International Journal for Vitamin and Nutrition Research

Int. J. Vitam. Nutr. Res. **2026**; 96(5): 52932
https://doi.org/10.31083/IJVNR52932

Review

Efficacy of Probiotic Supplementation on Gastrointestinal and Behavioral Outcomes in Children With Autism Spectrum Disorder: A Meta-Analysis

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Academic Editor: Torsten Bohn
Submitted: 15 April 2026 Revised: 24 June 2026 Accepted: 1 July 2026 Published: 28 August 2026

Abstract

Objective: This meta-analysis aimed to evaluate the efficacy of probiotic supplementation on both gastrointestinal (GI) and core behavioral symptoms in children with autism spectrum disorder (ASD). **Methods:** A systematic literature search was conducted in PubMed, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science from inception to February 5, 2026. We included randomized controlled trials (RCTs) and randomized crossover trials comparing oral probiotics with placebo or control arms (including no treatment and standard care) in children with ASD (aged ≤18 years). To minimize carryover bias, only the first-period data from crossover trials were extracted and analyzed as parallel-group data. Primary outcomes were overall GI symptoms (assessed using continuous and dichotomous measures). Secondary outcomes were core behavioral symptoms assessed by standardized scales (e.g., Autism Diagnostic Observation Schedule-Calibrated Severity Score (ADOS-CSS), Social Responsiveness Scale-2 (SRS-2)). Risk of bias was assessed using the Cochrane Risk of Bias tool for randomized trials (RoB 2.0). Random-effects meta-analyses were performed using Review Manager (RevMan) 5.4. **Results:** Six studies (four RCTs and two crossover trials) involving a total of 348 participants (330 from parallel-group RCTs and 18 from crossover trials; note that for crossover trials the reported sample sizes reflect participants who completed the first period and were included in the analysis). For GI symptoms, probiotics did not demonstrate a significant benefit on continuous outcome scores (standardized mean difference (SMD) = –0.09, 95% confidence interval (CI): –0.46 to 0.29); however, probiotics significantly increased the likelihood of achieving clinically meaningful improvement compared with placebo (relative risk (RR) = 1.28, 95% CI: 1.02–1.60; $p = 0.03$). Regarding core behavioral symptoms, a measure-specific effect was observed. Probiotic supplementation was associated with a small but statistically significant improvement when symptoms were evaluated using the ADOS-CSS (SMD = –0.42, 95% CI: –0.80 to –0.04; $p = 0.03$), but not when assessed using the parent-reported SRS-2 (SMD = –0.24, 95% CI: –0.54 to 0.05; $p = 0.11$). Heterogeneity was low for most analyses. **Conclusion:** This meta-analysis provides suggestive evidence that probiotic supplementation may confer clinically meaningful GI benefits as indicated by responder analyses, and may also improve certain directly observed core behavioral symptoms in children with ASD. However, the robustness of these pooled estimates is limited by several factors: only 2–3 studies contributed data to each pooled outcome, with the exception of SRS-2, which was based on a single study; probiotic formulations and outcome definitions varied considerably across trials; the risk-of-bias assessment revealed that only one study was low risk, one was at high risk, and four raised some concerns. Consequently, these findings should be interpreted as preliminary. Definitive conclusions will require larger, well-designed RCTs that incorporate standardized and comprehensive outcome measures. This PROSPERO Registration: CRD420261307184 (https://www.crd.york.ac.uk/PROSPERO/view/CRD420261307184).

Keywords: autism spectrum disorder; probiotics; gastrointestinal diseases; problem behavior; brain-gut axis; meta-analysis

1. Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by core deficits in social communication and interaction, alongside restricted, repetitive patterns of behavior, interests, or activities [1,2]. In recent years, the global prevalence of ASD has been rising significantly, posing a major public health challenge [3]. Beyond these core behavioral symptoms, a substantial body of research indicates that the co-occurrence of gastrointestinal (GI) symptoms is considerably higher in children with ASD compared to their typically developing peers [4,5]. These symptoms, including chronic constipation, diarrhea, bloating, and abdominal pain, severely compromise the quality of life for affected children and increase family burden [4].

Of particular interest is the growing evidence suggesting a potential link between the severity of GI symptoms and the core behavioral manifestations of ASD, often explained through the theoretical framework of the "gut-brain axis" [6]. This theory posits that alterations in the composition and function of the gut microbiota (i.e., dysbiosis) may influence central nervous system function via immune, neu-

roendocrine, and vagal pathways, thereby exacerbating or ameliorating neurobehavioral abnormalities [6,7]. It should be emphasized, however, that GI symptom improvement and core behavioral improvement represent separate clinical endpoints. While these two domains may be mechanistically interconnected through the gut-brain axis, they are not interchangeable, nor should they be assumed to necessarily improve in tandem. This hypothesis provides a rationale for targeting the gut microbiota as a potential intervention for ASD symptoms.

Probiotics, defined as live microorganisms that confer a health benefit to the host, are considered a potential strategy for modulating gut microbiota balance, improving intestinal barrier function, and reducing gut inflammation [8]. The proposed mechanisms through which probiotics may influence ASD symptoms are multifaceted: they can produce neuroactive metabolites (e.g., short-chain fatty acids, gamma-aminobutyric acid, and serotonin precursors) that act on the enteric nervous system and, via vagal afferent pathways, modulate central neural circuits involved in social behavior and stress response [6,7]. Additionally, probiotics may reduce systemic and neuroinflammation by restoring gut barrier integrity, thereby decreasing the translocation of bacterial lipopolysaccharides and subsequent microglial activation [6,8]. These mechanistic insights provide a biological rationale for probiotic interventions in ASD. Consequently, several randomized controlled trials (RCTs) have explored the efficacy of probiotic supplements in alleviating GI symptoms and potentially improving core behavioral symptoms in children with ASD [9,10]. However, the findings from these studies have been inconsistent. Some report significant improvements in both GI symptoms and behavioral scores, while others observe no significant benefits [10]. These discrepancies may be attributed to variations in study design, probiotic strains, dosage, treatment duration, and outcome measurement tools.

