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The effect of probiotic intervention on behavioral and gastrointestinal symptoms in children with autism: A systematic review and meta-analysis

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ABSTRACT

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition affecting children's physical and psychological well-being. Recent research highlights the gut microbiota's role in autism, with probiotic interventions gaining attention as a potential treatment.

Objective: This meta-analysis evaluates the impact of probiotics on behavioral and gastrointestinal (GI) symptoms in children with autism, focusing on clinical and crossover randomized controlled trials (RCTs).

Methods: We systematically searched PubMed, Embase, Web of Science, and EBSCOhost for relevant studies published in the past decade. Meta-analysis was performed using R software (version 4.4.1).

Results: A total of 8 studies were included in the Meta-analysis, and we found a significant overall effect size of the probiotic intervention on the severity of behavioral symptoms in children with autism (SMD = −0.251, 95 % CI:−0.466-−0.037,P = 0.04). Only three of the studies reported data related to gastrointestinal symptoms, and the analysis showed a non-significant intervention effect of probiotics in this area (SMD = −0.048, 95 % CI: −0.399 to 0.303, P = 0.41). Additional subgroup analyses showed no significant effect of region, duration of intervention, or probiotic type on the intervention effect.

Conclusion: Probiotic intervention reduced autism symptoms but had minimal effect on GI symptoms. Limitations include small sample sizes, short intervention durations, and variability in probiotics and measurement scales. Further high-quality RCTs are needed to confirm these findings.

Autism, fully known as autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by impaired social interaction and communication, in addition to restricted and repetitive patterns of behavior and interest (Diagnostic and statistical manual of mental disorders, 2013). The global prevalence of ASD has increased dramatically over the past three decades (Baio et al., 2018; Maenner et al., 2020; Kawa et al., 2017; Maenner et al., 2021), with the latest report from the United States (US) showing a prevalence rate of 1 in 44, and a prevalence rate of about 1 % in China, with 13 million patients, including 3 million children, making the disease a serious burden on families and society in China and around the world (Maenner et al., 2021; Lord et al., 2018). ASD primarily emerges in childhood, with a complex etiology that remains undefined. It is widely believed to result from the

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combined influence of genetic and environmental factors (Newschaffer et al., 2007; Ronemus et al., 2014; Boggess et al., 2016). Currently, there is no standardized treatment for ASD due to the intricacy of its causes. Treatment approaches are generally divided into behavioral interventions and pharmacological options. Common behavioral therapies include sensory integration training and Applied Behavior Analysis (ABA), which studies suggest may be effective across various age groups in children with ASD (Hume et al., 2021). While certain pharmacological studies suggest potential benefits, conclusive evidence supporting their effectiveness remains limited (Dean et al., 2017). The increased prevalence of ASD and the lack of significant efficacy of behavioral interventions and medications in children with autism spectrum disorders make the search for new treatments an urgent need.

In recent years, with the in-depth study of intestinal flora, it has been found that intestinal flora disorders can directly or indirectly lead to the development of ASD through the "microbe-gut-brain" axis (Góralczyk-bińkowska et al., 2022). Probiotics, on the other hand, are a type of intestinal flora, which are considered living microorganisms that, when given in sufficient amounts, can provide health benefits to their hosts (Hill et al., 2014), and they produce and transport neuroactive substances that act on the brain-gut axis, such as γ-aminobutyric acid produced by Lactobacillus stemloides, and dopamine produced by Staphylococcus aureus and Escherichia coli (Muhammad et al., 2022). It is worth noting that the commercial probiotics (e.g., Lactobacillus spp. and Bifidobacterium spp.) commonly used in clinical practice only cover a very small portion of the human intestinal microbiota (<0.1 %) (Derrien & Veiga, 2017), and their mechanisms of action are mostly limited to local bacterial regulation or metabolite secretion, which is fundamentally different from that of the Fecal Microbiota Transplantation (FMT) through the reestablishment of the overall microbial ecosystem (Choi & Cho, 2016). Various probiotic formulations, including single-strain and mixed-strain options, as well as products combined with dietary or behavioral interventions, have been explored in ASD treatment. Lactobacillus rhodochrous and Lactobacillus plantarum have been shown to be effective in intervening in the social behavior of mice with ASD (Choi & Cho, 2016; Sgritta et al., 2019), but there is insufficient evidence to demonstrate the effectiveness of probiotic interventions in children with ASD. Therefore, this paper carries out a Meta-analysis by including relevant studies on probiotic intervention in autism spectrum disorders (ASD) to systematically assess the efficacy of probiotics in improving ASD-related symptoms, with the aim of providing new research perspectives and theoretical basis for the intervention and treatment of ASD.

Methods

Both systematic reviews and meta-analyses were guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. This study protocol was registered with PROSPERO on October 18, 2024 (registration number: CRD42024585957).

Search strategy

As of June 10, 2024, a systematic literature search was conducted in PubMed, Cochrane labary, Web of Science, Embase, and EBSCOhost databases using subject and free word searches with search terms including ("autism spectrum disorder", 'autism', 'ASD', 'autistic disorder', "antibiotics, child", etc. The search terms in the same category were connected by 'AND', and the search terms in different categories were connected by 'OR', and the detailed search strategies are listed in the supplementary file.

