

REVIEW

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The therapeutic effects of probiotics on core and associated behavioral symptoms of autism spectrum disorders: a systematic review and meta-analysis

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Abstract

Background We aimed at investigating the efficacies of probiotics in alleviating the core and associated symptoms of autism spectrum disorder (ASD).

Methods Randomized placebo-controlled trials were identified from major electronic databases from inception to Nov 2023. The outcomes of interests including improvements in the total and associated symptoms of ASD were quantitatively expressed as effect size (ES) based on standardized mean difference (SMD) with 95% confidence interval (CI).

Results Ten studies with 522 participants (mean age = 8.11) were included in this meta-analysis. The primary results revealed significant improvement in total symptoms in the probiotics group compared with the controls (SMD = -0.19, $p = 0.03$, ten studies, $n = 522$) but not the core symptoms (i.e., repetitive restricted behaviors, As affiliations 3 and 5 are same, we have deleted the duplicate affiliations and renumbered accordingly. Please check and confirm.problems with social behaviors/communication). Subgroup analyses demonstrated improvement in total symptoms in probiotics users relative to their controls only in studies using multiple-strain probiotics (SMD = -0.26, $p = 0.03$, five studies, $n = 288$) but not studies using single-strain regimens. Secondary results showed improvement in adaptation (SMD = 0.37, $p = 0.03$, three studies, $n = 139$) and an improvement trend in anxiety symptoms in the probiotics group compared with controls (SMD = -0.29, 95% CI -0.60 to 0.02, $p = 0.07$, three studies, $n = 163$) but failed to demonstrate greater improvement in the former regarding symptoms of irritability/aggression, hyperactivity/impulsivity, inattention, and parental stress.

Conclusions Our study supported probiotics use against the overall behavioral symptoms of ASD, mainly in individuals receiving multiple-strain probiotics as supplements. However, our results showed that probiotics use

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was only associated with improvement in adaptation and perhaps anxiety, but not core symptoms, highlighting the impact of adaptation on quality of life rather than just the core symptoms. Nevertheless, the limited number of included trials warrants further large-scale clinical investigations.

Keywords Probiotics, Autism spectrum disorder, And meta-analysis

Introduction

Autism spectrum disorders (ASD) is a group of behavioral manifestations usually with an early onset characterized by restricted and repetitive patterns of interests, behavior, or activities, as well as core behavioral presentations of social and communication issues [1]. ASD is a global health issue with increases in prevalence, incidence, and disability-adjusted life-years (DALYs) from 1990 to 2019 [2], probably due to a change in diagnostic criteria and increase in public awareness [1]. However, current pharmacological treatments mainly focus on the associated behavioral and emotional symptoms of ASD (e.g., irritability or inattention), whereas no pharmacological intervention against the core symptoms has been approved by the U.S. Food and Drug Administration (FDA) [1]. On the other hand, core behavioral expressions of ASD may be viewed as normal manifestations of neurobiological variations within a population and should only be considered symptomatic when interfering with adaptation to daily lives [3]. Therefore, behavioral therapies aimed at developing strategies to reduce distress and functional impairment from the core symptoms of ASD remain the standard treatment [1]. However, limited access to behavioral therapies (e.g., distant location) [4], as well as questionable cost- and time-effectiveness [5] remained significant issues surrounding behavioral therapies. Such a lack of treatment options has contributed to the popularity of complementary and alternative medicine (CAM) among care providers of individuals with ASD, notwithstanding their unclear efficacy [6].

Dietary interventions are among the most popular CAM [6]. The rationale for dietary interventions stems from a proposed link between intestinal microbiota and the behavioral symptoms of ASD through the gut-brain axis (GBA) from previous animal and human studies [7] as well as the frequent gastrointestinal (GI) problems reported in those diagnosed with ASD [8]. In addition, previous experimental studies have shown not only an increase in intestinal mucosal permeability in a mouse model of ASD [9], but also an improvement in such ASD-related abnormal permeability through probiotics administration [10]. Consistently, a number of clinical trials have advocated the use of probiotic-related products as a therapeutic alternative for ASD symptoms [11–19]. Nevertheless, the efficacy of probiotics in this setting remains controversial as significant benefits in the treatment of ASD-related behavioral symptoms relative to placebos were only noted in one study [19], despite the apparently

favorable outcomes associated with probiotics in most other studies [11–18]. On the other hand, although probiotics are collectively defined as “live microorganisms which confer a health benefit on the host when administered in adequate amounts”, different formulations of probiotics may contain different strains of microorganism (e.g., *Lactobacillus* and *Bifidobacterium*), as well as different numbers of strains of microorganisms (i.e., single- vs. multi-strain) [20]. Interestingly, although a previous meta-analysis did not demonstrate a significant overall improvement in behavioral outcomes in probiotics users compared to the controls in those diagnosed with ASD, a significant improvement was noted when focusing on studies using probiotics blend compared with the controls on subgroup analysis [21]. In concert with this proposal, a previous experimental investigation showed a potential therapeutic advantage of multi-strain probiotics over single-strain regimens because of a higher chance of favorable microbiota attachment to the intestinal mucosa in the former [22]. Therefore, it is possible that formulations of probiotics may have different effects on symptoms of ASD.

Despite support of the use of probiotic blends against the symptoms of ASD [21], the effectiveness of probiotics in alleviating the core or associated emotional and behavioral symptoms of ASD remained unclear. Indeed, patients diagnosed with ASD frequently present with other behavioral problems (e.g., inattention, irritability, or anxiety) [23], which can be as challenging as the core symptoms to their caregivers [24]. Moreover, previous double-blind placebo-controlled randomized clinical trials have shown the efficacy of probiotics for symptom relief in individuals diagnosed with attention deficit hyperactivity disorder (ADHD) [25, 26] who are frequently comorbid with ASD [27]. Dietary supplementation with probiotics has also been reported to reduce the risk of ASD [28], and even improve neurocognitive functions [29, 30]. Given the anti-inflammatory properties of some probiotics [31, 32], the potential therapeutic effects of probiotics on ASD may be extended to other associated symptoms or comorbidities of ASD rather than confined to the core symptoms of ASD.

