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Systematic Review

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Posted Date: March 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8788605/v1

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Additional Declarations: No competing interests reported.

The Effect of Probiotics Supplementation on Mental Health Outcomes Among Children and Adolescents: A Systematic Review and Meta-Analysis of randomized controlled trials

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Abstract

Background: Interest is growing in microbiota-targeted approaches to support mental health in children and adolescents. Probiotics may influence brain function through the microbiota–gut–brain axis, but evidence in pediatric populations remains limited, and prior reviews often include non-RCT data, weakening conclusions. So, this study aimed to evaluate the effects of probiotic supplementation on mental health outcomes in children and adolescents using randomized controlled trials only.

Methods: This systematic review and meta-analysis followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines. Comprehensive searches of PubMed/MEDLINE, Embase, Cochrane CENTRAL, and ClinicalTrials.gov. Eligible studies included participants aged ≤18 years, compared probiotics with placebo or non-probiotic controls, and reported validated mental health outcomes. Risk of bias was assessed using the Cochrane Risk of bias 2 tool. Meta-analysis was conducted using standardized mean differences with 95% confidence intervals.

Results: Of 51 records identified, seven trials met inclusion criteria, focusing on attention deficit hyperactivity disorder (three studies), autism spectrum disorder (two studies), and depression or anxiety (two studies). Overall risk of bias was rated as having some concerns. Three trials (n = 215) were sufficiently comparable for meta-analysis. Probiotic supplementation was associated with a statistically significant improvement in mental health outcomes (pooled mean difference −0.4; 95% CI −0.69 to −0.01), indicating a moderate reduction in symptom severity across anxiety, depressive symptoms, attention deficit hyperactivity disorder -related difficulties, stress, and behavioral problems.

Conclusions: Probiotic supplementation has beneficial effects on mental health outcomes in children and adolescents. However, the limited number of randomized controlled trials warrant cautious interpretation. Larger, well-designed trials with standardized outcomes are needed to clarify clinical relevance and long-term effects.

Keywords: probiotics; mental health; children and adolescents; randomized controlled trials; gut–brain axis

1. Background

Probiotics are defined as live microorganisms, such as bacteria or yeasts, that confer health benefits to the host when consumed in adequate amounts (Hill et al., 2014). Probiotics act primarily within the digestive tract, where they may enhance gastrointestinal function and influence other aspects of health. In recent years, probiotic supplementation has gained significant attention for its potential benefits beyond digestive health, particularly in relation to mental well-being (Biswas et al., 2025). However, not all products labeled as probiotics provide clinically proven benefits; their effectiveness depends on factors such as the specific microbial strain, dosage, and viability.

Common probiotic genera include Lactobacillus, Bifidobacterium, Saccharomyces, and Streptococcus, among others. Probiotics, which can influence gut microbial composition, have been proposed to affect brain function through the microbiota–gut–brain axis (Biswas et al., 2025; Vijayaram et al., 2025). This axis is a bidirectional communication network between the central nervous system and the gastrointestinal tract that operates through neural, immune, endocrine, and metabolic pathways (Ramadan et al., 2025). Gut microbes influence brain function by modulating neural signaling via the vagus nerve, regulating immune responses and inflammation, producing microbial metabolites such as short-chain fatty acids, and influencing the synthesis of neuroactive compounds, including serotonin and gamma-aminobutyric acid (GABA) (Azevedo & Silva, 2025)

Probiotics as a potential adjunct therapy in the management of Mental health

Probiotics have received increasing attention for their potential therapeutic roles across a wide range of physical and neuropsychiatric conditions. Traditionally recognized for benefits in gastrointestinal and inflammatory disorders, probiotics are now understood to exert broader physiological effects through immunomodulation, antioxidative activity, and regulation of the bidirectional gut–brain axis. These mechanisms suggest that probiotics may influence central

nervous system functioning and emotional regulation, contributing to growing interest in their use as adjunctive treatments for mental health symptoms (Loh et al., 2024)

Evidence from randomized controlled trials and meta-analyses has shown that probiotics may alleviate depressive and anxiety symptoms, particularly among individuals with clinically diagnosed mood or anxiety disorders. These benefits, however, appear to depend on several factors, including age, baseline symptom severity, underlying medical or psychiatric conditions, probiotic strain composition, and duration of supplementation (El Dib et al., 2021)

Research examining the mental health effects of probiotics in children and adolescents' remains limited and inconsistent. Several meta-analyses have reported minimal or limited significant benefits of probiotics for cognitive outcomes, attention deficits, or symptoms associated with autism spectrum disorder (ASD). Yet, modest improvements have been observed within specific subgroups—such as those receiving multi-strain formulations or undergoing prolonged treatment—and in certain neurodevelopmental conditions. For example, evidence of increased intestinal permeability and microbiome disruption in ASD may partially explain responsiveness to probiotic therapy in this group, whereas similar benefits have not been consistently demonstrated in disorders such as ADHD (Mehra et al., 2023). Multi-strain or longer-term regimens may be more effective than single-strain, short-term interventions (Liang et al., 2024). In conditions like ASD, such as altered microbial communities or increased intestinal permeability may increase responsiveness to probiotics, whereas such mechanisms may be less pronounced in disorders like ADHD (Ligezka et al., 2021). A recent meta-analysis of five RCTs (total 692 youth) found no significant effect of probiotic supplementation on depressive symptoms in children and adolescents (standardized mean difference = 0.04, 95% CI: –0.33 to 0.41), although there was high statistical heterogeneity (I^2 = 77 %) (Chen et al., 2024). Similarly, in a meta-analysis of seven trials

with 379 participants (ages 4–18), probiotics did not significantly improve total ADHD symptoms, inattention, or hyperactivity/impulsivity compared to placebo (SMDs = 0.25, 0.14, and 0.08, respectively), though subgroup analyses suggested potentially greater effects in studies using multi-strain probiotics or adjunctive treatment with methylphenidate (Liang et al., 2024)

Moreover, studies in autism spectrum disorder (ASD) have yielded mixed results: a meta-analysis of 7 clinical trials found a non-significant overall effect on core behavioral symptoms (SMD = –0.24), but significant benefit in the subgroup receiving probiotic blends (He et al., 2023).

Another meta-analysis of probiotic treatment in ASD showed no statistically significant improvement in behavior or social responsiveness, though there was improvement in gastrointestinal symptoms (Zeng et al., 2024). A broader systematic review of psychobiotic interventions (probiotics and prebiotics) in children and adolescents (ages ~6–25) also concluded that evidence for improving stress, anxiety, and cognitive outcomes is minimal, and highlighted major methodological heterogeneity (in strains, populations, and outcomes) as a barrier to clear conclusions (Basso et al., 2022)

Despite growing interest in probiotics as modulators of mental health, their therapeutic potential in children and adolescents remains uncertain. Existing studies are fragmented and inconsistent, often involving participants with low baseline symptom severity rather than clinically diagnosed disorders.

While some RCTs report modest improvements in depressive or anxiety symptoms, most are limited by small sample sizes, short intervention durations, heterogeneous probiotic strains, and varied outcome measures, which prevents clear conclusions about their efficacy in youth populations (Hu et al., 2025). These inconsistencies may be influenced by moderating factors such as probiotic strain composition, treatment duration, and underlying neurodevelopmental

conditions, underscoring the need for a comprehensive synthesis across mental health domains including depression, anxiety, ADHD, stress, and behavioral problems.

Despite the documented biological plausibility of the microbiota–gut–brain axis, the translation of probiotic science into clinical mental health practice for children and adolescents remains inconclusive.