A number of systematic reviews and meta-analyses have assessed the effects of probiotic or nutritional interventions in individuals with ASD [11,12]. However, existing syntheses have important limitations. He et al. [12] focused on children and adolescents but did not specifically analyze core behavioral symptoms using standardized instruments. Díaz Vargas and Leonario Rodríguez [11] examined nutritional interventions in general rather than probiotics in particular, and their analysis included non-randomized study designs. Furthermore, none of the prior reviews offered a simultaneous evaluation of both GI symptoms (using continuous scores and dichotomous clinically meaningful endpoints) and core behavioral outcomes specifically within the pediatric age group (≤18 years). Thus, an updated and more comprehensive meta-analysis is needed to provide a more accurate estimate of the efficacy of probiotic supplementation on GI symptoms in children with ASD, and to rigorously assess its possible impact on core behavioral symptoms. This study aims, through a systematic review and meta-analysis, to achieve the following objectives: (1) to comprehensively evaluate the effectiveness of probiotic supplements compared to placebo in improving overall GI symptoms in children with ASD; (2) to investigate the effect of probiotic intervention on the core behavioral symptoms of ASD.

2. Methods

2.1 Study Registration and Protocol

This review was prospectively registered in PROSPERO (CRD420261307184, https://www.crd.york.ac.uk/PROSPERO/view/CRD420261307184) and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [13].

2.2 Eligibility Criteria

Studies were selected based on the following PICOS framework: population: children and adolescents (aged ≤18 years) diagnosed with ASD according to standardized diagnostic criteria (e.g., DSM-5, ICD-10, Autism Diagnostic Observation Schedule (ADOS)) [5]. Confirmation of ASD diagnosis by a trained clinician using validated tools (e.g., ADOS, ADI-R) or formally recognized diagnostic criteria (e.g., DSM-5, DSM-IV-TR, ICD-10/11) was mandatory for inclusion. Studies that depended exclusively on parent-reported diagnosis or medical records without explicit verification of diagnostic methods were not considered. It was not required that participants exhibit GI symptoms at baseline to be eligible for inclusion. No restrictions were placed on gender, ethnicity, or geographical location. Intervention: any oral probiotic supplementation (single- or multi-strain preparations), regardless of dosage or duration. Synbiotics were included only if the probiotic component was the primary intervention. Comparator: the comparator arm received placebo, no treatment, or standard care. Outcomes: primary outcomes: changes in overall GI symptoms, measured by validated GI symptom scales, questionnaires, or clinical assessments. Secondary outcomes: changes in core ASD behavioral symptoms (as assessed by standardized behavioral scales).

Exclusion criteria included: non-randomized studies (e.g., observational studies, case reports), reviews, conference abstracts (if insufficient data were reported), studies where the intervention was a synbiotic with a predominant prebiotic effect, studies involving adults (>18 years), and studies published in languages other than English or Chinese (due to resource limitations for translation). Studies that did not report quantifiable GI or behavioral outcome data were also excluded.

2.3 Information Sources and Search Strategy

A systematic literature search was performed from inception to February 5, 2026 across four major electronic