Therefore, the current meta-analysis, which included only randomized controlled clinical trials (RCT), aimed at providing reliable updated evidence regarding the efficacies of probiotics in alleviating the core and associated symptoms of ASD. Moreover, the effects of other factors

(e.g., number of probiotic strains) on therapeutic outcomes were investigated.

Methods

Protocol and registration

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [33] and registered in the international prospective register of systematic reviews (PROSPERO CRD42023483033).

Search strategy and selection criteria

Electronic databases, namely PubMed, Cochrane CENTRAL, Embase, and ScienceDirect, were searched for randomized controlled trials (RCTs) that studied the use of probiotics in the treatment of the core or associated symptoms of ASD from inception to November 21, 2023 without restrictions on language and country of origin using appropriate search strategies and keywords (eTable1). The reference lists of the retrieved literature were also scrutinized to avoid missing potentially eligible articles. Criteria pertaining to the population, intervention, comparator, and outcomes (PICO) of the current study were: (1) Population: participants diagnosed with ASD recruited in a RCT, (2) Intervention: probiotics or products using probiotics as a supplement or part of combination therapy, (3) Comparator: non-probiotic interventions or placebo, and (4) Outcome: changes in the core or associated behavioral symptoms of ASD or other related symptoms. On the other hand, studies whose (1) interventions did not include probiotics, (2) design was not RCT, or (3) outcome assessment did not provide data on core or associated behavioral symptoms of ASD were excluded.

Data extraction and quality assessment

According to the preset keywords and strategies (eTable1), the titles and abstracts of the acquired literature were independently screened by two authors (JC Lee and CM Cheng) who later extracted data on study characteristics and outcomes. Disagreements on study and data eligibility were resolved through discussion with a third author (CK Sun). Inter-rater reliability was evaluated with kappa coefficients [34]. In an attempt to retrieve missing data, the corresponding authors of articles without necessary information were contacted through electronic mails. The quality of a study and the level of evidence for each outcome were appraised with the Cochrane's "risk of bias" assessment tool [35], and the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) [36], respectively. Discrepancies in opinion between the two authors about the risk of bias or certainty of evidence ratings were settled by discussion.

Data synthesis and analysis

Primary outcomes of the present study were changes in the core symptoms of ASD, including overall behavioral symptoms, social behaviors, restricted repetitive behaviors (RRB), and communication, assessed with standardized rating scales or assessment tools such as the autism diagnostic observation schedule (ADOS), the Achenbach system of empirically based assessment (ASEBA), the clinical global impression–severity (CGI-S), the aberrant behavior checklist (ABC), and the social responsiveness scale (SRS). Secondary outcomes included all behavioral symptoms or issues associated with ASD such as inattention, hyperactivity/impulsivity, irritability, anxiety, adaptation, and parental stress. The outcomes of interest were quantitatively expressed as effect sizes (ES), which were presented as standardized mean differences (SMD) with 95% confidence intervals (CI) for continuous variables. Review Manager 5 (RevMan5.4; Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014) was adopted to conduct all data analyses. For the analysis of continuous data, the generic inverse-variance approach was used. To examine the robustness of study outcomes, the impact of individual studies was evaluated with sensitivity analysis using a leave-one-out approach. Heterogeneity and the probability of publication bias of the included studies were assessed with *I*-squared test and funnel plot inspection, respectively. Subgroup analyses were conducted focusing on the therapeutic strategies (supplementation vs. combination) and number of microbiome strains in probiotics (single- vs. multiple-strain) to scrutinize the possible effects on treatment outcomes. A *p* value of under 0.05 was deemed statistically significant for all study outcomes.

Results

Eligible studies and characteristics

In accordance with the PRISMA statement [33] (Fig. 1) 0.336 articles were initially identified from the electronic databases using predetermined search strategies (eTable2). Following the exclusion of 312 studies through title and abstract screening, 24 were subjected to full-text review that finally yielded ten eligible studies including 522 participants [11–18, 37, 38]. (Fig. 1) with a kappa coefficient for study eligibility being 1. Information from the eligible studies was extracted on November 23, 2023. All ten studies recruited children or adolescents except one that enrolled young adults with age ranging from 15 to 45 years with a mean age of 20 [18]. (Table 1). The mean age of the participants from the ten studies was 8.11 years (SD=4.86). In respect of the number of probiotic strains, five trials used multiple-strain probiotics [11–13, 17, 38] and five used single-strain regimens [14–16, 18, 38]. The median duration of treatment was ten weeks with a range of 4–24 weeks. The use of single