So, this research aims to evaluate the effect of probiotics supplementation on mental health outcomes (ADHD, ASD, anxiety, stress, depressive symptoms, and behavioral problems) among children and adolescents: a systematic review and meta-analysis of randomized controlled trials

2. Methods

2.1. Protocol and Registration

This systematic review and meta-analytic study was conducted in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009). The protocol was registered and published in the International prospective register of systematic review PROSPERO (CRD420251182440)

2.2. Literature Search Strategy

The Literature search strategy follows PICO criteria and employs a multi-database approach to ensure comprehensiveness. Based on the keywords pertinent to the study, major electronic databases including PubMed, Embase, ScienceDirect, Cochrane Central Register of Controlled Trials (CENTRAL), and ClinicalTrials.gov were searched from 2015 to November 17, 2025.

Database-Specific Strategies: PubMed: Articles published on PubMed data base were searched by using MeSH terms and free-text keywords. A structured search was conducted in CINAHL (EBSCO) using subject headings and keywords related to probiotics, mental health outcomes, and children/adolescents. The keywords were combined using Boolean operators as: (probiotic terms)

AND (mental health terms) AND (child/adolescent terms) AND (RCT terms). Filters were applied for randomized controlled trials and child/adolescent populations and within 10 years and written in English Language. A structured search was conducted in the Cochrane Central Register of Controlled Trials (CENTRAL) using keywords related to probiotics, mental health outcomes, and pediatric populations.

Selection Criteria and Screening (Eligibility)

2.2.1. Inclusion Criteria

This systematic review and meta-analysis included all full randomized controlled trials (RCTs) that evaluated the impact of probiotic supplementation on mental health outcomes in children and adolescents. Study eligibility was based on the criteria of PICO (i.e., population, intervention, comparator, and outcome): (1) Population: children or adolescents aged 18 or below, (2) Intervention: probiotics or products containing probiotics (any strain, dose, or duration), (3) Comparator: non-probiotic interventions or placebo, and (4) Outcome: Change in validated mental health outcomes (ADHD, ASD, anxiety, mood, stress, behavioral problems). Studies were eligible if probiotic supplementation (Intervention) was the primary exposure regardless of the strain, dose, or duration. All published RCTs written in English language within the last ten years regardless of geographic settings were included.

2.2.2. Exclusion Criteria

Studies that (1) did not include probiotics as interventions, (2) were not full RCTs, or (3) failed to provide data on symptoms of mental health as their outcome measures were excluded. Studies with insufficient information on adjusted effect (eg, unclear adjustment variable, missing CI estimates)

2.3. Data Extraction and Quality Assessment

All studies identified from different electronic database were imported into an EndNote library. Further screening of titles and abstracts and full text was accomplished by the investigators. Data extraction was independently performed by a reviewer using a pre-designed data extraction form. Extracted information included study characteristics (author, year, DOI, country, and study design), participant characteristics (age, sample size, Mean, SD), details of the probiotic intervention (strain, dose, duration), comparator or control conditions, and mental health outcomes measured (e.g., anxiety, mood, stress, ADHD, ASD, behavioral problems) along with the instruments used for assessment.

The corresponding authors of papers with missing data were contacted through emails in an attempt to retrieve the necessary information. Studies meeting inclusion criteria were abstracted into an abstraction table. The methodological quality of included studies was assessed using the Cochrane Risk of Bias 2 tool for randomized controlled trials. This tool evaluates potential sources of bias, including random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. Each domain was rated as low, Some concerns and high ("Cochrane handbook for systematic reviews of interventions | cochrane,"). The overall quality of evidence for each outcome was also determined by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool (Guyatt et al., 2008) Any discrepancies between reviewers were resolved through discussion.

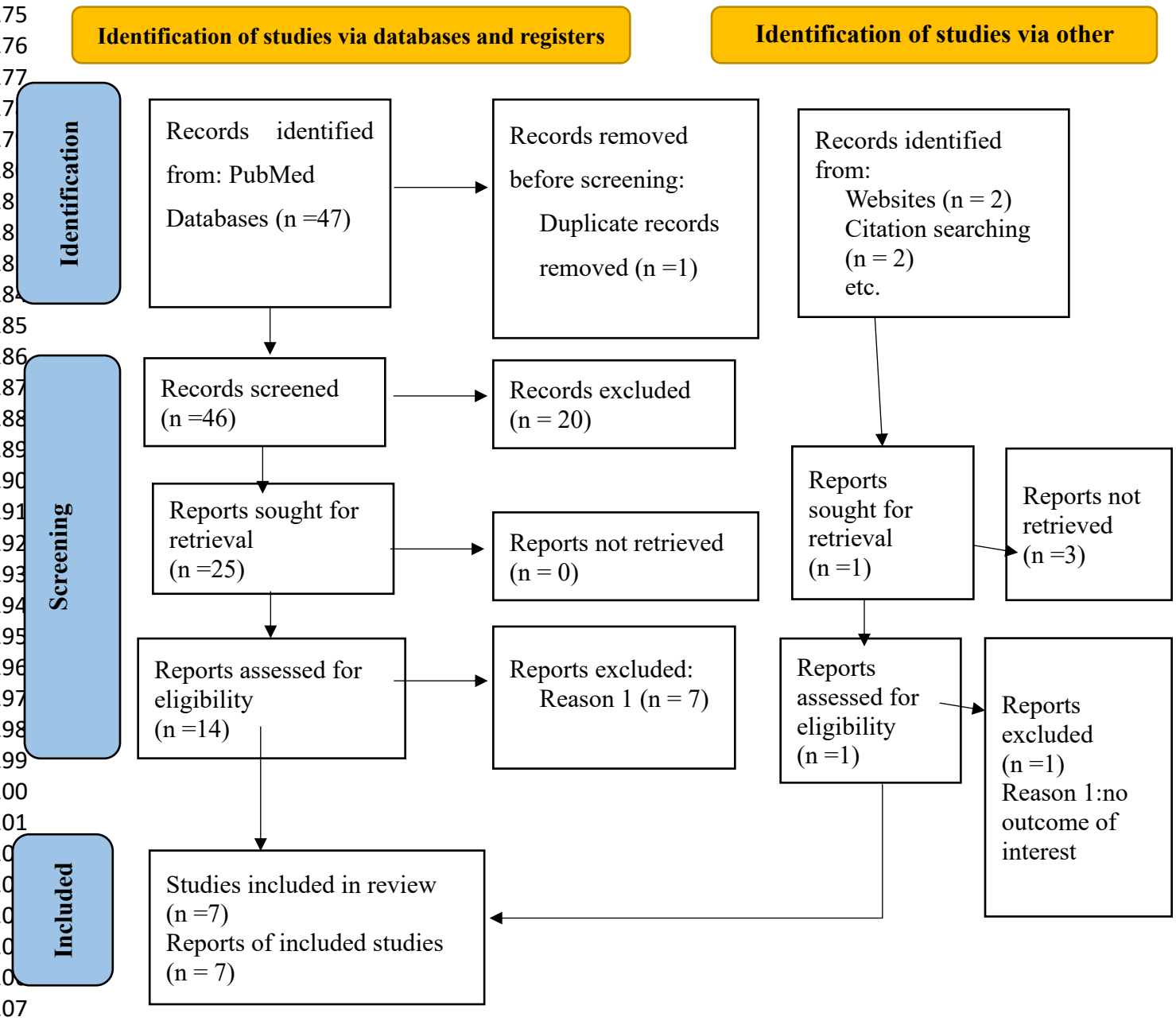


Figure 1. PRISMA 2020 flow diagram illustrating the systematic search procedure of databases, registers and other sources (Page et al., 2021)

2.5. Data Synthesis and Analysis

Data from included studies were synthesized both qualitatively and quantitatively. For quantitative synthesis, continuous outcomes such as scores on validated measures of anxiety, mood, stress,

ADHD, ASD, and behavioral problems were summarized using standardized mean differences (SMD) with corresponding 95% confidence intervals (CIs). Statistical heterogeneity was assessed using the I^2 statistic and Cochran's Q test, with I^2 values of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively. Statistical significance for all outcomes was declared as a probability value, p, less than 0.05. All statistical analyses were conducted using Stata Statistical Data Analysis software.