Inclusion and exclusion criteria

Inclusion criteria were established according to the PICOS principles (Participants, Intervention, Comparison, Outcome, Study). Inclusion criteria were as follows:(1) children and adolescents under 18 years of age diagnosed with autism or Asperger's syndrome or Asperger's disorder according to accepted criteria; (2) probiotics or probiotic preparations as the primary intervention in the trial group; (3) the trial did not limit control methods; (4) scores on autism-related behavioral symptoms measured by eligible questionnaires; and (5) randomized controlled trials (RCT) and crossover trials.

Exclusion criteria for systematic reviews: (1) Studies involving participants over 18; (2) Full-text articles that could not be obtained after contacting the authors. Additional exclusion criteria for the meta-analysis included: (1) Participants over 18; (2) Unavailable full-text articles after author contact; (3) Insufficient data for meta-analysis; (4) Lack of information on specific probiotic strains.

Study selection and data extraction

Initially, two researchers independently screened the retrieved literature by assessing titles and abstracts to select those that met our criteria. Then, the two researchers continued to independently assess these selected studies by reading the full-text articles and extracted data, including the basic characteristics of the study participants, sample size, interventions and controls, intervention duration, autism-related behavioral symptom scores, and gastrointestinal symptom scores. Any disagreements between the two researchers during the screening and data extraction process were resolved through discussion or consultation with a third reviewer.

Quality assessment of the literature

According to the PRISMA statement, the risk of bias in RCTs and crossover trials was assessed in seven dimensions: (1) randomized sequence generation; (2) allocation concealment; (3) blinding of participants and staff; (4) blinding of outcome assessment; (5) incomplete endpoint data; (6) selective reporting; and (7) other biases. Each bias was categorized into three levels: low risk, unclear

risk, and high risk.

Data analysis

RCTs and crossover trials were assessed for risk of bias using R4.4.1. All meta-analyses and visualizations were performed by R4.4.1. The mean (Mean) and standard deviation (SD) of the change in ASD-related behavioral symptom scores between baseline and endpoint were extracted from each intervention and control group of the included studies, and the Mean and SD of the change in gastrointestinal symptom scores were extracted from three additional studies.Standard deviations of the change in scores from a number of studies could not be obtained directly from the source or the authors; they were obtained based on the recommended formulae in the Cochran Handbook. The standard deviations of the baseline and endpoint scores and an assumed correlation coefficient of 0.5 were estimated according to the formula recommended in the Cochran Manual. Effect sizes were then assessed by calculating standardized mean differences (SMDs) and 95 % confidence intervals (CIs) using the Hedges method. Heterogeneity between studies was assessed using the I^2 statistic and p-value from Cochran's Q-test, with < 25 %, 25–50 %, and > 50 % representing low, moderate, and high levels of heterogeneity, respectively, and heterogeneity was considered statistically significant at $p < 0.05$. If $I^2 < 50$ %, a fixed-effects model was used; otherwise, a random-effects model was used. Publication bias was assessed using the Egger test. Subgroup analyses were performed to explore sources of heterogeneity, with country of study, scale used, intervention, intervention duration, and type of study considered as potential subgroup bases. Sensitivity analyses were performed to confirm the robustness of the results by removing one study and repeating the meta-analysis. All tests were two-sided and $p < 0.05$ was considered statistically significant.

Result

Search results

The process of literature search and screening is shown in Fig. 1. A total of 523 articles were obtained by first searching through five databases, and 16 articles remained after excluding non-requested articles by title and abstract. Among these 16 articles, 2 articles