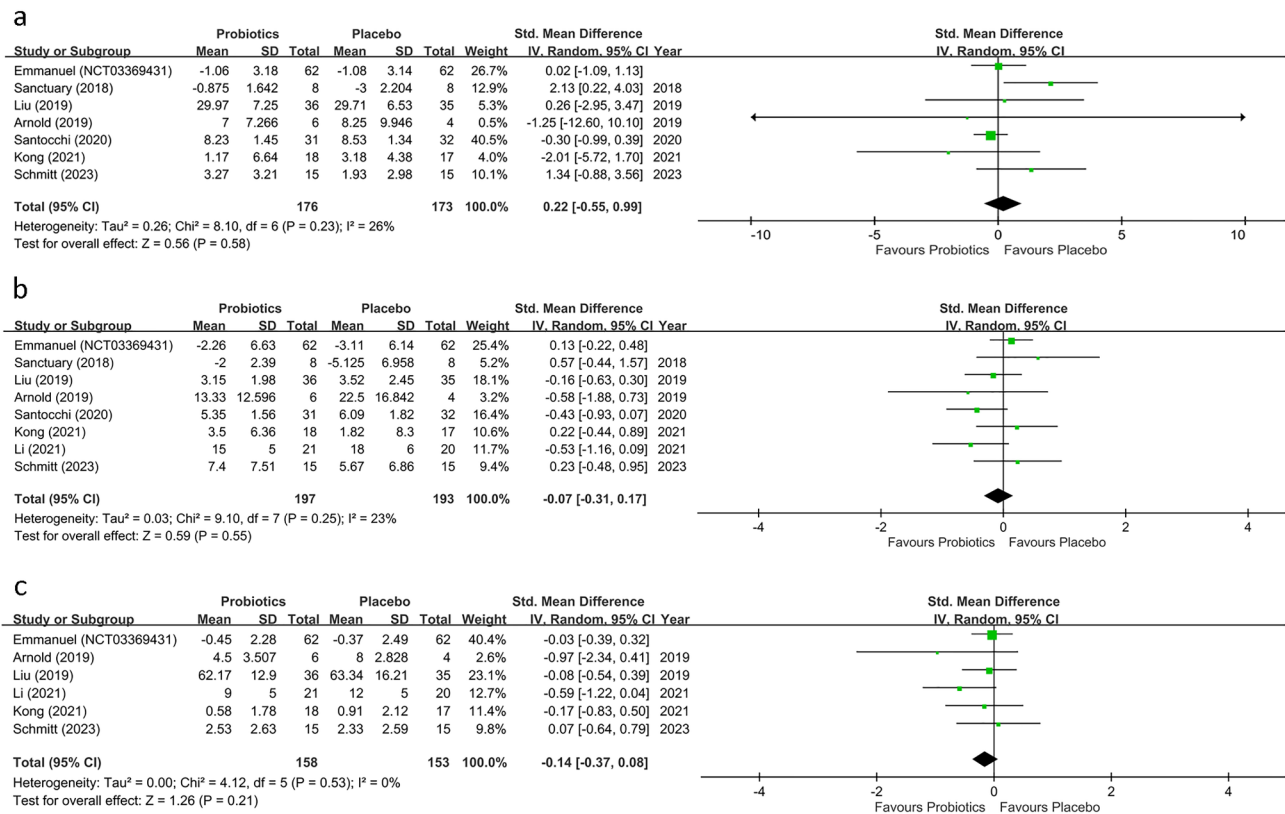


Fig. 1 PRISMA diagram of identifying eligible studies. ASD autism spectrum disorder

or multiple psychotropics was allowed in most studies regardless of their nature with the exception of one trial that excluded participants under psychotropic treatment other than methylphenidate [15]. Regarding study design, six trials used a parallel design [13–17, 38] with the other four being cross-over studies [11, 12, 18, 37]. While eight studies administered probiotics as diet supplements, one combined probiotics with behavioral intervention (i.e., applied behavioral analysis) [38] and the other used probiotics with another dietary supplement (e.g., bovine colostrum) [37]. The countries of origin of the included studies were USA [14, 17, 18, 37], Taiwan [15, 16], Italy [13, 17], China [38] and the UK [11]. With regard to adverse events, except two studies that did not provide relevant information [13, 38], all other trials showed that probiotics were well-tolerated and associated with either no or very mild side effects such as increased gassiness or loose stools [11, 12, 14–18, 37], without any serious adverse events being reported [11, 12, 14–18, 37].

Risk of bias appraisal

Risk of bias assessment with the Cochrane Collaboration's tool indicated a low risk of bias in allocation concealment and randomization sequence in the majority of studies. Similarly, with the exception of two trials that did not recruit placebo controls [37, 38], detection

and performance biases were considered low in other trials due to their double-blind design. Sensitivity analysis demonstrated no significant impact of the two studies [37, 38] on the primary outcome ($SMD = -0.19$, $95\%CI: -0.38 \sim -0.01$, $p = 0.04$). Relatively long follow-up periods in another two studies contributed to their high risk of attrition bias [13, 14]. (Fig. 2). With regard to reporting bias, it was considered low in all studies because all of them chose behavioral issues of ASD as the primary outcomes (Fig. 2). Nevertheless, a high risk of other bias was assigned to three studies due to sponsorship from private companies [15, 16, 18] (Fig. 2).

Primary outcome

The current study revealed a significant improvement in the overall behavioral symptoms of ASD in individuals receiving probiotics compared with the controls ($SMD = -0.19$, $95\%CI: -0.36 \sim -0.02$, $p = 0.03$, ten studies with 522 participants) (Fig. 3). Heterogeneity across the included studies was non-significant ($I^2 = 0\%$ and $p = 0.53$). Besides, funnel plot inspection demonstrated no notable asymmetry for the primary outcome (eFigure1). On the other hand, sensitivity analysis showed a loss of significance regarding the primary outcome in the probiotics group when either one of three studies was excluded [13, 17, 38], although the results were still

