10.1136/bmjgh-2022-010745.
6. Iddrisu I, Monteagudo-Mera A, Poveda C, Shahzad M, Walton GE, Andrews SC. A review of the effect of iron supplementation on the gut microbiota of children in developing countries and the impact of prebiotics. Nutr Res Rev. 2025;38(1):229-237. doi:10.1017/S0954422424000118.
7. Puga AM, Samaniego-Vaesken ML, Montero-Bravo A, Ruperto M, Partearroyo T, Varela-Moreiras G. Iron supplementation at the crossroads of nutrition and gut microbiota: the state of the art. Nutrients. 2022;14(9):1926. doi:10.3390/nu14091926.
8. Zakrzewska Z, Zawartka A, Schab M, Martyniak A, Skoczeń S, Tomasik PJ, et al. Prebiotics, probiotics, and postbiotics in the prevention and treatment of anemia. Microorganisms. 2022;10(7):1330. doi:10.3390/microorganisms10071330.
9. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506-514. doi:10.1038/nrgastro.2014.66.

10. Vonderheid SC, Tussing-Humphreys L, Park C, Pauls H, Hemphill NO, LaBomascus B, et al. A systematic review and meta-analysis on the effects of probiotic species on iron absorption and iron status. Nutrients. 2019;11(12):2938. doi:10.3390/nu11122938.
11. Apte A, Parge A, Nimkar R, Sinha A. Effect of probiotic and prebiotics supplementation on hemoglobin levels and iron absorption among women of reproductive age and children: a systematic review and meta-analysis. BMC Nutr. 2025;11:31. doi:10.1186/s40795-025-01015-3.
12. Hu Q, Liu Y, Fei Y, et al. Efficacy of probiotic, prebiotic, and synbiotics supplements in individuals with anemia: a systematic review and meta-analysis of randomized controlled trials. BMC Gastroenterol. 2024;24:472. doi:10.1186/s12876-024-03562-8.
13. Hu G, Yu Y, Yang X, Zhang D, Wang M, Li F. Efficacy and safety of probiotics in the treatment of iron deficiency anemia in Chinese children: a systematic review and meta-analysis. Front Nutr. 2026;13:1876092. doi:10.3389/fnut.2026.1876092.
14. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi:10.1136/bmj.n71.
15. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomized trials. BMJ. 2019;366:l4898. doi:10.1136/bmj.l4898.
16. Atkins D, Best D, Briss PA, et al. Grading quality of evidence and strength of recommendations. BMJ. 2004;328:1490. doi:10.1136/bmj.328.7454.1490.
17. Viechtbauer W. Conducting meta-analyses in R with the metafor package. J Stat Softw. 2010;36(3):1–48. doi:10.18637/jss.v036.i03.
18. Hartung J, Knapp G. On tests of the overall treatment effect in meta-analysis with normally distributed responses. Stat Med. 2001;20(12):1771–1782. doi:10.1002/sim.791.
19. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629–634. doi:10.1136/bmj.315.7109.629.
20. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557–560. doi:10.1136/bmj.327.7414.557.
21. Baryshnikova N, Uspenskiy Y, Novikova V. Microbiota and iron metabolism. Explor Dig Dis. 2025;4:100585.
22. IntHout J, Ioannidis JPA, Borm GF. The Hartung-Knapp-Sidik-Jonkman method for random-effects meta-analysis is straightforward and considerably outperforms the standard DerSimonian–Laird method. BMC Med Res Methodol. 2014;14:25.
23. Chethan S, Kumar R, Gulab CD, Kumar S, Mahesh M, Umapathy N. Effectiveness of Probiotic Supplementation in Enhancing Iron Therapy for Iron Deficiency Anemia in Children Aged 1 to 15 Years: A Randomized Controlled Trial. TPM Vol. 2025;32(S3):1591–98.
24. Palika R, Seeli H, Banjara SK, Challa SR, Pullakhandam R, Kulkarni B, Dasi T. Effect of combined probiotic and iron-folic acid supplementation on iron status and gut inflammation markers: a randomized controlled trial among anemic children. Eur J Clin Nutr. 2026;80:594–602.
25. Tremblay A, Xu X, Colee J, Tompkins TA. Efficacy of a Multi-Strain Probiotic Formulation in Pediatric Populations: A Comprehensive Review of Clinical Studies. Nutrients. 2021;13:1908.
26. García-Santos JA, Nieto-Ruiz A, García-Ricobaraza M, Cerdó T, Campoy C. Impact of Probiotics on the Prevention and Treatment of Gastrointestinal Diseases in the Pediatric Population. Int J Mol Sci. 2023;24(11):9427.
27. Rosen GM, Morrissette S, Larson A, Stading P, Griffin KH, Barnes TL. Use of a Probiotic to Enhance Iron Absorption in a Randomized Trial of Pediatric Patients Presenting with Iron Deficiency. J Pediatr. 2019;207:192–7.

28. Wu W. Prospective study of iron dextran combined with synbiotic probiotics in the treatment of nutritional iron deficiency anemia in children. Matern Child Health Care China. 2014;29:3284–6.
29. Yang Y. Efficacy analysis of the combined treatment of iron dextran oral liquid and Biostime probiotics in children with nutritional iron deficiency. Matern Child Health Care China. 2015;30:6390–2.
30. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Hematologica. 2020;105:260–72.
31. Suryawan R, Salekede SB, Ridha NR, Angriani H, Bahagia AD, Laompo A. Effect of Saccharomyces boulardii probiotic supplementation on ferritin levels in children with iron deficiency. Rom J Pediatr. 2025;74(4):253–9.
32. Sterne JAC, Sutton AJ, Ioannidis JPA, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomized controlled trials. BMJ. 2011;343:d4002.
33. Koker G, Sahinturk Y, Ozcelik Koker G, Coskuner MA, Eren Durmus M, Catli MM. Improved gastrointestinal tolerance and iron status via probiotic use in iron deficiency anemia patients initiating oral iron replacement: a randomized controlled trial. Br J Nutr. 2024;132(10):1308–16.

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