2.6. Trial registration: Clinical trial number: not applicable

3. Results

3.1. Eligible Studies and Characteristics

In compliance with the PRISMA 2020 statement (Page et al., 2021), 51 articles were initially retrieved from the electronic data bases based on the selected keywords and search strategies. After title and abstract screening, articles that were not RCTs, those included adults in their analysis, those in which depression was not an outcome measure, narrative reviews, pilot studies, and those that were duplicates from different databases were excluded. Finally, total of 7 studies met the inclusion criteria and were included in the final narrative analysis. Among the 7 studies included three of them were used for the metanalysis (Figure 1)

Interventions or exposures varied across studies but predominantly involved Bifidobacterium animalis Subsp. lactis (BPL1, CECT8145, 1010 colony forming units), Lactobacillus acidophi lus LB (Lacteol Fort), Lactobacillus rhamnosus HN001 (6Ã—10^9 CFU/day) or Bifidobacterium lactis HN019 (9Ã—10^9 CFU/day), Probiotic capsules (AB Biotics S.A.) and Lactobacillus plantarum PS128 (3Ã—10^10 CFU), and Bifidobacterium longum subsp. infantis YLGB-1496 among included studies, 2 trials focused on single-strain probiotics and the other adopted multiple strain regimens. Regarding the diagnosis, 3 studies enrolled infants and children without a disease

diagnosis, two focused on participants diagnosed with ADHD, one targeted those diagnosed with ASD, one targeted those diagnosed with both ASD and ADHD. The duration of treatment ranges from 12 weeks –11 years. In respect of study design, six studies adopted a parallel RCT design, whereas one study employed a cross over RCT. The countries of origin of the included studies were Catalonia, Egypt. Wellington and Auckland, Australia and New Zealand. Spain, Taiwan and Iran (Table1)

242 Table 1. Key features of eligible randomized clinical trials on the effect of probiotics on Children and Adolescents Mental health outcome

243 across different settings, 2025 G.C

Author	Year	Country	N1	Mean1	SD1	N2	mean2	SD2	Age	Health_Condition_of_subjects	Intervention	Duration_of_Intervention
Novau-Ferré et al	2025	Spain	41			39			5-14 Years	ADHD and ASD	Clostridia, Ruminococcaceae, and Lachnospiraceae in ADHD,Bacteroides, Bacilli and Actinobacteria in ASD	12 Weeks
John	2023	Taiwan	39	-2.95	5.58	40	-2.05	6.59	2.5-7 years	Autism spectrum disorder	Lactobacillus plantarum PS128 (PS128) contained 3 × 10 colony	4-month duration

											forming units (CFU) of live bacteria	
R F Slykerman	2018	Wellington and Auckland	207			115			Infants-11 years	No intial Diagnosis	Lactobacillus rhamnosus strain HN001, Bifidobacterium animalis subsp. lactis strain HN019	11 years
Susan E Jacobs,	2017	Australia and New Zealand.	373			362			2-5 years	No intial Diagnosis	Streptococcus thermophilus and Bifidobacterium lactis)	5 years
Montse Amat-Bou	2025	Catalonia	19			20			2 to 19 years	Prader_Willi_Syndrome	Bifidobacterium animalis Subsp. lactis (BPL1)	12 weeks,2 times
Zahra Sadat	2024	Iran	30	39.66	18.33	30	48.25	23.64	four to	ADHD	Lactobacillus plantarum PTCC 1896™	8 weeks

Sang sefi									sixte en.		(A7) and Bifidobacterium animalis subsp. Lactis (BB-12®)	
Reham M. Elhossiny	2023	Egypt	36	19.60	6.60	40	24.35	7.80	6–16 years,	ADHD	Lactobacillus acidophilus LB supplementation	12 eeks

244

3.2. Risk of Bias Appraisal

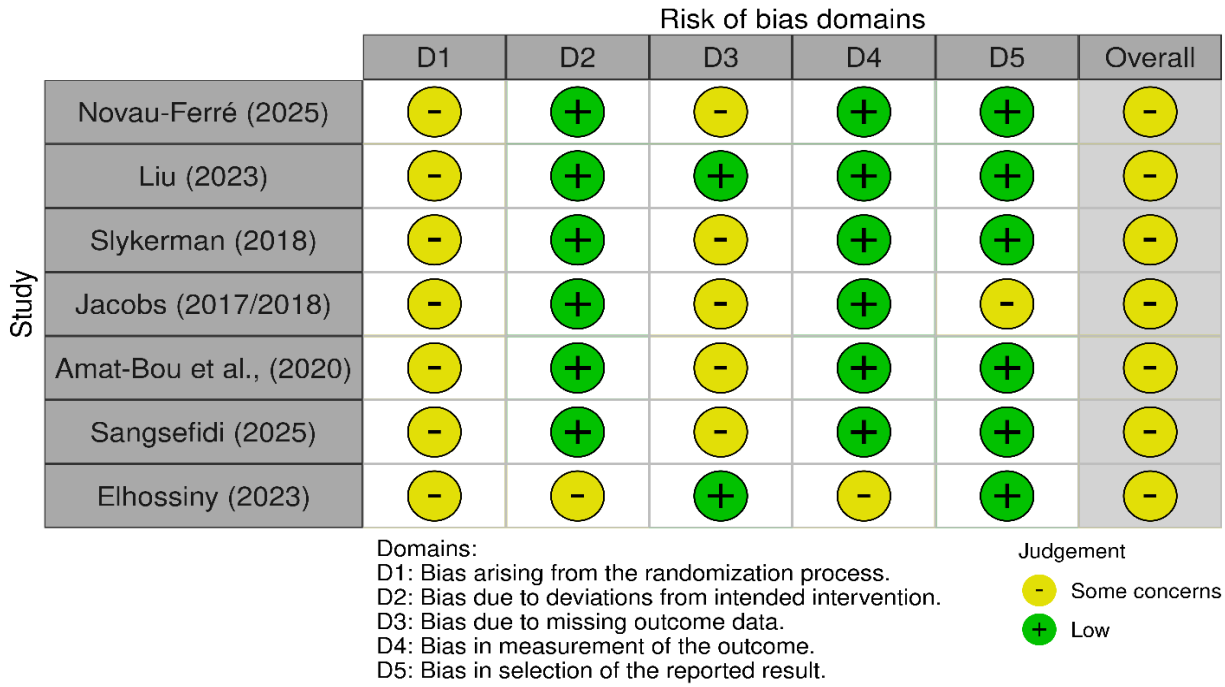


Figure 2. Illustrating the Risk of Bias for eligible studies per domain

The risk of bias (RoB) in the included studies was assessed using the Cochrane Risk of Bias Tool 2 (RoB 2). Assessments were conducted independently for each of the five RoB 2 domains which includes: (1) bias arising from the randomization process; (2) bias due to deviations from intended interventions; (3) bias due to missing outcome data; (4) bias in measurement of the outcome; and (5) bias in selection of the reported result. An overall RoB judgment was then derived for each study. Judgments were based on available full texts, abstracts, trial registrations and supplementary materials.

Bias Arising from the Randomization Process; Judgment: Some concerns (all 7 studies).