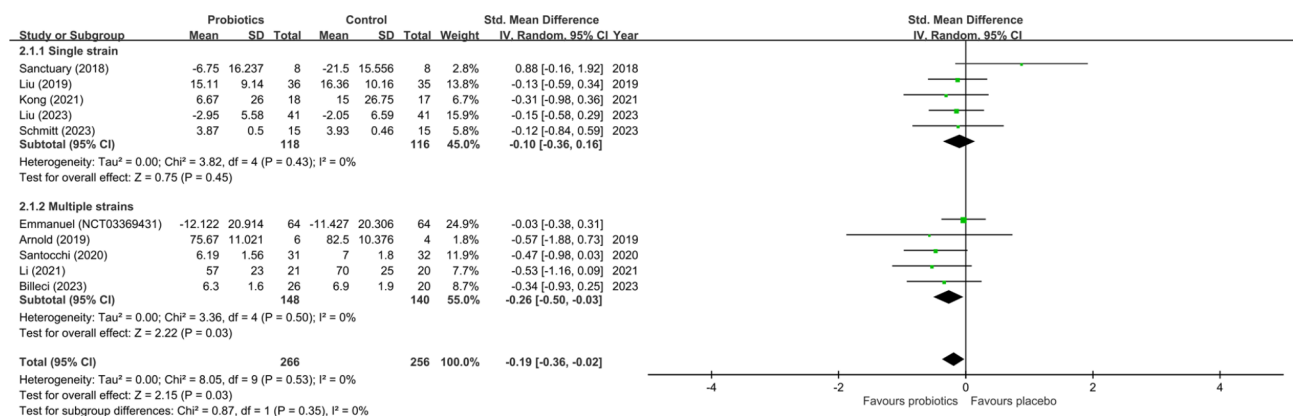
Table 1 Summary of characteristics of studies in the current meta-analysis

Study (year)	Diagnosis (Criteria)	Design	Comparison	N	Dura- tion (weeks)	Outcome	Psychotropic medications	Mean age (years)	Fe- male (%)	Country
Billeci (2023)	ASD (DSM-5)	RCT	Probiotics: multiple strains Placebo	20 26	24	1.Overall = ADOS CSS 2.Adaptation = VABS-II	Allow	3.88 (1.5–6)	23.9	Italy
Liu (2023)	ASD (DSM-5)	RCT	Probiotics: single strain Placebo	41 41	8	1.Overall = ASEBA 2.Irritability = ASEBA aggression 4.Hyperactivity = ADHDT 5.Inattention = ADHDT 6.Anxiety = ASEBA anxiety	Allow only psychostimulant	4.82 (2.5–7)	12.2	Taiwan
Schmitt (2023)	ASD (DSM-5)	RCT/ Crossover	Probiotics: single strain Placebo	15 15	4	1.Overall = CGI-S 2.RRB = ABC Stereotype 3.Social = ABC lethargy 4.Communication = ABC inappropriate speech 5.Irritability = ABC Irritability 6.Hyperactivity = ABC Hyperactivity 7.Adaptation = Vineland adaptive behavior com- posite score	Allow	20 (15–45)	0	USA
Kong (2021)	ASD (DSM-IV TR–5)	RCT	Probiotics: single strain Placebo	18 17	16	1.Overall = ABC total 2.RRB = ABC Stereotype 3.Social = ABC lethargy 4.Communication = ABC inappropriate speech 5.Irritability = ABC Irritability 6.Hyperactivity = ABC Hyperactivity	Allow	10.26 (3–25)	25.8	USA
Li (2021)	ASD (DSM-5)	RCT	Probiotics: multiple strains + ABA ABA only	21 20	12	1.Total = ATEC 2.Social = ATEC social behaviors 3.Communication = ATEC communication	Allow	4.55 (3–6)	24.4	China
Santocchi (2020)	ASD (DSM-5)	RCT	Probiotics: multiple strains Placebo	6 4	24	1.Total = ADOS CSS 2.Social = ADOS Social affect 3.RRB = ADOS RRB 4.Adaptation = VABS-II 5.Parental stress = PSI	Allow	4.14 (1.5–6)	16.4	Italy
Arnold (2019)	ASD (DSM-5)	RCT/ Crossover	Probiotics: multiple strains Placebo	31 32	8	1.Overall = SRS total 2.RRB = ABC Stereotype 3.Social = ABC lethargy 4.Communication = ABC inappropriate speech 5.Irritability = ABC Irritability 6.Hyperactivity = ABC Hyperactivity 7.Parental stress = PSI 8.Anxiety = PRAS-ASD total	Allow	8.85 (3–12)	40	USA

Table 1 (continued)

Study (year)	Diagnosis (Criteria)	Design	Comparison	N	Duration (weeks)	Outcome	Psychotropic medications	Mean age (years)	Female (%)	Country
Liu (2019)	ASD (DSM-5)	RCT	Probiotics: single strain Placebo	36 35	4	1.Overall = ABC-T total 2.RRB = SRS autism mannerism 3.Social = ABC-T social and self-help 4.Communication = SRS social communication 5.Irritability = CBCL aggressive behaviors 6.Hyperactivity = SNAP-IV 7. Inattention = SNAP-IV 8.Anxiety = CBCL anxiety	Allow	10.01 (7–15)	0	Taiwan
Sanctuary (2018)	ASD (ADOS)	RCT/ Crossover	Probiotics: single strain + BCP BCP	8 8	5	1.Overall = ABC total score 2.RRB = ABC Stereotype 3.Social = ABC lethargy 4.Irritability = ABC Irritability 5.Hyperactivity = ABC Hyperactivity	Allow	6.8 (2–11)	12.5	USA
Emmanuel NCT03369431	ASD (ADI-R, DISCO, ADOS)	RCT/ Crossover	Probiotics: multiple strains Placebo	64 64	12	1.Overall = ATEC total 2.RRB = ABC Stereotype 3.Social = ABC lethargy 4.Irritability = ABC Irritability 5.Hyperactivity = ABC Hyperactivity 6.Communication = ABC inappropriate speech 7.Parental stress = APSI	Allow	7.8 (3–16)	17.4	UK