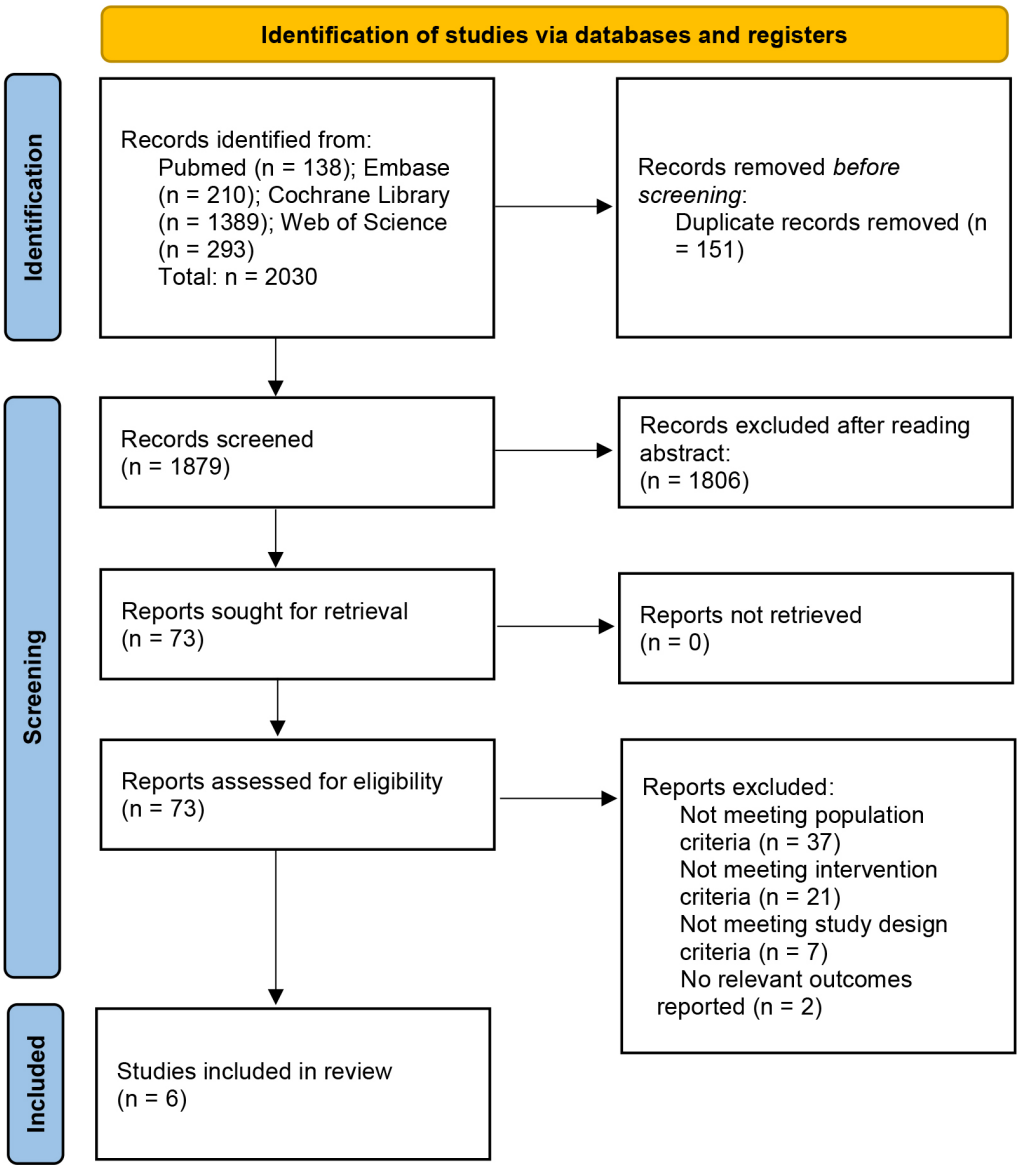

Fig. 1. PRISMA 2020 flow diagram. "n" denotes the number of records at each stage of the study selection process.

databases: PubMed, Embase (via Ovid), Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science. Additionally, a manual search of the reference lists of all included studies and relevant systematic reviews was conducted to identify any additional eligible trials.

The search strategy was developed by the research team in consultation with a medical information specialist. It combined controlled vocabulary and free-text keywords related to the three core concepts: (1) ASD, (2) probiotics, and (3) gastrointestinal symptoms. The search was tailored to the specific syntax of each database. The complete search strategies for all databases can be found in the **Supplementary Material**.

2.4 Study Selection Process

All retrieved records were imported into the reference management software Zotero (Corporation for Digital Scholarship, Vienna, Virginia, USA), and duplicates were removed. The study selection was performed independently by two reviewers in two stages. First, titles and abstracts were screened against the eligibility criteria. Second, the full texts of potentially relevant articles were retrieved and assessed in detail. Any disagreements at either stage were resolved through discussion or by adjudication from a third senior reviewer. The selection process, including reasons for exclusion at the full-text stage, is documented and presented in a PRISMA 2020 flow diagram (Fig. 1).

2.5 Data Collection Process

A standardized, pilot-tested data extraction form was developed in Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). Two reviewers independently extracted data from the included studies. Extracted data

included: study characteristics, participant characteristics, intervention details, control details, outcome data. In trials where probiotic treatment was combined with additional interventions such as behavioral therapy or routine care, we ascertained that these concomitant treatments were balanced across the intervention and control arms. This approach allowed us to attribute any intergroup disparities specifically to the probiotic regimen. For studies reporting only medians, interquartile ranges, or standard errors, appropriate conversions or estimations were planned following the Cochrane Handbook recommendations [14]. For dichotomous GI outcomes, we extracted from each study the specific cutoff criteria used to define clinically meaningful improvement. Narula Khanna et al. [9] adopted a reduction from severe Gastrointestinal Severity Index (GSI) (≥6) to below 6 as their threshold; Santocchi et al. (2020) [15] applied a ≥50% decrease in GSI score relative to baseline; and Billeci et al. (2023) [16] used a ≥2-point reduction in GSI score.

For continuous outcomes—including GI symptom scores and behavioral scale scores—our preference was to extract endpoint scores (means and standard deviations measured at the completion of the intervention). When such data were unavailable but change-from-baseline scores (means and standard deviations of the change) were reported, we extracted the latter. According to the Cochrane Handbook, the standardized mean difference (SMD) is suitable for pooling both endpoint and change scores. In the present meta-analysis, however, all continuous outcomes (GI continuous, ADOS-CSS, and Social Responsiveness Scale-2 (SRS-2)) were pooled using only endpoint scores, with no change scores incorporated.

Discrepancies in extracted data were resolved by consensus or by referring back to the original publication. The extracted data were subsequently entered into Review Manager (RevMan) software version 5.4 for analysis.

2.6 Risk of Bias in Individual Studies

The methodological quality of each included RCT was independently assessed by two reviewers using the revised Cochrane Risk of Bias tool for randomized trials (RoB 2.0) (The Cochrane Collaboration, London, UK) [17]. This tool evaluates bias across five domains: (1) randomization process, (2) deviations from intended interventions, (3) missing outcome data, (4) measurement of the outcome, and (5) selection of the reported result. For crossover trials, an additional domain regarding the crossover design was considered. Each domain was judged as "low risk", "some concerns", or "high risk". An overall risk of bias judgment for each study was generated based on the domain-level judgments. Disagreements were resolved by discussion or by consulting a third reviewer. The results of the RoB 2.0 assessment are presented graphically.

2.7 Data Synthesis and Statistical Methods

Statistical analysis was performed using RevMan version 5.4. For continuous outcomes, the SMD with 95% confidence interval (CI) was calculated as the summary effect measure. Hedges'g correction was applied in the calculation of all SMDs to account for potential bias in small samples, as this approach is advised when study sample sizes are limited. This was chosen because different scales were used to measure both GI and behavioral symptoms across studies. The SMD was interpreted as follows: <0.2 (negligible), 0.2–0.5 (small), 0.5–0.8 (medium), and >0.8 (large) effect. Heterogeneity among studies was assessed using the I^2 statistic and the Chi^2 test. I^2 values of 25%, 50%, and 75% were interpreted as low, moderate, and high heterogeneity, respectively [18]. A p value < 0.10 for the Chi^2 test was considered indicative of significant statistical heterogeneity. Given the anticipated clinical and methodological diversity among studies (e.g., different probiotic strains, durations), a random-effects model using the DerSimonian and Laird method was used for all primary meta-analyses [19].