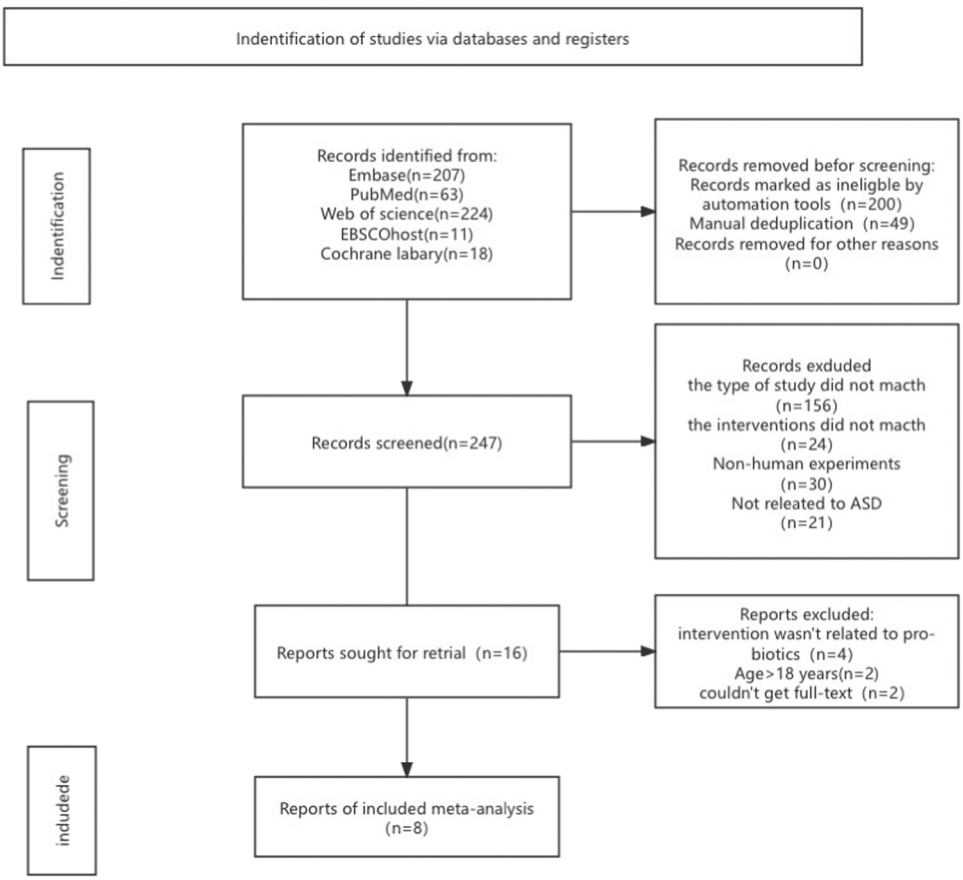

Fig. 1. PRISMA flow diagram.

could not be accessed in full text, 2 articles had subjects older than 18 years old, and the other 4 articles did not use probiotic intervention.

Study features

The basic information of the eight included literatures is shown in Table 1. A total of 365 children aged 1.5–15 years from three countries (four from China, two from the United States, and two from Italy) were included in the eight papers, including six randomized controlled trials and two crossover controlled trials. All eight studies validated the probiotic composition and dosage through laboratory testing to ensure that the probiotic types and dosages used in the interventions were accurate. Regarding the study population, only two studies explicitly limited the study population to children with autism with gastrointestinal symptoms, while the rest of the studies did not limit the gastrointestinal symptoms of the study population. In addition, none of the studies stated whether or not gastric acid-resistant capsule technology was used.

In all of the studies, probiotics were used as the intervention. Among them, four studies utilized single-strain probiotics (Guo et al., 2022; Mazzone et al., 2024; Liu et al., 2023, 2019) while the remaining four employed multi-strain probiotic formulations (Sanctuary et al., 2019; Li et al., 2021; Wang et al., 2020; Santocchi et al., 2020). Overall, the interventions involved 15 probiotic species, namely Bifidobacterium longum, Lactobacillus acidophilus, Enterococcus faecalis, Lactobacillus plantarum, Streptococcus thermophilus, Bifidobacterium shortum, Bifidobacterium infantis, Lactobacillus paracasei, Lactobacillus deuterococcus subsp. bulgaricus, Lactobacillus paracasei LPC-37, Bifidobacterium lactis BL-04, Lactobacillus acidophilus, Lactobacillus deuterococcus, and Lactobacillus fermentum. All interventions included Lactobacillus spp. with the exception of the study by Mazzone Guo et al. (2022), where Lactobacillus plantarum, Lactobacillus infantis and Bifidobacterium longum were the three most commonly used probiotics. All studies reported the dose of probiotics used. eight studies used probiotic doses ranging from 9–10^7colony forming units (CFUs) to 9–10^{12} CFUs, and more than half of the studies (n = 5) used tens of billions of doses of probiotics. In addition, the study by Arnold et al.↱ (Wang et al., 2020)divided patients into two different dose groups based on intervention duration, with daily probiotic doses ranging from 4.5–1012 CFU for the first four weeks and 9–1012 CFU for the last four weeks.

Of the eight studies, six focused solely on probiotics or probiotic formulations, while one combined probiotics with ABA therapy

Table 1
Basic information of included studies.

Author (Year)	Country/ Region	Type of trail	Age (Year)	Sample Size	Intervention Group	Compare Group	Intervention Duration	Scale on Autism	GI Symptoms
Liu YW (2023)	China	RCT	2.5 −7	79	n = 39(single probiotic) PS128 capsules 6×10^{10}CFU/day	n = 40 Placebo+PS128	4 months	ASEBA	
Li YQ (2021)	China	RCT	3 −6	71	n = 21(probiotic blend) Bifidobacterium triple live dispersion +ABA 9×10^7 CFU/day	n = 20 ABA	3 months	ATEC	
Arnold LE (2019)	America	crossover controlled trials	3 −12	10	n = 10 (probiotic blend) DSF $4.5–9 \times 10^{12}$ CFU/ day	n = 10 placebo	8weeks	SRS	PedsQL GI
Santocchi E (2020)	Italy	RTC	1.5 −6	63	n = 32(probiotic blend) DSF 9×10^{11} CFU/day	n = 31 placebo	6months	ADOS-CSS,	GI Severity Index Score
Sanctuary MR (2019)	America	crossover controlled trials	2–11	8	n = 8 (single probiotic) Bifidobacterium infantis +BCP 2×10^{10} CFU/day	n = 8 BCP	5months	ABC	
Liu YW (2019)	China	RTC	7 −15	80	n = 39(single probiotic) Bifidobacterium infantis 2×10^{10}CFU/day	n = 41 placebo	4 weeks	ABC-T	
Wang Y (2020)	China	RTC	2 −8	11	n = 7 (probiotic blend) probiotics + FOS 10^{10} CFU/day	n = 4 placebo	108days	ATEC	
Mazzone L (2024)	Italy	RTC	–	43	n = 21(single probiotic) L. reuteri	n = 22 placebo	6months	SRS	GSRS

(Sanctuary et al., 2019), and another incorporated prebiotics, such as fructooligosaccharides (Li et al., 2021). In the control groups, six studies administered a placebo, one used ABA therapy (Sanctuary et al., 2019), and one employed BCP (Liu et al., 2019). The intervention durations of these studies ranged from 4 weeks to 6 months, with 2 studies having intervention durations of 3 months or less and the remaining 6 studies having intervention durations of greater than 3 months.