ABA applied behavior analysis; ABC aberrant behavior checklist; ABC-T aberrant behavior checklist-Taiwan version; ADHDT attention-deficit/hyperactivity disorder test; ADI-R autism diagnostic interview-revised; ADOS autism diagnostic observation schedule; ADOS CSS autism diagnostic observation schedule calibrated severity score; APSI autism parenting stress index; ASD autistic spectrum disorder; ASEBA Achenbach system of empirically based assessment; ATEC autism treatment evaluation checklist; CBCL child behavior checklist; CGI-S clinical global impressions scale—severity; BCP bovine colostrum product; DISCO diagnostic interview for social and communication disorders; DSM-IV-TR diagnostic and statistical manual of mental disorders, fourth edition, text revision; DSM-5 diagnostic and statistical manual of mental disorders fifth edition; N number; PRAS-ASD total parent-rated anxiety scale for ASD; PSI parenting stress index; RCT randomized controlled trial; RRB restricted repetitive behaviors; SNAP-IV Swanson, Nolan and Pelham (SNAP)-IV-Taiwan version; SRS social reporting standard; VABS-II Vineland Adaptive Behavior Scales-II

**Fig. 2** Risk of bias for eligible studies. *Study retrieved from clinicaltrials.gov. ^XSponsored by pharmaceutical company.

in favor of probiotics use. Subgroup analyses supported an association between a significant improvement in the total symptoms of ASD and the use of multiple-strain probiotics relative to their controls (SMD = -0.26, 95% CI

-0.50 ~ -0.03, $p = 0.03$, five studies with 288 participants) but not when comparing between single-strain regimen group and the control group (SMD = -0.16, 95% CI -0.36 ~ 0.16, $p = 0.45$, five studies with 234 participants)

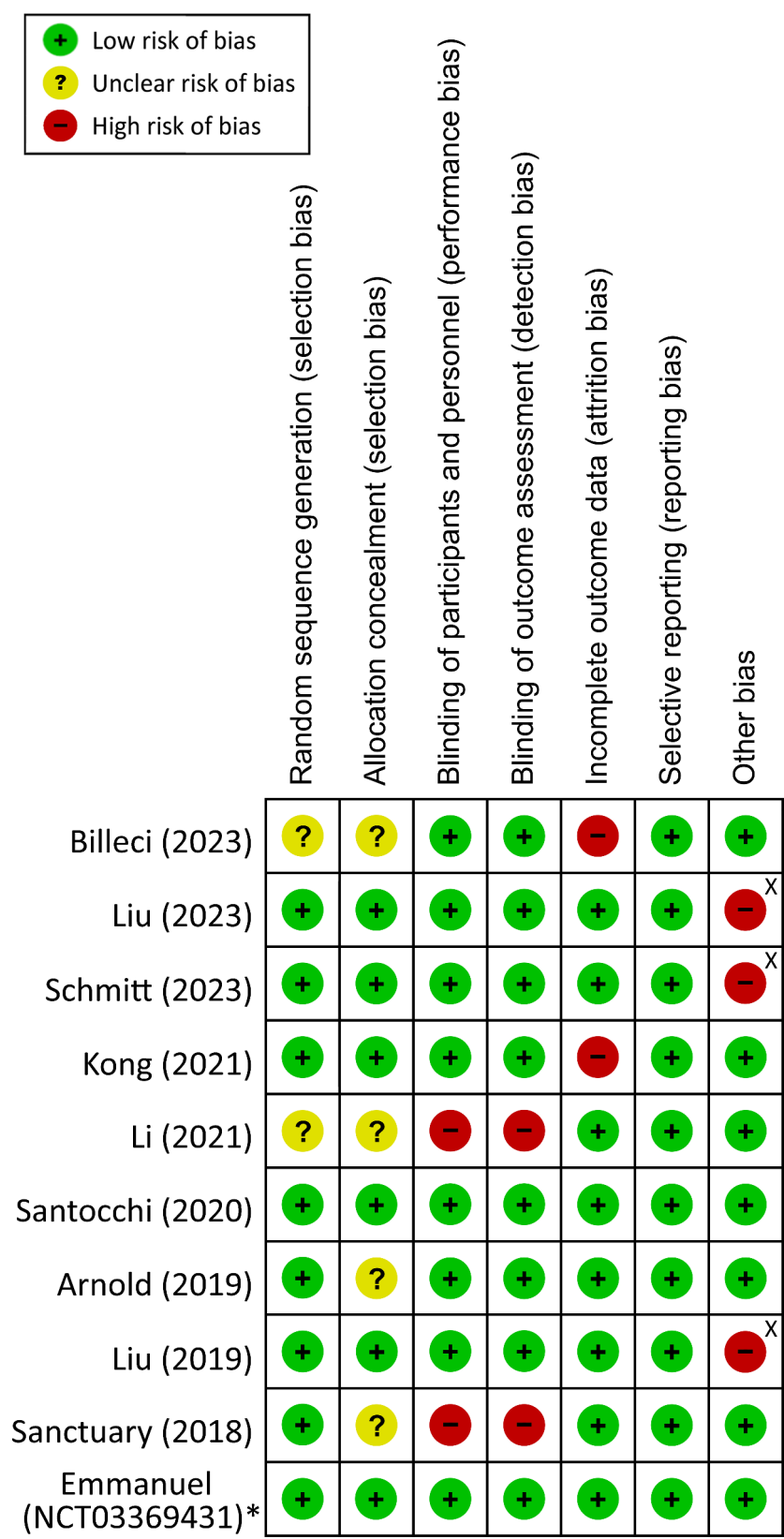


Fig. 3 Forest plot of effect size for comparing the difference in the overall behavioral symptoms of autism spectrum disorder between probiotics and control groups with subgroups comparison between single-strained and multiple-strained probiotics. *CI* confidence interval; *Std* standardized; *SE* standard error