For the single randomized crossover trial incorporated in this meta-analysis (Sanctuary et al. [20]), we extracted exclusively the first-period data—defined as measurements obtained at the conclusion of the initial intervention phase prior to crossover—and analyzed them as parallel-group data. Data from the second period (collected after washout and crossover) were not included. This strategy circumvents the need to model within-participant correlation, period effects, and carryover effects, which would be challenging and potentially unreliable given that only one crossover trial were available. Sanctuary et al. [20] employed a 2-week washout. Considering that probiotics may have sustained effects on gut microbiota and host physiology, this washout interval may not be adequate to completely eliminate carryover effects. Consequently, the more conservative first-period-only approach is justified. Furthermore, we conducted sensitivity analyses using a fixed-effect model to examine whether our findings were sensitive to the choice of statistical model; the results remained consistent with those of the primary analysis, reinforcing the validity of our crossover trial handling. Sensitivity analyses were conducted to test the robustness of the primary results by: (1) excluding studies judged to have a 'high' overall risk of bias, (2) using a fixed-effect model instead of a random-effects model, and (3) excluding individual studies one at a time to assess their influence on the pooled effect size.

Publication bias was assessed visually using funnel plots for outcomes with 10 or more studies and statistically using Egger's regression test [21]. Egger regression test would have been performed using Stata 17.0 (metabias command) or R software (meta package) if the number of studies had been adequate (≥10), as RevMan 5.4 (The Cochrane Collaboration, Copenhagen, Denmark) does not

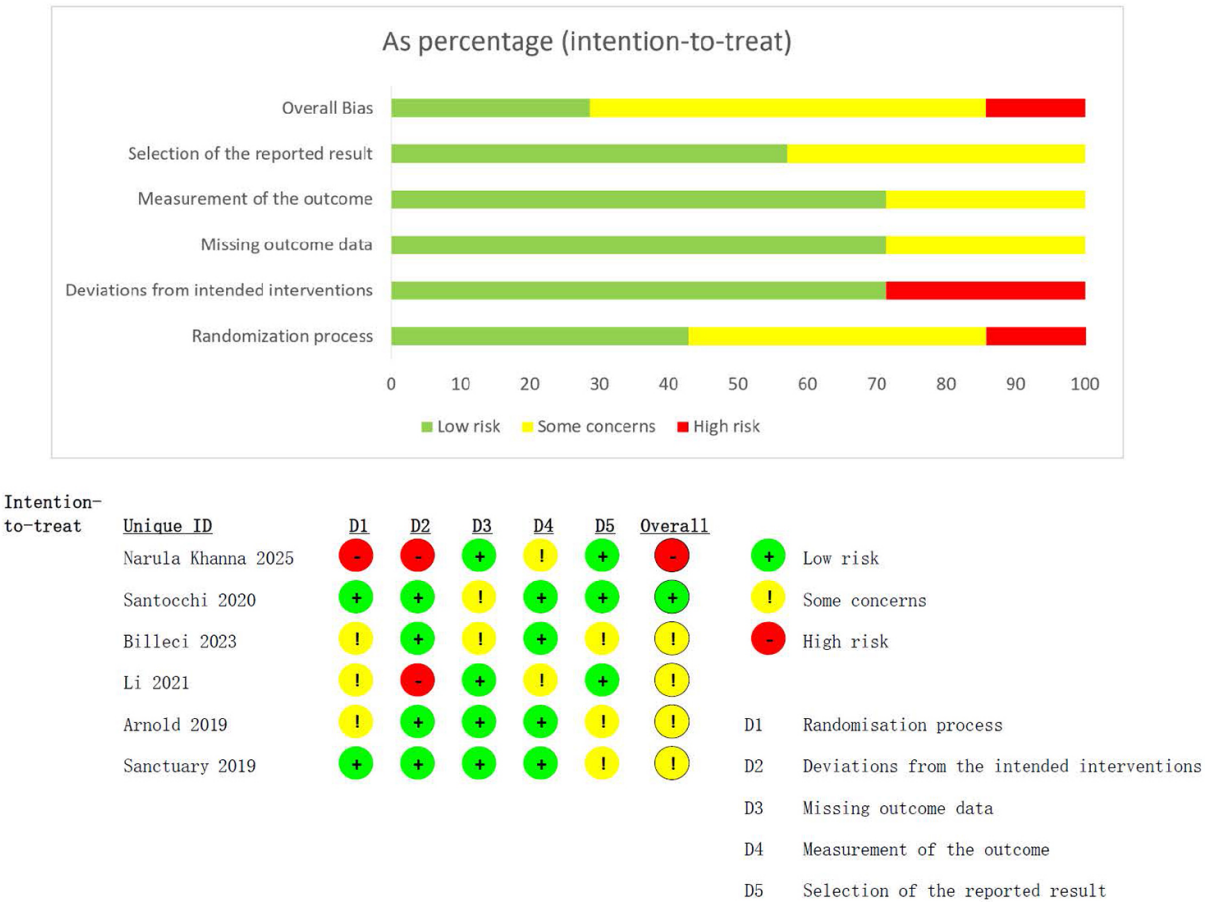

Fig. 2. Risk of bias summary. Risk of bias assessments for each included study using the Cochrane RoB 2.0 tool. Domains evaluated: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. Judgments are at the study level for the primary outcomes.

provide native support for such an analysis. A p value < 0.05 for Egger's test was considered indicative of potential small-study effects.

3. Results

3.1 Study Selection

Our systematic search of PubMed (n = 138), Embase (n = 210), CENTRAL (n = 1389), and Web of Science (n = 293) initially yielded 2030 records. Following the removal of 151 duplicates, 1879 unique records proceeded to title and abstract screening, from which 1806 were discarded as evidently irrelevant. The remaining 73 full-text articles were retrieved and assessed against our eligibility criteria. After detailed full-text evaluation, 67 articles were excluded for the following reasons: inappropriate population (n = 37), ineligible intervention (n = 21), unsuitable study design (n = 7), and unreported relevant outcomes (n = 2). Finally, 4 randomized controlled trials and 2 randomized crossover trials were included; of these, 4 RCTs and 1 crossover trial contributed to the quantitative meta-analyses, while 1 RCT (Li et al. [22]) and 1 crossover trial (Arnold et al. [23]) were summarized qualitatively.