Two studies used the Autism Treatment Evaluation Checklist (ATEC) to measure changes in symptom severity and assess treatment effects in children with ASD (Sanctuary et al., 2019; Li et al., 2021), and the ATEC consists of four subscales, language, social competence, sensory/cognitive awareness, and health/physical/behavioral (Eugene arnold et al., 2019). Two studies used the Social Responsiveness Scale (SRS) to measure the degree of social impairment in children with ASD (Guo et al., 2022; Santocchi et al., 2020), and the SRS assessment encompasses social awareness, social information processing, reciprocal social communication skills, social anxiety/avoidance, and autistic concerns and characteristics (Mahapatra et al., 2020). The remaining four studies used, respectively, the Autism Behavior Checklist Taiwan (ABC-T), the Autism Diagnostic Observation Scale Calibrated Severity Score (ADOS-CSS), and the Achenbach System of Empirically Based Assessment of Heart Behavior Problems (ASEBA) (Mazzone et al., 2024; Liu et al., 2023, 2019; Wang et al., 2020). The ABC is used to assess the effects of medications and other treatments on individuals with severe developmental disabilities and contains five subscales, namely irritability, hyperactivity/non-compliance, lethargy/social withdrawal, stereotyped behaviors, and inappropriate speech. The ABC-T is a questionnaire containing 47 questions that assesses behavioral problems in children with intellectual and developmental disabilities and is divided into five subscales: sensory (feeling and perception), Relationships (relationships and connections), Body and Object Use (physical activity and rigorous use of objects), Language (communication and interaction), and Socialization and Self-Help (adaptability and self-care) (Constantino, 2013). The ADOS-CSS is used to quantify symptoms of autism and is a standardized calibrated severity score of the Autism Diagnostic Observation Scale (ACOS) (Kaat et al., 2021). The ASEBA is used to assess child and adolescent mental and behavioral health problems, and the system includes a variety of instruments and questionnaires covering emotional, behavioral, and social issues (Janvier et al., 2022). Three studies evaluated the assessment of gastrointestinal symptoms in subjects using the Pediatric Quality of Life Inventory GI module (PedsQL GI), the GI Severity Index Score, and the Gastrointestinal Symptom Rating Scale (Maenner et al., 2020; Kawa et al., 2017; Maenner et al., 2021). The PedsQL GI includes 10 symptoms such as abdominal distension, vomiting, and dysphagia, which are scored on a 5-point scale (0 =never, 4 =every day) according to frequency of occurrence, and the total score is standardized to be 0–100, with a lower score indicating a more severe symptom. The GI Severity Index Score covers frequency of bowel movements, fecal character, and duration of abdominal pain, and is based on a combination of symptom frequency and intensity, with a total score ranging from 0–20, with higher scores indicating more severe symptoms. GSRS test items include epigastric pain, chest discomfort, acid reflux, hunger pain, nausea, bowel sounds, abdominal distension, throat discomfort, bad breath, urine odor, constipation, diarrhea, loose stools, dry stools, need to defecate immediately when having bowel movements, urgency and heaviness, etc., The scale is divided into 7 levels (1 means no symptoms at all; 2 means slightly; 3 means a little; 4 means moderately; 5 means more obvious discomfort; 6 means more serious; 7 means especially serious).

A total of eight studies assessed gastrointestinal symptoms or the composition of gut microbiota. Among these, two studies reported adverse effects, while six documented reasons for participant withdrawals. The sample sizes in most studies were small to moderate,