All seven studies were double- or triple-blind RCTs with generally robust designs, However, none provided explicit details on random sequence generation (e.g., computer-generated random

numbers) or allocation. This lack of detail is common in smaller or non-tertiary trials and raises potential for selection bias.

Bias Due to Deviations from Intended Interventions (Performance Bias)

Studies conducted by Novau-Ferré et al. (2025), Liu et al. (2023), Slykerman et al. (2018), Jacobs et al. (2017/2018), (Amat-Bou et al., 2020), and Sangsefidi et al. (2025) were explicitly double- or triple-blind. So, the Judgment for those (6 studies) is "Low risk". One study conducted by Elhossiny et al. (2023) judged as "Some concerns" as blinding was not clearly described.

Overall: Predominantly low risk due to strong blinding protocols.

Bias Due to Missing Outcome Data (Attrition Bias) Judgment: Two studies were judged as Low risk whereas 5 studies had Some concerns. Supporting Evidence: Low risk: Liu et al. (2023) (high completion rate implied, intention-to-treat analysis); Elhossiny et al. (2023) (low dropout: 76/80 analyzed, reasons reported). Some concerns: Novau-Ferré et al. (2025), Slykerman et al. (2018), Jacobs et al. (2017/2018), (Amat-Bou et al., 2020), and Sangsefidi et al. (2025) due to moderate or unclear attrition rates, particularly in long-term follow-ups (e.g., 27–33% loss in Slykerman and Jacobs studies). Reasons for dropout often unreported. Higher losses in vulnerable subgroups (e.g., preterm infants) noted in Jacobs et al. Overall: Some concerns predominate, reflecting challenges of follow-up in pediatric populations.

Bias in Measurement of the Outcome (Detection Bias) Judgment: Low risk (6 studies); Some concerns (1 study). Supporting Evidence: Low risk: Novau-Ferré et al. (2025), Liu et al. (2023), Slykerman et al. (2018), Jacobs et al. (2017/2018), (Amat-Bou et al., 2020), and Sangsefidi et al. (2025) – objective outcomes (e.g., microbiota sequencing, standardized neurocognitive tests) or blinded assessors for parent-reported/behavioral measures. Some concerns: Elhossiny et al. (2023)

unclear blinding of outcome assessors. Overall: Predominantly low risk due to effective blinding of assessment.

Bias in Selection of the Reported Result (Reporting Bias) Judgment: Low risk (6 studies); Some concerns (1 study). Supporting Evidence: Low risk: Most studies transparently reported positive, negative, and non-significant results.

Overall Patterns. No study was judged as high risk in any domain or overall. The most consistent concerns relate to the randomization process (universal) and attrition (common in long-term trials). A risk of bias plot was generated by using the robvis Software, ROB2 Method (McGuinness & Higgins, 2020)

3.3. Qualitative Synthesis of Probiotic Intervention on Mental Health Outcomes (Primary Outcomes)

3.3.1 Effects of Probiotic Intervention in ADHD

A randomized, double-blind, placebo-controlled trial involving children with ADHD and ASD found no significant changes in alpha or beta diversity of the gut microbiota following a 12-week probiotic intervention in the ADHD group, indicating a weaker microbiome response compared to ASD (Novau-Ferré et al., 2025). Within the ADHD subgroup, the intervention increased the abundance of Odoribacter, a butyrate-producing bacterium linked to reduced inflammation and improved cognitive function, while decreasing Eggerthellaceae (uncultured genus, associated with anxiety, depression, and stress) and Escherichia–Shigella (potentially pathogenic taxa) (Novau-Ferré et al., 2025). These targeted changes suggest probiotics may reduce inflammatory or pathogenic taxa in ADHD, though without broader microbiota restructuring (Novau-Ferré et al., 2025)

Other trials focused on preschool children with ASD, the psychobiotic Lactobacillus plantarum PS128 showed no significant effects on hyperactivity scores across time points (Liu et al., 2023). Adjunctive probiotic supplementation with Ritalin (containing Lactobacillus plantarum PTCC 1896 and Bifidobacterium animalis subsp. lactis BB-12) for 8 weeks led to a significant decrease in Conners' Parent Rating Scale (CPRS) total score after 4 weeks and improved auditory response control at study end compared to placebo (Zahra Sadat Sangsefidi et al., 2025). Similarly, Lactobacillus acidophilus LB combined with atomoxetine for 3 months significantly improved CPRS-R-L and Child Behavioral Checklist total T scores, as well as focus attention and executive function measures (lower perseverative and non-perseverative errors on Wisconsin Card Sort Test) compared to atomoxetine alone (Amat-Bou et al., 2020)

3.3.2. Effects of Probiotic Intervention in ASD

In a trial examining children with ADHD and ASD, probiotic supplementation significantly increased alpha diversity only in the ASD group, suggesting improved microbial richness and evenness(Novau-Ferré et al., 2025). Several genera linked to adverse outcomes were downregulated in ASD, including Coprococcus (associated with neurotransmitter synthesis for depression), Phocea (correlated with comorbid anxiety and motor deficits), and Angelakisella (part of Clostridia, linked to GI symptoms and neurotoxin production), implying potential reductions in anxiety, and neurotoxic processes (Novau-Ferré et al., 2025). The psychobiotic Lactobacillus plantarum PS128 in young children with ASD (ages 2.5–7 years) showed mixed effects on hyperactivity, with no significant differences between early and late use groups across time points (Liu et al., 2023)

3.3.3. Effects of Probiotic Intervention on Anxiety and Depressive Symptoms

In young children with ASD, immediate administration of Lactobacillus plantarum PS128 significantly reduced anxious/depressed symptoms on the ASEBA scale after the first 2-month blinded period (P = 0.044; independent t-test P = 0.033), though not significant after multiple comparison correction (Liu et al., 2023)

When both groups received PS128 in months 2–4, the delayed group showed significant improvement (P = 0.017), replicating anxiolytic effects; however, continued use in the early group showed no further reduction, possibly due to an outlier (Liu et al., 2023). In a subgroup of children older than 6 years with Prader-Willi syndrome (often involving mental health symptoms), Bifidobacterium animalis subsp. lactis BPL1 induced modest but significant improvements in withdrawn/depression symptoms and a trend toward reduced attention problems on the Child Behavior Checklist compared to placebo (Amat-Bou et al., 2020)

3.3.4. Effects of Probiotic Intervention on Behavioral Problems

Early-life perinatal and infant probiotic supplementation (Lactobacillus rhamnosus HN001 or Bifidobacterium animalis subsp. lactis HN019) showed no significant effects on neurocognitive outcomes, behavior, or mood (including depression/anxiety) at 11-year follow-up in children at risk of allergic disease (Slykerman et al., 2018). Probiotic administration (combination of Bifidobacterium infantis, Streptococcus thermophilus, and Bifidobacterium lactis) to very preterm infants from soon after birth until discharge or term-corrected age had no impact on neurodevelopmental outcomes or behavior in early childhood, though rates of deafness were lower (Jacobs et al., 2017). Overall, while some adjunctive probiotic trials showed benefits in ADHD symptomology and cognition (Amat-Bou et al., 2020; Zahra Sadat Sangsefidi et al., 2025), others

found no effects on behavioral or neurodevelopmental outcomes in general or preterm populations (Jacobs et al., 2017; Slykerman et al., 2018).