3.2 Characteristics of Included Studies

This meta-analysis ultimately included 4 RCTs [9,15,16,22] and 2 randomized crossover trials [20,23]. The key characteristics of these studies are summarized in Table 1 (Ref. [9,15,16,20,22,23]).

3.3 Risk of Bias Assessment

The methodological quality of the included trials was assessed using the Cochrane RoB 2.0 tool. A summary of the judgments is presented in Fig. 2. Our risk-of-bias evaluation using RoB 2.0 classified Santocchi et al. [15] as having a low overall risk. Narula Khanna et al. [9], however, was deemed to be at high risk, mainly because of problems with the randomization process and deviations from intended interventions. The other four included studies (Billeci et al. [16], Li et al. [22], Arnold et al. [23], and Sanctuary et al. [20]) were judged to have some concerns. For Li et al. [22], although the lack of blinding led to a high-risk rating for Domain 2, the study's overall assessment remained "some concerns", as the co-intervention was equally provided to both arms and its outcome contributed only to the descriptive synthesis. The most prevalent con-

Table 1. Characteristics of included studies.

Study	Country	Design	Sample size (I/C)	Age (years)	Baseline GI symptoms	Probiotic strains	Duration	GI outcome	Core behavioral outcome
Narula Khanna et al. (2025) [9]	India	RCT	90/90	2–9	All had GI symptoms	12-strain patented blend	3 months	The proportion of severe GSI (≥6) decreased: 27.8% (probiotic group)	The total score change of SRS-2: I: –6.10; C: –3.10 (p = 0.01)
Billeci et al. (2023) [16]	Italy	RCT	26/20	2.2–6.1	Grouped by GSI	De Simone Formulation (8 strains)	6 months	6-GSI score: I: 1.2 ± 2.5; C: 1.5 ± 1.1	ADOS-CSS: I: 6.3 ± 1.6; C: 6.9 ± 1.9
Santocchi et al. (2020) [15]	Italy	RCT	31/32	1.5–6	Grouped by GSI	De Simone Formulation (8 strains)	6 months	6-GSI score: I: –0.83; C: –0.08	ADOS-CSS: I: –0.65; C: +0.03 (p = 0.08)
Li et al. (2021) [22]	China	RCT	21/20	3–6	Included children with GI symptoms	*Bifidobacterium*, *L. acidophilus*, *E. faecalis*	3 months	Specific GI symptom scores not reported	ATEC total score: I: 57 ± 23 (n = 21) C: 70 ± 25 (n = 20)
Arnold et al. (2019) [23]	USA	Randomized Crossover Trial	10 (completed crossover)	3–12	All had GI symptoms, low PedsQL GI scores	VISBIOME (8 strains: 4 types of lactic acid bacteria, 3 types of *bifidobacteria*, 1 type of thermophilic streptococcus)	8 weeks per phase, 3-week washout	PedsQL GI score: not directly listed	Social Responsiveness Scale (SRS) T-Score: Model-estimated difference: –3.07 (SE 2.68), Effect size d = 0.36.
Sanctuary et al. (2019) [20]	USA	Randomized Crossover Trial	8 (completed analysis)	2–11	87.5% gas/bloating, 62.5% met IBS criteria	*Bifidobacterium longum* subsp. infantis (UCD272)	5 weeks per arm (with 2-week washout)	87.5% showed improvement	ABC total score (BCP group): –21.5 ± 15.556

Abbreviations: 6-GSI, Six-item Gastrointestinal Severity Index; RCT, randomized controlled trial; I/C, intervention/control; GSI, Gastrointestinal Severity Index; SRS-2, Social Responsiveness Scale-2; ADOS-CSS, Autism Diagnostic Observation Schedule-Calibrated Severity Score; ATEC, Autism Treatment Evaluation Checklist; ABC, Aberrant Behavior Checklist; PedsQL, Pediatric Quality of Life Inventory; BCP, Bovine Colostrum Product; GI, Gastrointestinal; IBS, Irritable Bowel Syndrome; SE, Standard Error.

These two studies (Santocchi et al., 2020 [15] and Billeci et al., 2023 [16]) used independent participant samples with no overlap.

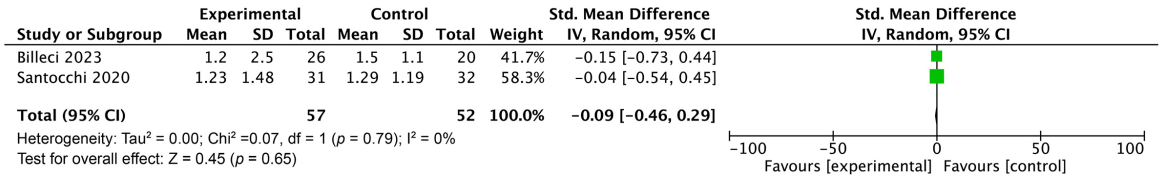

Fig. 3. Forest plot of the effect of probiotic supplementation on overall gastrointestinal symptom scores (continuous outcome). CI, confidence interval; SD, standard deviation; Std., standardized mean difference.

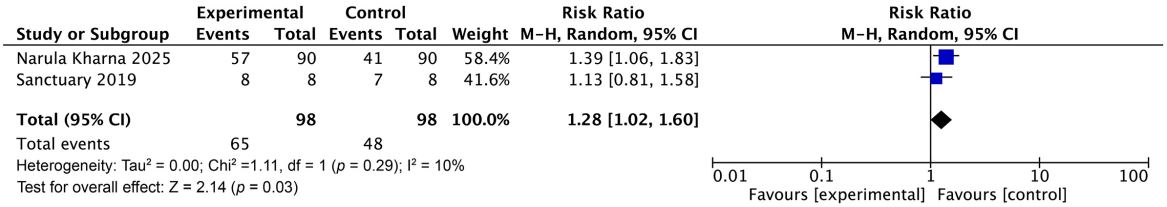

Fig. 4. Forest plot of the effect of probiotic supplementation on clinically significant improvement in gastrointestinal symptoms (dichotomous outcome). M-H, Mantel-Haenszel.