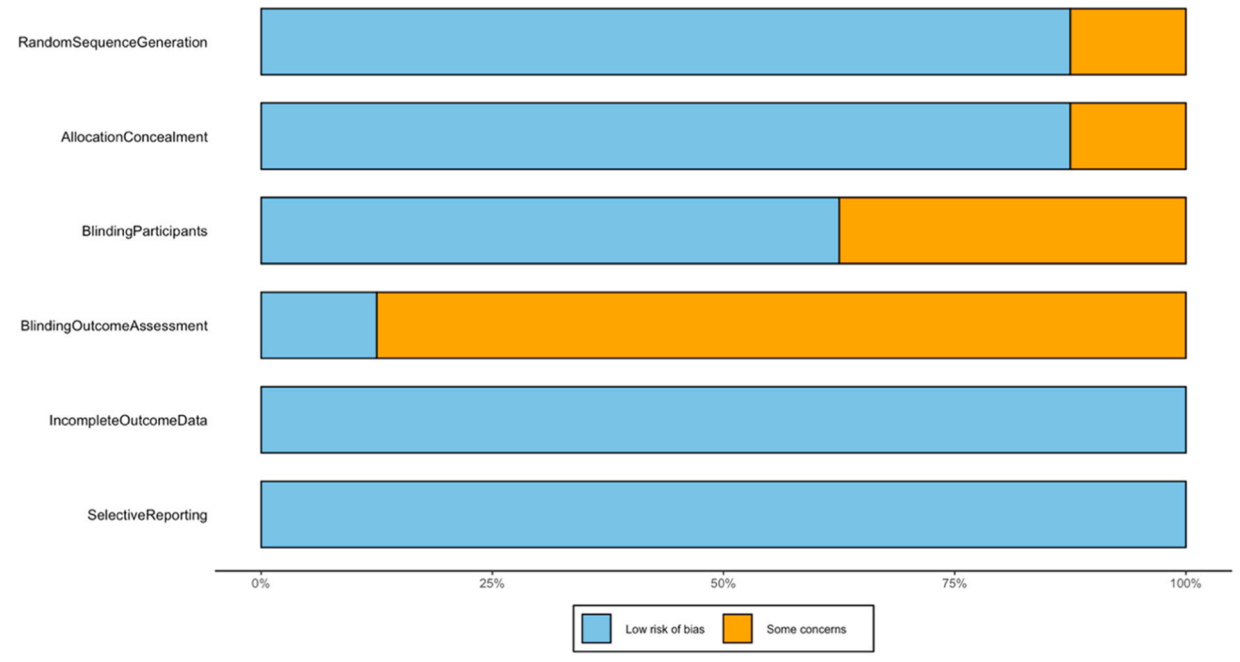

Fig. 2. Risk of bias graph.

ranging from 8 to 100 participants, and all studies were published within the last ten years.

Study quality

Six randomized controlled trials and two cross-over randomized controlled trials were assessed according to the seven dimensions recommended by the PRISMA guidelines. The eight included papers were at low risk of bias for outcome data bias, selective reporting bias, and other biases; only one study was at low risk of bias for outcome metrics. Overall only one study out of the eight papers was of high quality, and the rest of the studies were of average quality. Specific information is shown in Figs. 2 and 3.

Results of the meta-analysis

Effects of a probiotic intervention on behavioral symptoms in children with autism.

A total of eight studies were included in the meta-analysis, all published within the last 10 years. A total of 357 children with ASD aged 1.5 to 15 years from three countries, China (n = 3), Italy (n = 2), and the United States (n = 3), were included in these studies. In addition, a total of 177 children with ASD were assigned to the intervention group and 186 children to the control group. The characteristics of the eight studies are shown in Table 1.

oderate heterogeneity was present between the studies ($p < 0.05$, $I^2 = 53\%$), and therefore a random-effects model was used. The results of the meta-analysis showed a statistically significant difference between the behavioral symptoms of the experimental group and the intervention group (SMD = −0.251 with a 95% CI:−0.466-−0.037,P = 0.04). The forest plot of the combined analysis results is shown in Fig. 4.

Effect of probiotic intervention on gastrointestinal symptoms in young children

Three of the eight studies included in the Meta-analysis compared data on intestinal symptoms in young children after probiotic