3.4. Secondary Outcomes

One RCT (Novau-Ferré et al., 2025) did not directly measure psychiatric symptom change, its findings provide indirect qualitative evidence of how probiotics may influence biological pathways linked to mental health in ADHD and ASD via biological pathways (e.g., increased Odoribacter for butyrate production/SCFA-linked anti-inflammatory and cognitive benefits; reduced pathogenic/inflammatory taxa). No clinical symptom scales were used, so mental health implications remain secondary (Novau-Ferré et al., 2025). Another RCT which primarily examined neurodevelopmental outcomes and behavior in preterm infants supplemented with probiotics found that a notable secondary outcome was significantly lower rates of deafness in the probiotic group, representing an unexpected sensory benefit unrelated to the primary neurodevelopmental focus (Jacobs et al., 2017)

3.5. Certainty of Evidence

This assessment draws from principles of GRADE (Grading of Recommendations Assessment, Development and Evaluation), considering factors such as study design, risk of bias, inconsistency, indirectness, imprecision, and publication bias. The evidence begins with a high certainty rating.

1. Starting Level: High Certainty - All included studies were RCTs, which provide high-quality evidence for intervention effects.

2. Downgrading Factors

Risk of Bias (-1 level): All seven studies were rated as "some concerns" overall using Cochrane RoB 2, primarily due to unclear randomization processes (lack of details on sequence generation and allocation concealment) across all studies. Additional concerns included attrition in long-term

follow-ups (e.g., Slykerman et al., 2018; Jacobs et al., 2017/2018) and potential blinding issues in one study (Elhossiny et al., 2023). No study was judged high risk, but the consistent "some concerns" in key domains justifies downgrading by one level.

Inconsistency (-1 level): Small heterogeneity was observed in narrative synthesis and limited quantitative synthesis (e.g., $I^2 = 17.4\%$). Effect sizes varied widely, likely due to differences in probiotic strains (single vs. multi-strain), populations (ADHD vs. ASD vs. Prader-Willi vs. preterm/perinatal), intervention type (monotherapy vs. adjunctive to stimulants), and outcome measures (ADHD-specific scales vs. broader behavioral or neurocognitive assessments). Not all outcomes showed consistent magnitude of effect. Positive effects in some ADHD trials (Amat-Bou et al., 2020; Zahra Sadat Sangsefidi et al., 2025), but null in other for behavior/neurodevelopment (Jacobs et al., 2017; Slykerman et al., 2018)

Indirectness (-1 level): Some interventions were adjunctive rather than standalone probiotics, and outcomes included surrogate or broader measures. One RCT focus on microbiota (no direct clinical symptoms measured) (Novau-Ferré et al., 2025); Two papers target general/preterm populations, not neurodevelopmental disorders specifically (Jacobs et al., 2017; Slykerman et al., 2018).This limits direct applicability to typical children/adolescents with common mental health concerns.

Imprecision (-1 level): Total participant numbers were moderate, but individual studies were small (n=19–735), leading to wide confidence intervals in many cases. The meta-analysis subset (three studies) included fewer participants (215), and pooled estimates had confidence intervals that approached or included minimal clinically important differences. Optimal information size was not met for most outcomes

Publication Bias: Unable to assess reliably due to <10 studies

Final GRADE Certainty: Low

This indicates that our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of effect. Further high-quality research is very likely to have an important impact on our confidence and may change the estimate.

3.6. Effect Size

3.6.1. Model Assumptions

The inverse variance (IV) random-effects model was used, assigning greater weight to larger and more precise studies. A random-effects approach was chosen due to heterogeneity among the included trials, including differences in probiotic formulations, intervention duration, and outcome definitions. Standardized mean differences were calculated using Hedges' g, which provides a more accurate estimate of the true effect in small samples compared with Cohen's d.

Table 2. Meta-analysis pooling of standardized mean differences using the random-effects inverse-variance model with Der Simonian-Laird (DL) estimate of tau²

Primary Author (year of publication)	Intervention Group (N, Mean, SD)	Control Group (N, Mean, SD)	Effect Size (Hedges' g)	SE	[95% Conf. Interval]	% Weight
John (2023)	39, -2.95, 5.58	40, -2.05, 6.59	-0.15	0.225	-0.589 to 0.294	36.87
Zahra Sadat Sangsefi (2024)	30, 39.66, 18.33	30, 48.25, 23.64	-0.41	0.261	-0.918 to 0.105	28.92
Reham M. Elhossiny (2023)	36, 19.60, 6.60	40, 24.35, 7.80	-0.65	0.236	-1.117 to -0.192	34.21
Overall, DL			-0.40		-0.694 to -0.097	100.00

Test of overall effect = 0: z = -2.598 p = 0.009

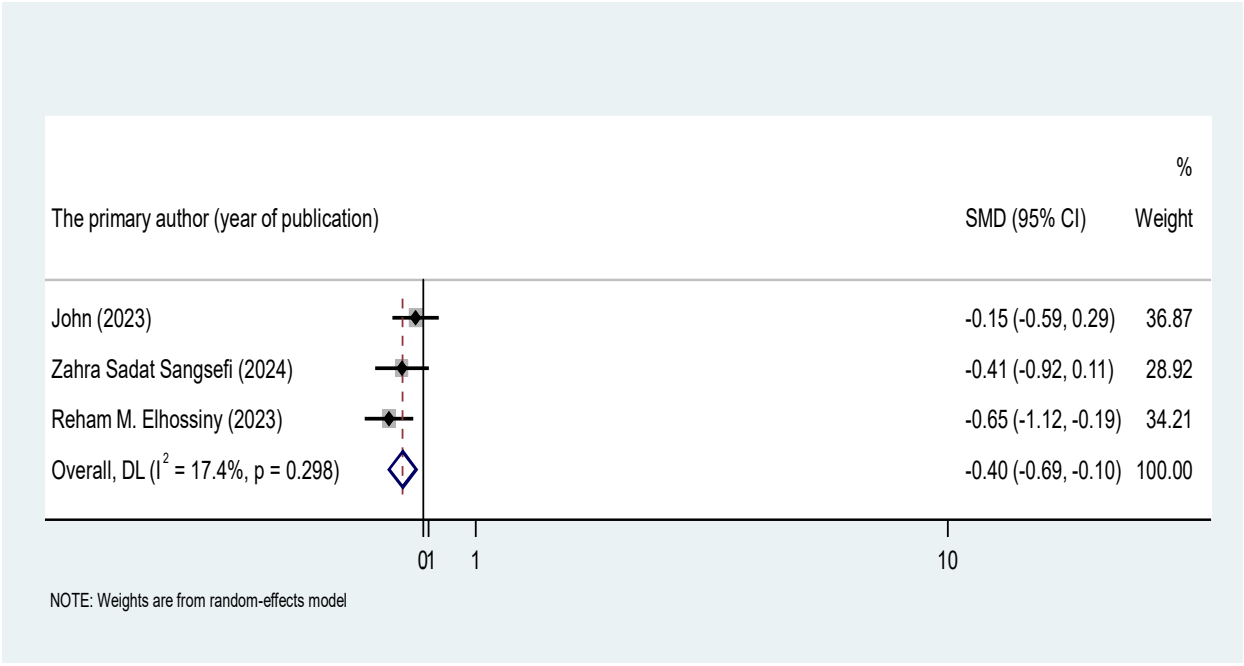


Figure 3. Forest plot of the meta-analysis examining the effect of probiotic supplementation on mental health outcomes in children and adolescents.

3.6.2. Pooled Analysis of Probiotic Interventions on Mental Health Outcomes

The meta-analysis included only three randomized controlled trials that reported complete mean and standard deviation data for both groups, limiting the analysis to John (2023), Sangsefi (2024), and Elhossiny (2023). The remaining studies had missing outcome data and were excluded from the quantitative synthesis. These three randomized controlled trials, conducted in Taiwan (2023), Iran (2024), and Egypt (2023), evaluated probiotic-based interventions in neurodevelopmental conditions, primarily autism spectrum disorder and ADHD, and provided sufficient quantitative data for meta-analysis**.** Individual effect sizes, calculated as standardized mean differences using Hedges' g, ranged from −0.15 to −0.65, with all point estimates favoring probiotic supplementation. This consistent direction of effect suggests a trend toward reduced symptom

severity or improved outcomes among children and adolescents receiving probiotics. The observed effect sizes fall within the small-to-moderate range, which is typical for behavioral and neurodevelopmental interventions.