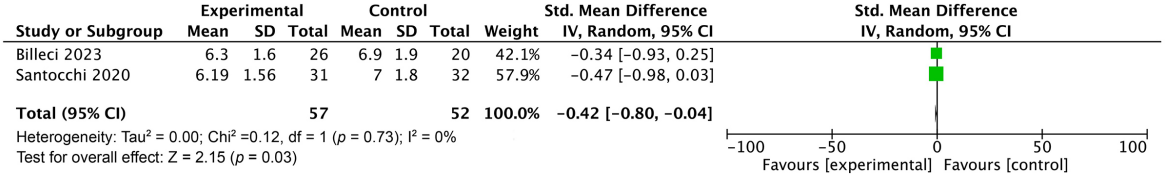

Fig. 5. Forest plot of the effect of probiotic supplementation on core behavioral symptoms as measured by the ADOS-CSS.

cerns arose from the randomization process and deviations from the intended interventions, largely due to insufficient reporting of allocation concealment and blinding procedures.

3.4 Results of Meta-Analysis

3.4.1 Primary Outcome: Overall Gastrointestinal Symptoms

Fig. 3 presents the pooled effect of probiotic supplementation on overall GI symptom scores (continuous outcome) from two studies that provided mean and standard deviation data at endpoint. The random-effects meta-analysis showed a non-significant reduction in GI symptom scores with probiotics compared to placebo (SMD = –0.09, 95% CI: –0.46 to 0.29, $p = 0.65$). Heterogeneity was low ($I^2 = 0\%$, $Tau^2 = 0.00$), indicating consistency across studies in this analysis subset.

Fig. 4 illustrates the effect of probiotics on the proportion of participants experiencing clinically significant GI symptom improvement (dichotomous outcome). The pooled analysis of two studies demonstrated a statistically significant benefit of probiotics over placebo (RR = 1.28, 95% CI: 1.02 to 1.60; $p = 0.03$). Heterogeneity was low ($I^2 = 10\%$, $Tau^2 = 0.00$).

3.4.2 Secondary Outcome: Core Behavioral Symptoms

As shown in Fig. 5, the pooled analysis of two trials assessing behavioral symptoms via the ADOS-CSS scale at endpoint revealed a statistically significant improvement in the probiotic group (SMD = –0.42, 95% CI: –0.80 to –0.04; $p = 0.03$), indicating a small effect size consistent with our predefined thresholds. Heterogeneity was low ($I^2 = 0\%$, $Tau^2 = 0.00$), suggesting consistent findings across the included studies.

Fig. 6 summarizes data from one study using the SRS-2 to evaluate social responsiveness and core behavioral symptoms of ASD at endpoint. The single-study estimate yielded a small, non-significant effect (SMD = –0.24, 95% CI: –0.54 to 0.05; $p = 0.11$). This indicates that probiotic supplementation did not substantially impact SRS-2-assessed behaviors in the studied populations.

3.5 Heterogeneity and Sensitivity Analyses

Heterogeneity was low across all pooled analyses ($I^2 = 0\%$ for GI continuous and ADOS-CSS; $I^2 = 10\%$ for dichotomous GI outcome). The SRS-2 result was based on a single study and is therefore presented as an individual-study estimate without an I^2 value. Sensitivity analyses that removed high-risk-of-bias studies produced pooled estimates identical to the primary findings (RR = 1.28, 95% CI: 1.02 to 1.60; $p = 0.03$, $I^2 = 10\%$, $Tau^2 = 0.00$), with no change in effect direction or significance. Funnel plots were not constructed due to the limited number of studies per outcome (<10), precluding formal assessment of publication bias.

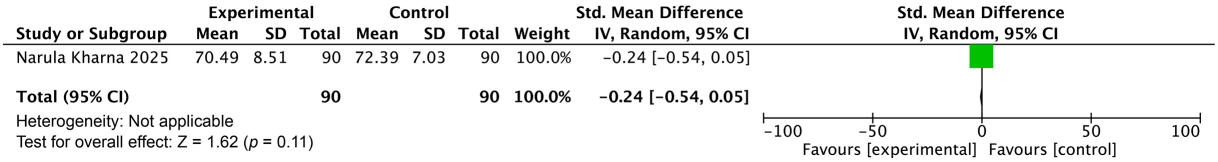

Fig. 6. Forest plot of the effect of probiotic supplementation on social responsiveness as measured by the SRS-2.

3.6 Qualitative Synthesis of Studies Not Included in Meta-Analysis

Two eligible randomized controlled trials—Li et al. [22] and Arnold et al. [23]—met the inclusion criteria but could not be incorporated into the quantitative meta-analysis for specific methodological reasons. Li et al. [22] used the Autism Treatment Evaluation Checklist (ATEC) total score as their behavioral outcome measure. Because this tool captures diverse treatment effects across language, behavior, and social domains—and is conceptually distinct from the prespecified core behavioral instruments (ADOS-CSS and SRS-2)—we considered that pooling ATEC data with the other measures would not yield an interpretable summary effect. Arnold et al. [23] reported the SRS T-score but furnished only a model-estimated group difference (−3.07, SE 2.68) and an effect size ($d = 0.36$), with no endpoint means or standard deviations reported for either group. Because the SMD could not be derived from the available data, this study could not be incorporated into the meta-analysis. Accordingly, a narrative summary of both studies is provided below. Li et al. [22] conducted a 3-month parallel-group RCT involving 41 Chinese children with ASD (aged 3–6 years) to evaluate the effect of *Bifidobacterium*- and *Lactobacillus*-based probiotic supplementation combined with applied behavior analysis (ABA) versus ABA alone. Both the probiotic group and the control group received the ABA intervention under the same conditions, thereby ensuring that any observed differences between the two arms could be ascribed to the probiotic treatment. Although no gastrointestinal outcome data were reported, the study demonstrated a significantly greater reduction in ATEC total scores in the probiotic group compared to controls (57 ± 23 vs. 70 ± 25; $p = 0.007$), alongside favorable shifts in gut microbiota composition at the genus level. Although these results trend in the same direction as those from the ADOS-CSS meta-analysis, the ATEC and ADOS-CSS assess fundamentally different behavioral constructs, so direct comparability cannot be assumed. Nevertheless, these observations offer indirect support for the microbiota-gut-brain axis hypothesis.