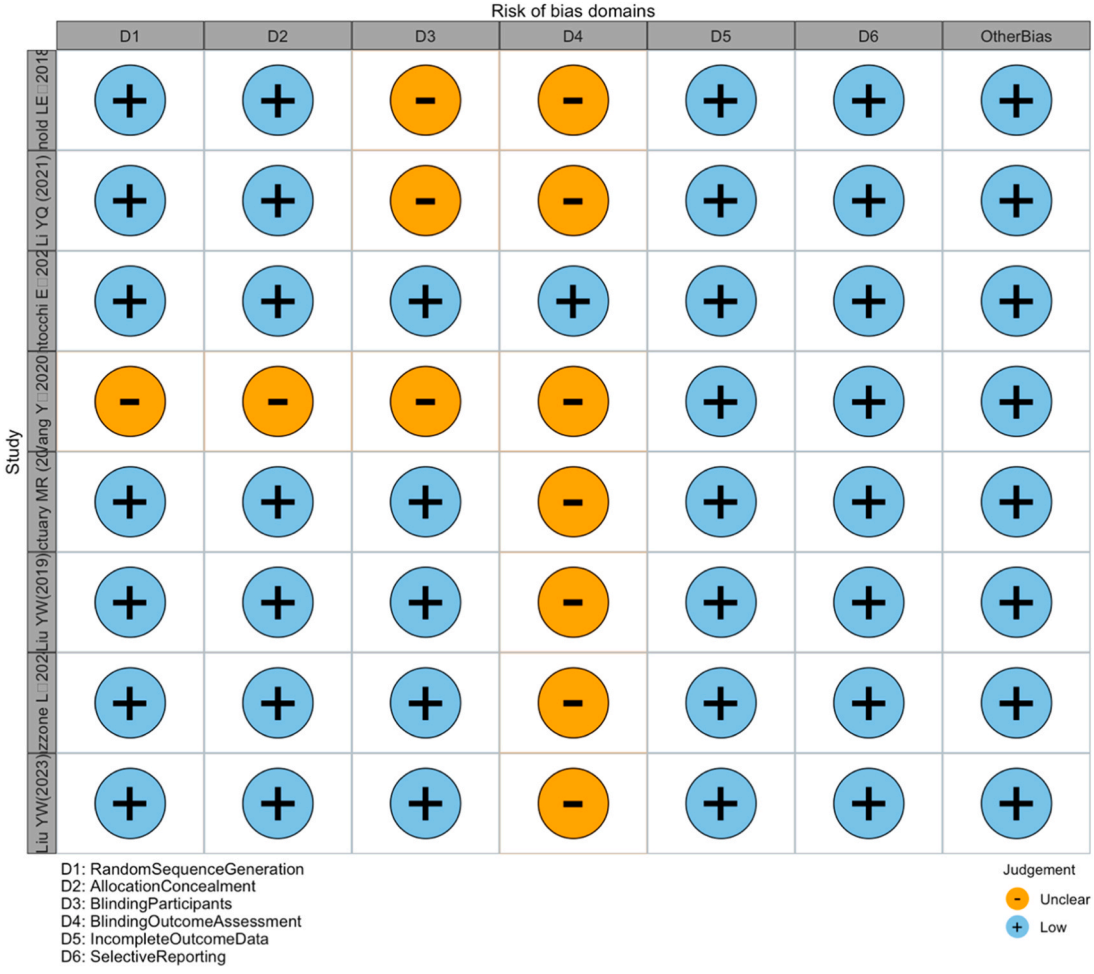

Fig. 3. Summary of risk of bias for randomized controlled trials and cross-over trials.

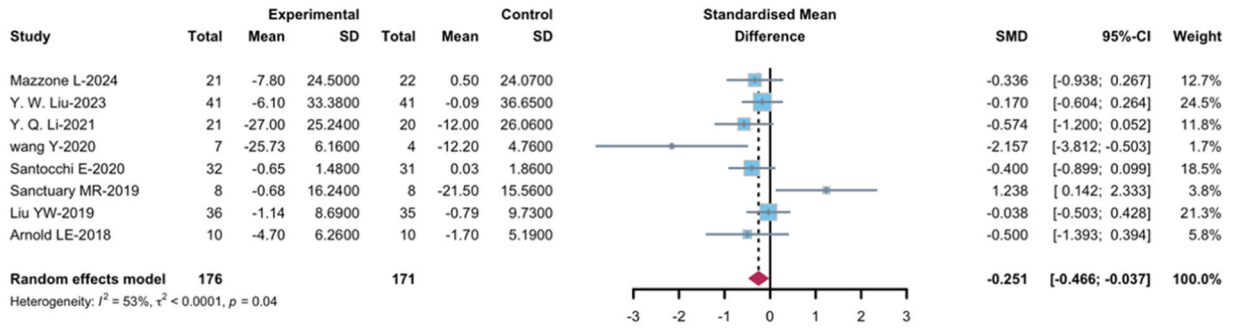

Fig. 4. Forest plot of the effects of probiotics on behavioral symptoms in children with autism.

interventions, so the combined effect size of the effects of probiotic interventions on the gastrointestinal tract in young children was analyzed, and the results showed that the changes in the beneficial test and control groups were not statistically significant. The forest plot of the combined analysis results is shown in Fig. 5.

Subgroup analysis

A subgroup analysis was conducted to determine if variations in region, probiotic strains, or intervention duration influenced the efficacy of probiotic treatments. Results showed no significant impact from these factors on treatment outcomes. Detailed subgroup analysis figures are provided in the supplementary files S1–3.

Publication bias and sensitivity analysis

The meta-analysis included fewer than 10 studies (n = 8), and publication bias was assessed using the Egger test rather than the funnel plot, indicating a low likelihood of publication bias ($p > 0.05$). To assess the stability of the results of the meta-analysis, a sensitivity analysis was performed on the eight included studies. There was no significant change in the overall effect size after the exclusion of any of the studies, as shown in the figure; therefore, the results of this meta-analysis were relatively robust..

Discussion

Probiotics may positively impact autism interventions by helping to balance the gut microbiota. Many children with autism experience gut microbiota imbalances, leading to gastrointestinal issues and chronic low-grade inflammation, which may, in turn, affect central nervous system function through the gut-brain axis (Yang et al., 2000). Probiotics can help improve gut health and reduce inflammation by promoting beneficial bacteria and suppressing harmful bacteria. Additionally, they produce neuroactive compounds like short-chain fatty acids and serotonin, potentially improving behavioral and emotional symptoms in children with autism (Fattorusso et al., 2019). This review included eight studies published within the past decade, analyzing various probiotics and formulations used to assess their effects on ASD symptoms. To capture a comprehensive range of studies on the use of probiotics in treating ASD, we expanded our literature search. Ultimately, eight studies were identified that met the inclusion criteria. These consisted of six randomized controlled trials (Guo et al., 2022; Mazzone et al., 2024; Liu et al., 2023; Sanctuary et al., 2019; Li et al., 2021; Wang et al., 2020) and two crossover studies (Liu et al., 2023; Wang et al., 2020). Among these, one study was deemed high quality (Wang et al., 2020), while the rest were of moderate quality. Differences in probiotics, dosages, intervention durations, and assessment tools for ASD symptoms contributed to the study's heterogeneity, underscoring the need for high-quality, standardized research to explore the therapeutic potential of probiotics in ASD. In addition, all of the included studies did not specify whether the gastric acid-resistant capsule technology was used, as well as some of the study subjects were not limited to those with gastrointestinal symptoms, which may have had an impact on the results of the study. Gastric acid-resistant capsule technology can ensure that probiotics can reach the intestine smoothly, and if this technology is not used in the studies, the actual number of probiotics reaching the intestine may be insufficient, which may affect the effectiveness of the intervention. In addition, whether the study population was limited to those with gastrointestinal symptoms may also affect the assessment of the effect of probiotics on the improvement of