Study-Specific Effects

John (2023) reported a small effect size (g = −0.15; 95% CI: −0.59 to 0.29), indicating a trend favoring the intervention, although the confidence interval crossed zero. Zahra Sadat Sangsefi (2024) demonstrated a moderate effect (g = −0.41; 95% CI: −0.92 to 0.11), also favoring the intervention but with some statistical uncertainty. Reham M. Elhossiny (2023) reported the largest effect (g = −0.65; 95% CI: −1.12 to −0.19), with the confidence interval fully below zero, indicating a statistically significant benefit**.** Although only one study reached statistical significance individually, all three studies showed effects in the same beneficial direction, highlighting coherence across geographically and clinically diverse samples.

Pooled Effect and Interpretation

Using a random-effects model, the pooled standardized mean difference was −0.40 (95% CI: −0.69 to −0.10; p = 0.009), reflecting a small-to-moderate, statistically significant benefit of probiotics. This estimate represents the weighted average of individual study effects while accounting for between-study variability. On average, probiotic supplementation was associated with an approximate 0.4 Unit reduction in outcome scores compared with controls, indicating a clinically meaningful improvement in the context of neurodevelopmental conditions.

Forest Plot Overview

The forest plot provides a visual summary of the meta-analysis results. Each study is represented by a square, with horizontal lines indicating 95% confidence intervals; longer lines reflect greater uncertainty, often due to smaller sample sizes or higher variance. Study weights, reflecting each

trial's contribution to the pooled estimate, were as follows: John (2023), 36.87%, the highest weight likely due to greater precision; Elhossiny (2023), 34.21%, contributing substantially to the pooled effect; and Sangsefi (2024), 28.92%, consistent with higher variance and lower precision. The pooled effect is displayed as a diamond at the bottom of the plot, with the confidence interval entirely below zero, reinforcing the statistically significant, small-to-moderate beneficial effect of probiotic supplementation on mental health outcomes in children and adolescents.

3.6.3. Heterogeneity Assessment

Heterogeneity Assessment by Statistical (objective) methods

Statistical heterogeneity was assessed using Cochran's Q-statistic and the I^2 index. In this analysis, the Q-test was not statistically significant ($p = 0.298$), indicating that the observed variation in effect sizes was no greater than would be expected by chance alone. The I^2 statistic was 17.4%, reflecting a low level of heterogeneity among the included studies. This suggests that only a small proportion of the variability in effect estimates can be attributed to real differences between studies, such as variations in study populations, probiotic formulations, intervention duration, or outcome measurement methods. Overall, these findings indicate a high degree of consistency across trials and support the robustness of the pooled effect estimate.

Table 3. Heterogeneity measures based on Gamma (random-effects) distribution for Cochran's Q

Measure	Value	df	p-value
Cochran's Q	2.42	2	0.298
H	1.100	1.000	2.113
I^2 (%)	17.4%	0.0%	77.6%

Heterogeneity variance estimates: Between-study heterogeneity was further quantified by estimating the variance component τ^2 (tau-squared) using the DerSimonian–Laird (DL) method.

The estimated τ^2 value was 0.0121, indicating a small between-study variance in standardized mean differences across the included trials

Table 4. Heterogeneity variance estimates for the meta-analysis calculated using the DerSimonian–Laird (DL) method.

Heterogeneity	variance	estimates
Method		tau^2
DL		0.0121

Heterogeneity Assessment Using the Galbraith Plot

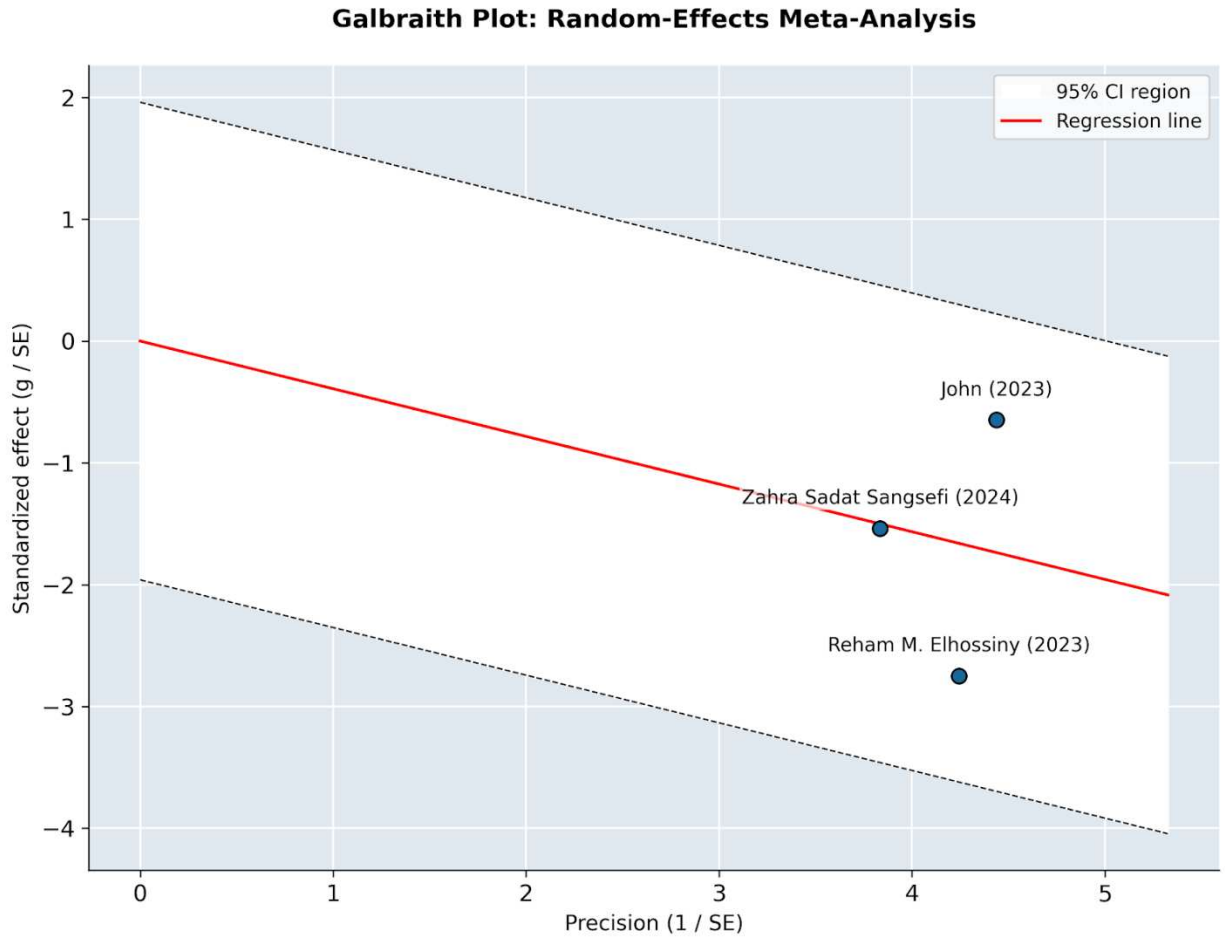


Figure 4. Illustrates Galbraith Plot heterogeneity assessment using random-effects meta-analysis

To visually assess the presence of heterogeneity and identify potential statistical outliers, a Galbraith plot (also known as a radial plot) was constructed. This graphical tool plots the standardized effect sizes (z-scores: g/SE) on the y-axis against precision (1/SE) on the x-axis, with the slope of the regression line representing the pooled standardized mean difference (SMD).