Arnold et al. [23], in a U.S.-based randomized crossover pilot trial of 10 children with ASD, GI symptoms, and anxiety, assessed the 8-strain probiotic formulation VISBIOME (ExeGi Pharma, LLC, Rockville, MD, USA) over two 8-week phases. While the primary outcome (Pediatric Quality of Life Inventory (PedsQL) GI module) and anxiety measures did not reach statistical significance—likely due to limited sample size—a significant improvement was observed in parent-selected GI target symptoms ($p = 0.02$, $d = 0.79$). Notably, the relative abundance of *Lactobacillus* was significantly correlated with PedsQL scores (Spearman's $\rho = 0.57$, $p = 0.022$). A potential connection between *Lactobacillus* colonization and GI-related quality of life is suggested by this exploratory correlation. Nevertheless, the small sample size and the preliminary nature of the trial caution against interpreting this finding as proof of a dose-dependent effect. The study also reported sustained improvement through a 3-week washout, indicating potential carryover effects that warrant further investigation in parallel-group designs.

Together, these two studies provide convergent, albeit non-quantifiable, support for the potential benefits of probiotic supplementation in children with ASD—particularly in improving behavioral symptoms and GI-related target complaints. Their findings are consistent with the direction of effect observed in the main meta-analysis and underscore the need for future trials to adopt harmonized, meta-analyzable outcome measures.

4. Discussion

The present meta-analysis systematically evaluated the efficacy of probiotic supplementation on both gastrointestinal and core behavioral symptoms in children with ASD. This meta-analysis is characterized by the simultaneous evaluation of GI symptoms through both continuous and dichotomous (responder) measures, as well as the assessment of core behavioral outcomes. We further confined our analysis to children and adolescents (≤18 years) and adhered to the PRISMA 2020 and Cochrane RoB 2.0 frameworks for methodological rigor. These design choices collectively facilitate a more nuanced understanding of probiotic interventions in this population.

Our primary analyses showed that the observed effect of probiotics differed according to the type of outcome measure employed. For GI symptoms measured on continuous scales, the probiotic group did not show a significant improvement relative to placebo (SMD = –0.09, 95% CI: –0.46 to 0.29, $p = 0.65$). In contrast, when the outcome was expressed as the proportion of responders achieving clinically significant improvement, a statistically robust and clinically meaningful benefit was observed in favor of probiotics (RR = 1.28, 95% CI: 1.02 to 1.60, $p = 0.03$). This discrepancy underscores a critical methodological consideration in intervention studies for ASD: the choice of end-

point may significantly influence the interpretation of efficacy. Continuous symptom scales may be less sensitive to detect changes that are subjectively meaningful to patients and caregivers, whereas responder analyses based on predefined clinically important differences may better capture real-world therapeutic benefits. The low heterogeneity observed in the dichotomous analysis ($I^2 = 10\%$) suggests that the beneficial effect of probiotics on clinically meaningful GI improvement was consistent across the included studies, despite variations in baseline symptom severity, probiotic strains, and the specific cutoff criteria used to define improvement.

Regarding core behavioral symptoms, our results revealed a measure-specific effect. Probiotic supplementation was associated with a small and statistically significant improvement in symptoms as measured by the ADOS-CSS (SMD = –0.42, $p = 0.03$). It should be noted, however, that this result is preliminary in nature and is confined to the ADOS-CSS, which is a clinician-administered observational instrument. This finding should not be interpreted as evidence of a global improvement in core ASD symptomatology, and confirmation through larger, adequately powered trials is necessary before definitive conclusions can be reached. In contrast, no significant effect was found on the parent-reported SRS-2 (SMD = –0.24, $p = 0.11$). One possible explanation for this divergence lies in the fundamental differences between the two instruments, though this remains speculative and requires direct empirical testing. The ADOS-CSS, which is administered by trained clinicians in a standardized observational context, may offer greater objectivity and sensitivity to subtle behavioral changes. The SRS-2, by contrast, relies on parental reports across naturalistic settings and may be more prone to expectation or reporting biases, especially when blinding is not maintained. However, these considerations are only hypothetical at this stage; only studies that apply both instruments within the same trial can clarify whether measurement discrepancies account for the observed divergence. This pattern aligns with the findings by Mazzone et al. [10], who reported improvements in specific social behaviors but not in overall autism severity scores, suggesting that probiotics may modulate particular behavioral domains rather than exerting a global effect on ASD symptomatology. Moreover, although ABA was balanced across groups in the Li et al. [22] trial, the possibility of synergistic or interactive effects between probiotics and behavioral therapy cannot be entirely ruled out, meaning that the observed behavioral gains should not be ascribed solely to probiotic treatment. We also emphasize that the ADOS-CSS, SRS-2, Aberrant Behavior Checklist (ABC), CARS, and ATEC evaluate different conceptual domains. The ADOS-CSS is designed to capture core ASD features through clinician observation, whereas the SRS-2, ABC, and ATEC are parent-reported measures that may encompass broader behavioral issues not necessarily specific to autism. Therefore, our results should be interpreted with reference to each individual instrument rather than as evidence of a global effect on core behavioral symptoms.