(Fig. 3). However, a direct comparison between the subgroup of studies using multiple-strain probiotics and that adopting single-strain products showed no significant difference ($SMD=0.26$ v.s. -0.16 , $p=0.35$) (Fig. 3). Our subgroup analysis focusing on the choice of probiotics administration strategies on treatment outcome demonstrated significant therapeutic benefits in studies adopting probiotics as supplements when compared to the controls ($SMD=-0.19$, $95\%CI -0.38\sim-0.01$, $p=0.03$, eight studies with 465 participants) (eFigure2). With regard to the core symptoms of ASD, the current study showed no significant improvement in ASD-associated RRB, social behaviors, or communication problems in the probiotics group compared to the control group ($SMD=0.22$, $95\%CI -0.55\sim0.99$, $p=0.58$, seven studies with 349 participants, $SMD=-0.07$, $95\%CI -0.31\sim0.17$, $p=0.55$, eight studies with 390 participants, $SMD -0.14$, $95\%CI -0.37\sim0.08$, $p=0.21$, six studies with 311 participants, respectively) (Fig. 4a, b and c). No significant heterogeneity was noted for RRB ($I^2=26\%$ and $p=0.23$), social behaviors ($I^2=23\%$ and $p=0.25$), and communication ($I^2=0\%$ and $p=0.53$). In addition, the results demonstrated neither inconsistency on leave-one-out sensitivity analysis nor notable asymmetry on funnel plot inspection for the outcomes of RRB, social behaviors, and communication (eFigure3-5).

Secondary outcomes

Our analysis showed no significant difference in the associated problems of ASD between the probiotics and control groups in terms of irritability/aggression (eFigure6), hyperactivity/impulsivity (eFigure7), parental stress (eFigure8), and inattention (eFigure9). Besides, no significant heterogeneity, inconsistency on leave-one-out sensitivity analysis, or notable asymmetry on funnel plot inspection was found for the symptoms of irritability/aggression, hyperactivity/impulsivity, parental stress and inattention (eFigure10-13). Despite the lack of statistical significance, there was a trend of greater improvement in symptoms of anxiety in the probiotics group than that in the control groups ($SMD=-0.29$, $95\%CI:-0.60\sim0.02$, $p=0.07$, three studies with 163 participants) (eFigure14). No significant heterogeneity or asymmetry on funnel plot inspection (eFigure15) was discernible. Sensitivity analysis with the leave-one-out approach showed a significantly higher degree of improvement in the symptoms of anxiety in the probiotics group than that in the control group after excluding the study by Liu et al. [16]. Moreover, our findings revealed a significantly greater improvement in adaptation mainly assessed with the vineland adaptive behavior scales in the probiotics group than that in the control groups ($SMD=0.37$, $95\%CI:0.03\sim0.71$, $p=0.03$, three studies with 139 participants) (eFigure16) without significant heterogeneity or asymmetry on funnel plot inspection (eFigure17).

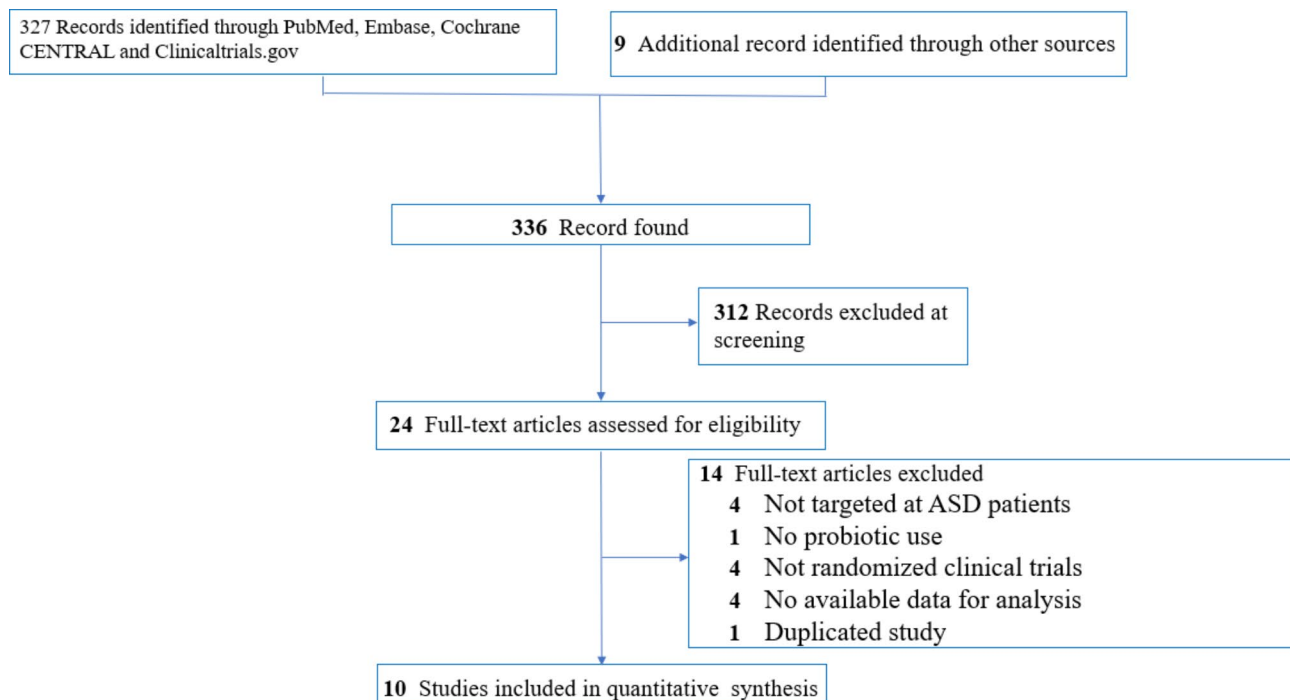


Fig. 4 **a** Forest plot of effect size for comparing the difference between probiotics and control groups for the restricted repetitive behaviors; **b** social behaviors; **c** communication. ASD autism spectrum disorder; CI confidence interval; Std standardized; SE standard error