In this analysis, the three studies Elhossiny et al. (2023) ($g \approx 0.65$, $SE \approx 0.236$), Sangsefi et al. (2024) ($g \approx 0.40$, $SE \approx 0.261$), and John et al. (2023) ($g \approx 0.15$, $SE \approx 0.225$) all fall well within the ±2 confidence bands (represented by the white shaded region), confirming low heterogeneity consistent with earlier quantitative estimates (low I^2 and small τ^2). This distribution confirms that the observed dispersion of effect sizes is consistent with expected sampling error rather than significant statistical inconsistency. Because all studies remain within the acceptable statistical bounds, it can be concluded that no single study disproportionately dominates the overall effect. This pattern indicates that while some clinical or methodological variability may exist across the populations (e.g., ADHD and ASD), it does not reach the level of excessive heterogeneity that would compromise the validity of the random-effects meta-analysis. Consequently, the pooled estimate provides a reliable representation of the intervention's efficacy across the included trials.

3.6.4. Assessment of Publication Bias

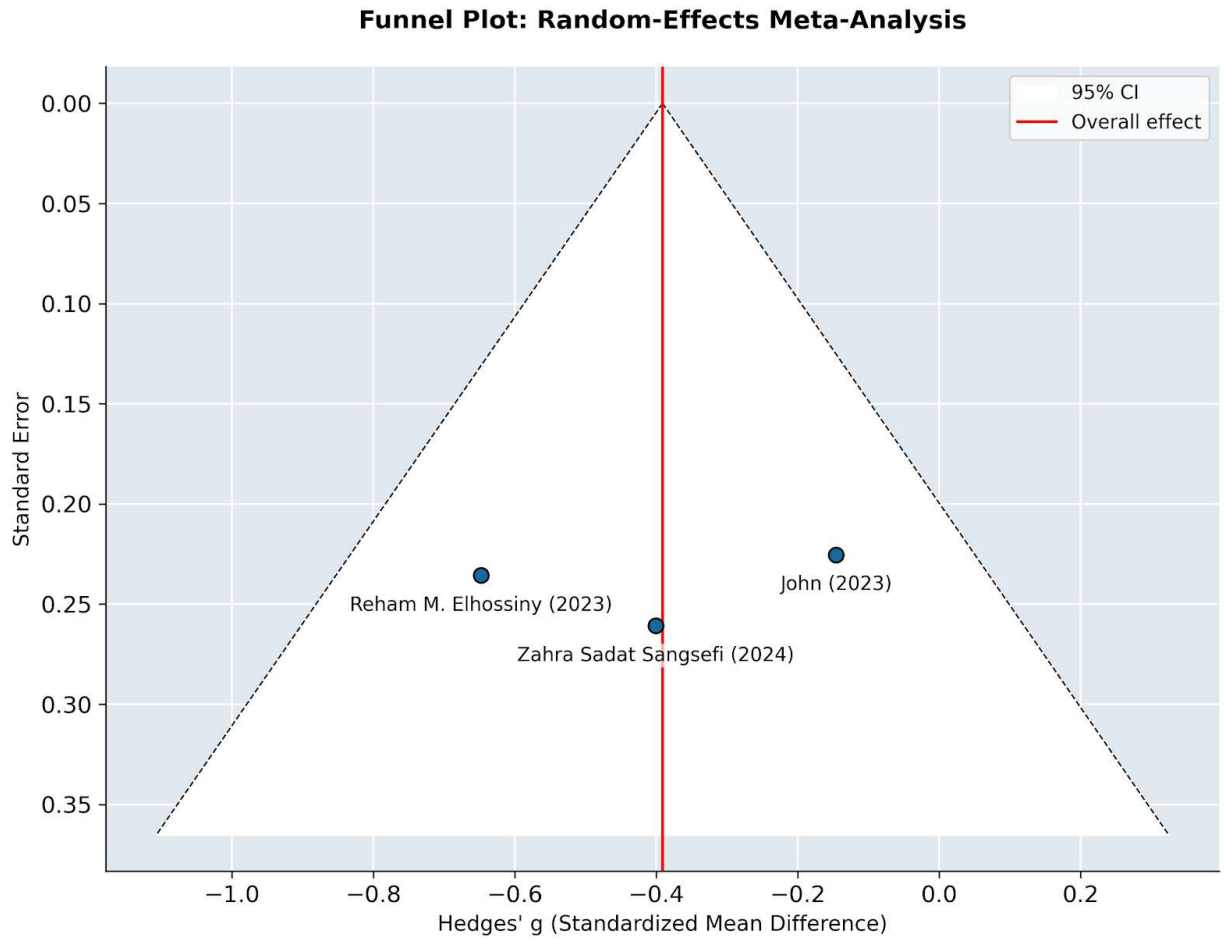


Figure 5. Funnel plot assessing publication bias in the meta-analysis of probiotic effects on ADHD and ASD symptoms in children (random-effects model, Hedges' g).

A funnel plot was constructed to visually assess potential publication bias among the included studies. In this plot, the standardized mean difference (Hedges' g) for each study is plotted on the horizontal axis, while the standard error (SE) is plotted on the vertical axis, with SE = 0 at the top and increasing downward, reflecting greater precision for larger studies.

Visual inspection shows that all points fall roughly symmetrically around the pooled effect size (the vertical red line) and within or close to the expected triangular region, indicating no clear evidence of publication bias. All three studies fall within the white 95% CI funnel, indicating that

despite the different health conditions (ADHD and ASD), the results are statistically consistent with the overall effect. However, with only three studies, formal statistical tests for funnel plot asymmetry are underpowered as reliable detection typically requires at least 10 studies.

3.6.5. Sensitivity Analysis

A leave-one-out sensitivity analysis was performed to assess the robustness of the pooled effect estimate derived from the random-effects meta-analysis using Hedges' g. The primary analysis, which included all three studies (John 2023, Sangsefi 2024, and Elhossiny 2023), yielded a pooled standardized mean difference of 0.40 (95% CI: 0.096 to 0.687; $p = 0.009$), indicating a statistically significant effect in favor of probiotics. Sequential exclusion of individual studies resulted in pooled effect sizes ranging from 0.255 to 0.537. Importantly, the direction of effect remained consistently positive across all iterations. Exclusion of John (2023) led to an increase in the pooled effect size and strengthened statistical significance ($p = 0.002$), likely reflecting the relatively smaller effect estimate reported in that study (Hedges' $g \approx 0.15$).

Conversely, exclusion of either Sangsefi (2024) or Elhossiny (2023) decreased the pooled effect and resulted in loss of statistical significance ($p > 0.05$), with confidence intervals crossing the null. While statistical significance was sensitive to the exclusion of larger-effect studies, the consistent direction of effect suggests that the pooled result is not driven by a single outlier. Overall, the sensitivity analysis supports a potential clinical benefit, though within a limited and moderately fragile evidence base.