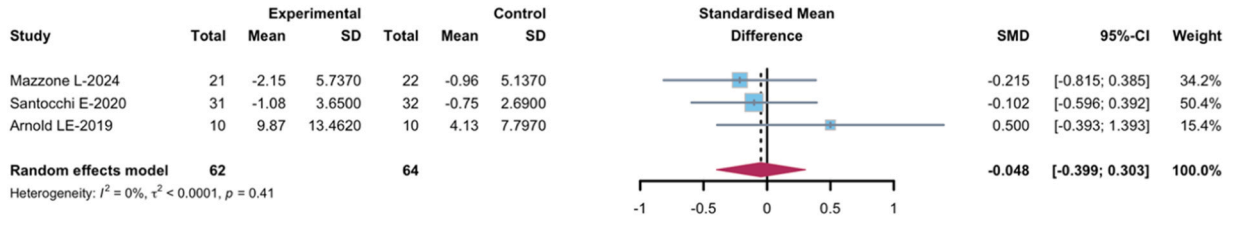

Fig. 5. Forest Plot of the Effects of Probiotics on Gastrointestinal Symptoms in Children with Autism.

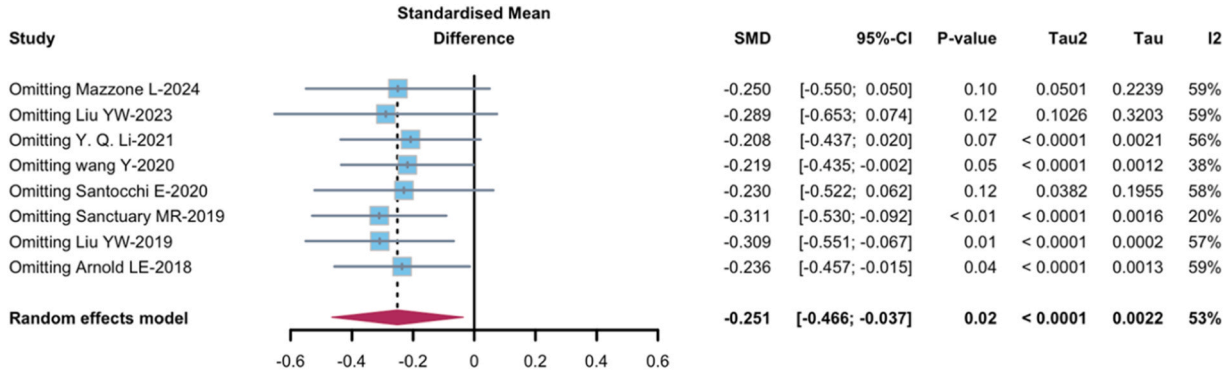

Fig. 6. Leave-One-Out Sensitivity Analysis Forest Plot.

gastrointestinal symptoms, and if the study population was not limited to those with gastrointestinal symptoms, the effect of probiotics on the improvement of gastrointestinal symptoms may be masked. Therefore, future studies should make this information more explicit to improve the quality of the study and the reliability of the results.

Previous meta-analyses have suggested probiotics do not significantly impact ASD symptom severity (Tweedie-cullen et al., 2024; He et al., 2023), contrasting with this review's findings. In contrast to the study of Song.w (He et al., 2023)et al., which concluded through meta-analysis that probiotics and prebiotics did not significantly improve the severity of symptoms, gastrointestinal problems and co-morbid psychopathology in ASD patients, similar conclusions were reached in the gastrointestinal symptoms as in the present study, which showed insignificant improvement, but in behavioral symptoms, the present study concluded that there was a significant improvement, while this English-language literature concluded that there was no significant improvement. HE.X (Tweedie-cullen et al., 2024) and others concluded that probiotics had no significant effect on the overall effect size of behavioral symptoms in children with ASD, which is different from the conclusion of significant improvement in behavioral symptoms in the present study, although they found significant improvement in the subgroup analysis of probiotic blends, which has some echoes of the present study's discussion of the effect of interventions with different probiotic species. In addition, in terms of study heterogeneity and limitations, both the present study and previous studies were mentioned, but with differences in specific focus and analysis perspectives. These comparisons not only highlight the uniqueness of this study, but also provide a multidimensional reference direction for subsequent studies. The meta-analysis identified a statistically significant but modest negative effect favoring the intervention group over the control group, with a standardized mean difference (SMD) of −0.251 (95% CI: −0.466 to −0.037, P = 0.04). A moderate level of heterogeneity was noted ($I^2 = 53\%$), which could be attributed to variations in participant demographics, intervention protocols, or assessment tools across studies. Some studies, such as Wang et al., (1), showed significant negative effects, while others, like Sanctuary et al., showed positive effects, suggesting that probiotic efficacy may vary by context. Future research should aim to standardize intervention strategies, improve study consistency, and explore subgroup factors to provide more targeted recommendations.