Certainty of evidence

Details regarding the certainty of evidence for individual outcomes according to the GRADE guidelines are summarized in eTable3. The evidence of our primary outcomes focusing on improvement in the overall behavioral symptoms of ASD, RRB and social behaviors of ASD were downgraded to moderate because of the limited number of eligible studies. Our primary outcome pertinent to improvement in communication was further downgraded to low on account of even more limited data availability. In respect of secondary outcomes, the certainty of evidence regarding irritability/aggression and hyperactivity/impulsivity was downgraded to low due to the limited number of eligible trials and the fact that such outcomes were not direct targets of those studies. With regard to the secondary outcomes of parental stress, inattention, anxiety, and adaptation, the level of evidence was downgraded to very low on the ground of notably limited data availability for a precise analysis.

Discussion

To our best knowledge, this is the first meta-analysis that investigated the effects of probiotics on the core symptoms as well as the behavioral and emotional problems of ASD. A previous meta-analysis, which only focused on the overall behavioral symptoms of ASD, failed to show significant improvement in subjects with ASD treated with probiotics compared to the control group [21]. By contrast, the results of the present meta-analysis, which included ten RCTs with 522 participants, demonstrated significant improvement in the overall behavioral symptoms in the probiotics group compared with the control group but without notable beneficial impact of probiotics on the core symptoms of ASD including social interaction, communication, and RRB. Our secondary analyses further showed significant improvement in the adapted behaviors in the probiotics groups compared to the controls as well as a non-significant trend of probiotics-related improvement in anxiety symptoms. On the other hand, no significant difference was noted in other secondary outcomes including irritability/aggression, hyperactivity/impulsivity, inattention, and parental stress between the two groups. Overall, our study supported an association of probiotics use with improvement in the behavioral but not the core symptoms of ASD. Besides, the use of probiotics correlated with significantly improved adapted behaviors but only a trend of improvement in anxiety.

In contrast to our finding of significant improvement in the overall behavioral symptoms of ASD in the probiotics group compared to the controls, a previous meta-analysis did not show a significant difference between the two groups despite demonstration of a trend in favor of the former [21]. The inclusion of up to ten RCTs in our

investigation compared to seven studies in the previous meta-analysis [21] may contribute to the difference in findings. Several mechanisms may explain the association between probiotics use and the observed alleviation of ASD symptoms [7]. One of the key hypotheses involves the gut-brain-axis involving a network of neuroendocrine pathways that enable a bidirectional communication between intestinal microbiome and the central nervous system [7]. Consistently, a previous study has identified the vagus nerve as the route of communication between intestinal microbiome and the central gamma-aminobutyric acid system [39]. Moreover, given the known association between systemic inflammation and neurocognitive impairment [40, 41], the anti-inflammatory properties of certain probiotics [31, 32] may be beneficial to the maintenance of neurocognitive functions. Besides, a prior experimental study using a mouse ASD model has demonstrated a correlation of an abnormal increase in intestinal mucosal permeability (e.g., dysbiosis) with systemic inflammation and abnormal neurotransmitter signaling in the brain [42]. Adopting the same ASD model, another study further showed a normalization of such an increased mucosal permeability through the oral administration of the probiotic *B. fragilis* [10]. Nevertheless, although our results are supported by prior studies that suggested a possible modulating role of probiotics in GBA which has been reported to be associated with the behavioral symptoms of ASD, evidence derived from the current study is still not solid enough given the limited number of available trials.

Consistent with the results of a previous meta-analysis that showed significant improvements in the overall behavioral symptoms in subjects diagnosed with ASD treated with probiotic blends compared with controls [21], our subgroup analysis focusing on the use of single-versus multiple-strain probiotics demonstrated a significant improvement in the overall symptoms of ASD only in the multiple-strain probiotics group compared to the control group. Consistently, a prior animal study suggested the merit of multiple-strain probiotics due to an increased opportunity of adhesion of beneficial microbiota to intestinal mucosa [22]. Additionally, another meta-analysis reported the effectiveness of using multiple- instead of single-strain probiotics in the prevention of necrotizing enterocolitis and mortality in preterm infants [43]. Taken together, our findings and those from a previous meta-analysis [21] supported the use of multiple-strain probiotics for the alleviation of ASD symptoms. Nevertheless, the fact that only five studies were available for subgroup analysis suggests the need for further large-scale studies to address this issue.

Because most RCTs in our study used probiotics as dietary supplements except two that combined probiotics with other therapeutic approaches (i.e., probiotics

plus bovine colostrum product or probiotics plus applied behavior analysis) [37, 38], we conducted subgroup analysis focusing on studies using probiotics only as supplements that consistently showed greater improvement in the overall behavioral symptoms of ASD in probiotics users than that in the controls. The results, therefore, suggested that probiotic supplementation without combining with other treatments may be effective in this setting, although our findings await further validation due to the limited number of RCTs and the small ES.

Despite the significant overall behavioral improvement in subjects diagnosed with ASD treated with probiotics compared with the controls, no significant difference in improvement was noted in the three core symptoms of ASD (i.e., social functioning, communication, and RRB) between the two groups. There are several possible reasons for this observation. First, the overall improvement in behavioral symptoms may be attributed to the collective minor improvements in each core domain of behavioral problems. However, statistically significant differences may be obscured by the small number of RCTs available for analyzing each core behavioral symptom. Second, the lack of available information about changes in the core behavioral symptoms in two RCTs that provided findings in favor of probiotics in the treatment of the overall symptoms of ASD [13, 38] may render the improvement in core symptoms non-significant because of the absence of their probable positive contributions. Third, the observed behavioral improvement may stem from other associated symptoms of ASD such as mood or irritability. In summary, our study could not provide robust evidence either to support or dismiss the effectiveness of probiotics for each core behavioral symptom of ASD due to the small numbers of available trials (i.e., a maximum of eight RCTs for each core behavioral symptom). Future studies are warranted to elucidate this issue.