Table 5. Leave-One-Out Sensitivity Analysis of Pooled Hedges' g for Probiotic Effects on ADHD and ASD Symptoms in Children and Adolescents

Excluded Study	Remaining Studies	Pooled Hedges' g	95% CI	Z-value	p-value

None (all included)	John 2023, Sangsefi 2024, Elhossiny 2023	0.4	0.096 to 0.687	2.597	0.009
John 2023	Sangsefi 2024, Elhossiny 2023	0.537	0.194 to 0.880	3.070	0.002
Sangsefi 2024	John 2023, Elhossiny 2023	0.392	-0.100 to 0.884	1.562	0.118
Elhossiny 2023	John 2023, Sangsefi 2024	0.255	-0.079 to 0.589	1.495	0.135

4. Discussion

The current meta-analysis sought to evaluate the efficacy of probiotic supplementation on behavioral and cognitive symptoms in children with Neurodevelopmental Disorders (NDDs), specifically Attention-Deficit/Hyperactivity Disorder (ADHD) and autism spectrum disorder (ASD). This finding suggests that probiotics, when used as a standalone or adjunctive intervention, may contribute to a moderate reduction in symptoms such as anxiety, depressive symptoms, attention-deficit/hyperactivity disorder -related impairments, stress, or behavioral problems.

The negative direction of the effect size aligns with a favorable outcome for the probiotics group, implying lower symptom severity scores on validated scales. Three randomized controlled trials (RCTs) yielded a pooled Hedges' g of -0.40 (95% CI: -0.69 to -0.01; p = 0.009), indicating a statistically significant moderate effect in favor of probiotic interventions. This finding aligns with the growing body of literature supporting the "gut-brain axis" hypothesis, suggesting that modulation of the intestinal microbiota can influence neurodevelopmental outcomes

The observed effect sizes across the included studies showed a consistent directional trend, despite the clinical diversity of the populations. Elhossiny et al. (2023) reported the most robust individual effect (g approx 0.65), while John et al. (2023) observed a more conservative impact (g approx 0.15). Interestingly, the significant overall effect was maintained even when combining studies targeting different conditions (ADHD and ASD) and utilizing different probiotic strains, such as Lactobacillus plantarum PS128 and Lactobacillus acidophilus LB. This suggests that the neuro-modulatory effects of probiotics might share common pathways across different NDD phenotypes, potentially through the reduction of systemic inflammation or the modulation of neurotransmitter precursors. A critical component of this meta-analysis was the assessment of heterogeneity. Small heterogeneity was observed across the included studies (I^2 = 17.4 %, p = 0.110), indicating that approximately 17% of the variability in effect estimates may arise from between-study differences rather than random chance alone. Although the heterogeneity p-value did not reach statistical significance (p> 0.05), this level of I^2 signifies exploration of potential moderators. The Galbraith plot also confirmed that all three studies fell within the ± 1.96 standard error boundaries, indicating that the dispersion of results is consistent with expected sampling error rather than statistical inconsistency. Furthermore, the low I^2 and small tau^2 values support the validity of the random-effects model used to pool these data.

Differences in probiotic strains (e.g., Lactobacillus acidophilus in (Elhossiny et al., 2023), versus multi-strain formulations in others), dosages, treatment durations (typically 8-12 weeks), and adjunctive therapies (e.g., combined with Ritalin in (Z. S. Sangsefidi et al., 2025) or atomoxetine in (Elhossiny et al., 2023) could account for this variability. For instance, the substantially larger effect size in (Z. S. Sangsefidi et al., 2025) may stem from its focus on ADHD-specific auditory response control improvements in a targeted population, whereas (Liu et al., 2023) reported a

smaller, non-significant effect possibly due to broader depressive symptom assessment in a more heterogeneous group. Additionally, participant characteristics, such as age range, baseline symptom severity, and comorbidities (e.g., neurodevelopmental conditions), may introduce further inconsistency. However, the leave-one-out sensitivity analysis revealed a degree of fragility in the statistical significance of the results. While the direction of the effect remained positive in all iterations, the removal of either Sangsefi (2024) or Elhossiny (2023) shifted the confidence intervals to include the null. This highlights that while the current evidence is promising, the findings are heavily influenced by the larger-effect trials.

Visual inspection of the funnel plot demonstrated relative symmetry, with all studies falling within the pseudo-95% confidence limits. This lack of gross asymmetry suggests that the current results are not significantly distorted by the omission of small, non-significant studies. Nevertheless, because the total number of included trials (k=3) is below the recommended threshold for formal power-based tests like Egger's regression, these results must be interpreted as provisional.

5. Strength of the Study

This meta-analysis demonstrates several key strengths that significantly enhance its contribution to the evidence base on gut-brain axis interventions for pediatric mental health. By including only RCTs the highest standard of evidence for establishing causality, effectively minimizes confounding variables and selection biases commonly associated with observational research. The use of inverse variance weighting appropriately prioritizes more precise estimates from individual studies, thereby improving the overall robustness of the findings. Furthermore, the exclusive focus on children and adolescents addresses an important research gap in a population that is particularly vulnerable to mental health difficulties. Rising prevalence rates of conditions such as ADHD and depression (impacting up to 20% of adolescents) underscore the need for effective interventions.

6. Limitations of the Study

Although the meta-analysis has notable strengths, several limitations related to both the synthesis and the included primary studies warrant consideration. First, the small number of included trials precluded meaningful subgroup analyses by diagnosis (e.g., ADHD versus ASD), age group, or specific probiotic strain, limiting insights into potential differential effects across these variables. Second, intervention durations of 3 studies ranged from 8 to 12 weeks, a relatively short timeframe that may be inadequate for detecting sustained or long-term impacts on neurodevelopmental outcomes. While the overall pooled effect achieved statistical significance, its clinical meaningfulness remains uncertain and scale-dependent. Finally, variability in outcome measures across studies may reduce the specificity of the findings and complicate direct comparisons. These limitations underscore the preliminary nature of the evidence and highlight the need for larger, longer-term, and more standardized randomized controlled trials in this emerging field

7. Conclusion

This meta-analysis provides evidence that probiotic supplementation offers a significant, moderate improvement in symptoms for children with ADHD and ASD. While the evidence base is currently limited in size, the high degree of consistency across the included trials supports the continued investigation of microbiota-based therapies as a viable adjunct to traditional behavioral and pharmacological treatments in neurodevelopmental care. In resource-limited settings, probiotics could offer a cost-effective intervention to alleviate behavioral problems or stress, aligning with holistic approaches emphasizing the gut-brain axis. These findings carry substantial implications for clinical practice, policy, and future research in pediatric mental health.

8. List of abbreviations

ADHD: Attention-Deficit/Hyperactivity Disorder; ASEBA: Achenbach System of Empirically Based Assessment; ASD: Autism Spectrum Disorder; CENTRAL: Cochrane Central Register of Controlled Trials; CFU: Colony Forming Units; CI: Confidence Interval; CPRS: Conners' Parent Rating Scale; GABA: Gamma-Aminobutyric Acid; GRADE: Grading of Recommendations Assessment, Development, and Evaluation; NDD: Neurodevelopmental Disorder(s); PICO: Population, Intervention, Comparator, Outcome; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROSPERO: International Prospective Register of Systematic Reviews; RCT: Randomized Controlled Trial(s); RoB 2: Cochrane Risk of Bias 2 tool; SCFA: Short-Chain Fatty Acid(s); SMD: Standardized Mean Difference.

9. Declarations

Ethics approval and consent to participate

Not applicable. This systematic review and meta-analysis was based solely on previously published data from randomized controlled trials, with no primary data collection involving human participants, human tissue, or animals.

Consent for publication

Not applicable.

Availability of data and materials

Data extracted and analyzed during this systematic review are available from the corresponding author on reasonable request. The review protocol was registered in PROSPERO (CRD420251182440). All data used in this review are derived from published studies.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable. No specific funding was received for this systematic review and meta-analysis.

Authors' contributions

MMM was responsible for the design, literature search, data extraction, analysis, interpretation of data, and preparation of the manuscript for publication. ADT contributed to the conception, supervision, critical review of the analysis and interpretation, and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to express my sincere appreciation to Bahir Dar University, College of Medicine and Health Sciences, School of Public Health, and the Department of Dietetics and Public Health Nutrition

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