Children with ASD frequently experience gastrointestinal issues, such as chronic constipation, diarrhea, or irritable bowel syndrome (IBS), which are often associated with disruptions in gut microbiota balance. Children experiencing these issues may display greater anxiety and behavioral reactivity, with symptom severity linked to heightened anxiety and reactivity (Song et al., 2022). While probiotics may help relieve gastrointestinal symptoms by balancing the gut microbiota, this review's combined effect sizes from three studies showed no statistically significant difference in gastrointestinal symptom improvement between intervention and control groups (SMD = 0.048, 95% CI: −0.399 to 0.303) (Guo et al., 2022; Liu et al., 2019; Sanctuary et al., 2019; Li et al., 2021; Wang et al., 2020; Santocchi et al., 2020). However, the scoring differences among studies likely contributed to this lack of statistical significance. For example, Sanctuary et al. (2020) used a reverse scoring system, where higher scores indicated symptom improvement, differing from the other studies. Despite inconclusive findings in this meta-analysis, further research on specific subgroups and probiotics is needed to examine the impact of probiotics on gastrointestinal symptoms in children with autism.

Subgroup analysis explored whether region, probiotic strains, and intervention duration influenced outcomes. Results showed that region, probiotic strain, and intervention duration did not significantly affect outcomes, as shown in supplementary figures. Regional differences in diet, environment, lifestyle, and genetics may lead to gut microbiota variations, potentially impacting probiotic efficacy. However, this meta-analysis did not find significant regional effects. Possible reasons include small sample sizes in certain regions, inconsistent intervention methods, and high variability between studies ($I^2 = 74\%$), which may have masked potential regional differences. These results highlight the need for large-scale, multicenter studies to explore probiotic efficacy in ASD treatment across diverse populations.

In examining intervention duration, we found that studies with interventions shorter than three months showed no significant effect. In studies with interventions longer than three months, there was no significant improvement in autism symptoms, with an effect size (MD) of −2.72 (95% CI: −10.64 to 5.19), high heterogeneity ($I^2 = 79\%$), and $p < 0.01$. Although prolonged interventions may be more likely to demonstrate probiotic effects, the current studies lacked significant findings, potentially due to limited sample sizes, intervention designs, or duration constraints.

In analyzing probiotic strains, single strains and mixed strains were examined separately. The mixed strains group showed a

potentially positive, though statistically insignificant, effect on improving behavioral issues, with high heterogeneity ($I^2 = 84$). For instance, the study by Li et al., 2021 reported significant results with mixed strains (MD = −13.53, 95% CI [−20.06, −7.00]). Conversely, the single strains group showed a consistent, albeit not statistically significant, trend of improvement (MD = −3.46, 95% CI [−9.37, 2.44]) with lower heterogeneity ($I^2 = 66$). The high variability suggests study design, strain selection, and intervention duration may affect results.

Compared to prior studies, this review incorporated recent literature and new analytical tools, offering a broader exploration of probiotic effects on ASD symptoms. Nonetheless, limitations include considerable variation in study design, participant characteristics, and outcome measurement methods, potentially affecting data aggregation and interpretation. Additionally, small sample sizes may limit statistical power and result generalizability. Missing data in some studies also required estimates based on Cochrane Handbook recommendations, affecting result reliability.

Despite these limitations, this review has important clinical and theoretical implications. The results suggest that probiotic interventions may serve as an adjunctive therapy to help improve behavioral symptoms in children with autism, but it is not yet possible to clarify their ameliorative effect on gastrointestinal symptoms, and these findings also provide support for further exploration of the role of the gut-brain axis in autism. Future research should prioritize high-quality, large-scale randomized controlled trials to validate the efficacy and mechanisms of probiotics in ASD treatment. Standardizing probiotic types, dosages, and extending intervention durations will allow for a more comprehensive assessment of long-term effects. Additionally, exploring individual differences such as age, gender, and baseline symptom severity may enhance understanding of factors influencing treatment outcomes.

Conclusion

Based on the results of this Meta-analysis, we found that the probiotic intervention showed some effectiveness in improving symptoms in children with autism. This suggests that probiotics have potential clinical applications as a safe and effective intervention. However, in this study, no significant effect of region, intervention time and probiotic type on the intervention effect could be observed. This may be related to the small number of included studies, insufficient sample size, and differences in intervention modalities. Therefore, future studies should further expand the sample size and consider additional variables to more fully assess the role of probiotics in autism interventions. Although current evidence suggests that probiotics have a potential ameliorative effect on autism symptoms, caution is needed in their practical application in conjunction with an individualized treatment plan, and more high-quality studies are expected to validate these findings.

CRediT authorship contribution statement

Yong Tang: Writing – review & editing. **Yongting Li:** Methodology. **Tingting Wang:** Data curation. **Xiaolong Chen:** Formal analysis. **Zhigang Li:** Supervision, Formal analysis. **Wanlin Zou:** Software.

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Declaration of Competing Interest

They have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reia.2025.202630.

Data availability

The data that has been used is confidential.

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