Despite the lack of significant probiotics-associated improvement in the core behavioral symptoms of ASD, our results on secondary outcomes showed a significant improvement in adaptive behaviors assessed mainly by the Vineland Adaptive Behavior Scales in subjects treated with probiotics compared with controls. Consistently, prior RCTs have reported an association between the use of probiotics and cognitive function improvement [44, 45], which may be attributed to the systemic anti-inflammatory properties of certain probiotics [40, 41], that may be protective against neurocognitive impairment given the known negative effect of systemic inflammation on neurocognitive functions [31, 32]. Although we were unable to evaluate the result of cognitive function with standardized tools such as Wechsler Intelligence Scale for Children (WISC) due to a lack of available data, our finding of improved adaptive functions may imply a

beneficial influence of probiotics on overall adaptation. However, the limited number of available RCTs ($n=3$) suggests the need for further studies based on more objective cognitive assessment (e.g., WISC) to verify our findings.

Analyses of our secondary outcomes focusing on other associated problems of ASD showed an apparent trend of improvement in anxiety symptoms in individuals treated with probiotics compared with the controls despite a lack of statistical significance, while no difference was noted in other behavioral issues of ASD including irritability/aggression, inattention, hyperactivity/impulsivity, and parental stress between the two groups. Previous research has demonstrated a correlation between intestinal microbiome and mood regulation [46]. Compared with the intestinal microbiota composition in healthy individuals, prior investigations have also shown increased levels of the phyla *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* but a reduced level of *Firmicutes* in those diagnosed with major depressive disorders [47]. One proposed hypothesis is the mood-modulatory effect of probiotics through the microbial-gut-brain axis [48]. Despite the reported superiority of probiotics over placebo in anxiety relief from previous RCTs [49, 50], a recent meta-analysis showed effectiveness of probiotics only against depressive symptoms but not anxiety in patients with anxiety- or depression-related diagnoses [51]. Since one out of the three included RCTs used a measurement tool for both depression and anxiety [15] but the remaining two used tools for only anxiety [12, 16], the mixed results from both depression and anxiety may influence the specificity of our outcomes. Nevertheless, given our finding of a nearly significant effect and the positive results of a previous meta-analysis for mood symptoms [51], more investigations into the efficacy of probiotics in improving mood symptoms specifically targeting anxiety or depression in patients with ASD are warranted.

Several limitations in this study need to be taken into consideration. First, one of the major limitations of the current study is that most of our included trials did not investigate the effect of probiotics on GI symptoms. For instance, chronic constipation, which is noted in a significant number of children diagnosed with ASD and can adversely affect their quality of life [52], was not specified in the included studies. Taking into account the therapeutic potential of probiotics against chronic constipation [53], it is difficult to determine whether the observed improvement in symptoms of ASD was associated with alleviation of GI symptoms (e.g., constipation). Therefore, we strongly recommend the survey of changes in GI symptoms in future probiotics-related trials to rule out the potential confounding effects in this clinical setting. Second, notwithstanding our inclusion of up to ten trials and a total of 522 participants, the results were still

not robust enough to provide solid evidence. Moreover, our results on some core or associated symptoms of ASD, which were derived from limited numbers of available trials (i.e., only two trials for inattention), require validation from future studies. Third, certain heterogeneities in treatment strategies such as probiotics used as supplements or part of combination therapy may be potential confounding factors that influence the therapeutic outcomes. Nevertheless, our consistent findings on subgroup analysis after excluding studies using probiotics as part of combination therapies indicated minimal influence of this confounder. Forth, a number of other factors that may affect the therapeutic outcomes of probiotics (e.g., dietary habits, use of other nutritional supplements) were unaccounted for due to a lack of relevant information for meta-regression or subgroup analysis. Finally, two studies that did not use placebo control may be more susceptible to performance and detection bias [37, 38]. Nevertheless, our sensitivity test demonstrated consistent results after excluding those two studies.

Conclusions

The current study showed a significantly greater improvement in the overall behavioral symptoms of ASD in participants treated with probiotics than that in the control groups. Our subgroup analyses further demonstrated a significant alleviation of the behavioral symptoms of ASD in those receiving multiple-strain probiotics compared to controls. Moreover, except a significant improvement in adaptation and an apparent trend of improvement in mood symptoms, the use of probiotics was not associated with significant mitigation of core or other associated symptoms of ASD. Nevertheless, all double blinded placebo-controlled studies failed to address probiotics-related improvement in gastrointestinal (GI) symptoms (e.g., chronic constipation) that may be a significant confounder in the assessment of study outcome. Our results, which were derived from a limited number of available trials that provided limited information on changes in GI symptoms warrant further large-scale clinical investigations to shed light on the mechanisms underlying the observed improvement in the total symptoms of ASD and verify our findings.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13034-024-00848-3>.

Supplementary Material 1

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Not applicable.

Author contributions

JC Lee, CM Chen and CK Sun conceived and designed the study; YS Cheng, HJ Chiu, MY Wang, IT Tsai, and YC Cheng contributed to data extraction, YS Cheng, YH Tang and HJ Chiu analyzed the data, JC Lee, CM Chen, and CK Su and KC Hung were major contributors to writing the manuscript. All authors contributed sufficiently to this work. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed the possible publication of our article on *Child and Adolescent Psychiatry and Mental Health*.

Competing interests

The authors declare no competing interests.

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