The gut-brain axis provides a plausible mechanistic framework for observations [6,7]. The significant improvement in dichotomous GI outcomes suggests that probiotics can effectively modulate gut homeostasis in a subset of children with ASD. This reduction in gastrointestinal symptom burden may, in turn, influence neural function through reduced inflammation, improved intestinal barrier integrity, or modulation of microbial-derived neuroactive metabolites [24]. The positive signal on the ADOS-CSS, but not the SRS-2, could imply that such gut-mediated effects may preferentially impact observable social-communicative behaviors rather than broader trait-like characteristics assessed by parent questionnaires. The probiotic interventions in the included trials varied considerably in their composition, ranging from single-strain products such as *Bifidobacterium longum* subsp. infantis to multi-strain formulations containing up to 12 distinct organisms. This heterogeneity carries important implications for the biological interpretation of our results. Different bacterial genera and species may act on the gut-brain axis via diverse mechanisms: *Lactobacillus* and *Bifidobacterium* species—present in several of the included studies—have been reported to reduce systemic inflammation, strengthen intestinal barrier function, and generate neuroactive compounds including gamma-aminobutyric acid and short-chain fatty acids. Nevertheless, it remains uncertain which specific strains, dosages, or combinations are responsible for the observed clinical effects. Given the wide variation in formulations across studies, our summary estimates cannot be linked to any single probiotic composition, and no strain-specific guidance can be offered at present. Future studies incorporating biomarker correlates (e.g., inflammatory cytokines, microbiota composition, metabolomic profiles) are essential to elucidate these pathways and identify predictors of response [25].

5. Limitations

Several limitations of this analysis warrant consideration. First, the total number of included RCTs remains modest (n = 6), limiting the statistical power for subgroup analyses and increasing the vulnerability of findings to individual study characteristics. Furthermore, the majority of included trials are small-scale, exploratory, and single-center in design, which raises the concern that positive results may be inflated by small-study effects. Accordingly, the pooled estimates warrant cautious interpretation. Second, considerable clinical heterogeneity exists across studies with respect to probiotic strains (single versus multi-strain, varying genera and species), dosages (ranging from 10^8 to 10^{10} CFU/day), and treatment durations (spanning 5 weeks to 6 months). Combining such biologically diverse interventions under a single meta-analytic framework

implicitly assumes—likely incorrectly—that all probiotics exert comparable effects on GI and behavioral outcomes. This assumption neglects strain-specific mechanisms, including immunomodulatory properties and neurotransmitter synthesis. Thus, our pooled effect sizes should not be construed as evidence supporting probiotics as a uniform therapeutic class, and this meta-analysis does not permit recommendations regarding any specific strain, dose, or treatment duration. Future investigations should employ strain-specific meta-analyses or network meta-analyses to better address this heterogeneity. Third, as noted, the risk of bias was a concern in several studies, primarily due to inadequate reporting of blinding and allocation concealment. Fourth, for some studies, outcome data (e.g., mean and standard deviation) were not directly reported and had to be derived through secondary transformation or estimation from available statistics (e.g., medians, interquartile ranges, or standard errors), which may introduce measurement error and affect the precision of pooled effect estimates. Fifth, the thresholds used to define clinically meaningful improvement for the dichotomous GI outcome differed across studies (as outlined in the Results), which may have contributed to the low heterogeneity (I^2 = 10%) detected in that pooled analysis. Sixth, our search strategy was confined to English- and Chinese-language publications, which may have introduced language bias, thus limiting the global representativeness of our conclusions. Seventh, the initial search did not include microbiome-related keywords such as gut microbiota or microbiome, potentially compromising search sensitivity. To address this, we manually searched the reference lists of included studies and of recent systematic reviews on probiotics in ASD published within the past three years. However, we cannot rule out the possibility that eligible trials using only microbiome-related terminology without explicitly referring to gastrointestinal symptoms were overlooked. Eighth, the DerSimonian-Laird random-effects model can generate unstable variance estimates when the meta-analysis includes few studies, as was the case for outcomes like ADOS-CSS (based on two trials) and SRS-2 (based on a single study). Although fixed-effect sensitivity analyses yielded results consistent with the primary analysis (direction and significance unchanged), the precision of these estimates must be interpreted with care. Future meta-analyses with a larger evidence base should consider the Hartung-Knapp adjustment or more sophisticated random-effects approaches. Ninth, co-interventions (ABA or standard care) were given alongside probiotics in the included trials. Although these co-interventions were balanced between groups, the observed effects can be attributed primarily to probiotic supplementation rather than to the combined approach, as the co-interventions were equally applied to both arms. However, potential synergistic or interactive effects between probiotics and behavioral therapies cannot be fully excluded, and future studies should explicitly assess probiotic–behavioral interactions. Tenth, publication bias could not be formally evaluated using funnel plots or Egger's regression test, as each outcome included fewer than 10 studies. Thus, the presence of publication bias or small-study effects cannot be excluded, and our results should be read with this caveat. Finally, five of the six included studies recruited children with baseline GI symptoms. Therefore, our findings are most applicable to ASD children with concurrent GI issues, and their extrapolation to ASD children without GI symptoms is uncertain.

6. Conclusions

In summary, this meta-analysis offers preliminary evidence that probiotic supplementation may be associated with GI symptom improvement as measured by responder analysis, and may also yield benefits in certain core behavioral domains in children with ASD as assessed by clinician-administered instruments. However, these conclusions must be qualified by the small number of available studies, the lack of significant findings on continuous GI scales and the parent-reported SRS-2, and the substantial clinical heterogeneity across trials. The observed effects appear to be measure-specific and may not be generalizable to the entire ASD population. Accordingly, these results should be viewed as hypothesis-generating rather than definitive. Confidence in the pooled estimates is further constrained by the fact that only one of the six included studies was rated at low risk of bias, with the remainder showing some concerns or high risk. Large-scale, methodologically rigorous RCTs incorporating standardized, multi-dimensional outcome assessments—including clinician-rated, parent-reported, and biomarker-based endpoints—are needed to confirm or challenge these preliminary findings. Future studies should prioritize the identification of predictive biomarkers to enable personalized probiotic regimens, as well as head-to-head comparisons of distinct probiotic strains and formulations.

Availability of Data and Materials

Not applicable.

Author Contributions

Conception and design: CLF and WYQ. Acquisition of data: All authors. Collection and assembly of data: All authors. Data analysis and interpretation: YS. Manuscript writing: All authors. Final approval of manuscript: All authors. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

We would like to express our gratitude to all those who helped us during the writing of this manuscript. Thanks to all the peer reviewers for their opinions and suggestions.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJVNR52932.

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