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REVIEW ARTICLE

Benefits of Probiotics in Autism Spectrum Disorders: A Meta-Analysis of Randomized Controlled Trials

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Abstract

Using probiotics as a pharmaceutical intervention is based on the fact that dysbiosis affects many people with autism spectrum disorders (ASD). This study aimed to quantify various probiotics' overall and individualized benefits in treating ASD. Randomized or cross-over trials comparing the efficacy of placebo or active control vs. probiotics in patients of any age diagnosed with ASD based on DSM IV/V criteria were considered under inclusion cri- teria. An electronic database search in PUBMED and Cochrane Library was conducted using MeSH search terms "probiotics" AND "Autism." Mean change in the total score of clinical parameters used to assess ASD symptom severity was the primary outcome measure analyzed. All the outcome measures were estimated by calculating the Standardized Mean Difference (SMD) values and their 95% Confidence Intervals (CI), considering the different clinical parameters used to assess the change in ASD symptoms in identified clinical tri- als. An insignificant decrease in the total score value of primary outcome measure by -0.14 (SMD: 0.14, 95% CI:-0.45 to 0.17) in patients treated with probiotics was observed. The quantity of decrease remained insignificant in subgroup analyses also. Observed insignifi- cant clinical benefits of probiotics in ASD patients could be due to the presence of gastroin- testinal symptoms as co-morbidity. We hypothesize that intolerance to dietary components is responsible for gastrointestinal symptoms and inflammation. Perhaps probiotics are still beneficial in ASD patients without gastrointestinal symptoms, while their combination with prebiotics is effective in patients with gastrointestinal symptoms.

Keywords: Probiotics, ASD, Autism.

Introduction

Autism spectrum disorder (ASD) is a spectrum of neurological and developmental disorders diagnosed primarily by the presence of impaired social interaction, social communication, and stereotypical behaviors.[1-3] It includes autistic disorder, Asperger's syndrome, pervasive developmental disorder-not otherwise specified (PDD-NOS), Rett's disorder, and childhood disintegrative disorder.[1,2] Increased screening, awareness, and changing diagnostic criteria have been attributed to its increased incidence rate.[1-3] The unclear etiology and pathogenesis of ASD have been due to the complex interplay of multiple genetic and environmental factors.[1,2]

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The involvement of multiple genes, the presence of multiple genetic deficits in ASD patients, the higher incidence in twins and male gender, the variation in clinical manifestations, and the association with other genetic disorders all support the genetic basis as a major etiological agent responsible for its pathogenesis. Early diagnosis and intervention with non-pharmacological behavior therapy is the treatment of choice for ASD.[3-4] However, it is costly and requires significant time and resources.[3-5] Thus, adopting behavioral therapy as a universal treatment strategy is unacceptable in all countries.[3-4]

Risperidone and aripiprazole are the only drugs approved by the FDA to treat irritability and disruptive symptoms of ASD.[2,5] Their selective efficacy on disruptive symptoms, minimal efficacy on core ASD symptoms, and high incidence of ADRs necessitate the development of better pharmacological agents.[3-5]

Using probiotics as a pharmaceutical intervention is based on the fact that dysbiosis or altered gastrointestinal microbial flora affects many people with ASD.[6] Dysbiosis has been attributed to leaky gut epithelium, systemic inflammation, and thus altered neurotransmitter signaling in the brain.[6] Results of clinical trials analyzing the benefits of probiotics in ASD are insignificant and inconclusive.[7–14]

The insignificant reduction is not only of ASD symptoms but also of the severity of gastrointestinal symptoms in ASD patients.[9,12] However, these trials are either pilot or small-scale trials, and the authors of these trials opine on the need for large-scale trials. Results of a meta-analysis study also do not support the benefits and use of probiotics for ASD.[15]

Moreover, this meta-analysis study included either a few trials or low-quality trials testing the efficacy of probiotics and prebiotics plus probiotics. Hence, there is no clarity on the actual benefits of probiotics alone in ASD. In addition, there is a lack of consistency in the type of probiotics used in individual clinical trials and meta-analysis studies published so far. Consequently, there was a need to analyze the efficacy of probiotics alone and by including recently published clinical trials. We also felt the need for subgroup analysis based on the type of probiotic agent used. Hence, the present meta-analysis was conducted to quantify the overall and individual benefits of various probiotics in reducing ASD symptoms.

Methods

The study is being reported by PRISMA statement consisting of a 27-item checklist and a 4-phase flow diagram.

Protocol and Registration

Protocol not registered and does not exist

Inclusion and Exclusion Criteria

Articles included in this study were restricted to randomized or cross-over trials. Only those trials comparing the efficacy of placebo or active control vs. any probiotic agent in patients of any age diagnosed with ASD based on DSM IV/V criteria were considered under inclusion criteria. The exclusion criterias adopted were: trials published with incomplete data required for statistical analysis, trials published in a language other than English, and trials published as abstracts. No restriction was applied based on phase, sample size used in the trials, or the year of publication. We didn't plan to contact the corresponding authors to access missing or other required data.

Information Source and Literature Search

A literature search in PUBMED and Cochrane Library was conducted using MeSH search terms "probiotics" AND "Autism". We limited electronic database searches to articles published or available online up to 19th May 2022 without restriction on the beginning or oldest year of publication. An additional manual search of some relevant articles was also conducted to identify any missed trials by reviewing their references. Two authors were independently involved in conducting both electronic database and manual searches.

Study Selection, Data Collection Process, and Data Items Collected

Both authors independently went through the standard process of article selection and data collection of all required data in a prior designed data extraction sheet. The screening process for the eligible articles was conducted by going through the titles and abstracts of all articles retrieved from the literature search. Potential articles selected by this method were then screened in their full-text form for the availability of required data on population, intervention, comparator, and outcome apart from trial design and other parameters to assess their eligibility for inclusion as per preset eligibility criteria.

Trials meeting all eligibility criteria were selected, and data on baseline demographic, clinical data, characteristic study data, intervention and data required to estimate outcome measures were collected by both authors individually. The mean change (baseline-final) and standard deviation (SD) values of any clinical parameter used to assess changes in ASD symptoms were extracted to compare efficacy. Those trials which did not report SD values were excluded from quantitative analysis. However, for those trials publishing baseline (day 0) and final (day 90) values, we used a mathematical formula to calculate SD values from baseline and final mean values. The following formula was used to calculate mean change SD value: square root of (baseline SD^2 + final SD^2 + 2 × 0.6 × baseline SD × final SD).[16] Differences in opinions between the authors on the trial selection and data extracted/calculated were resolved after achieving consensus between the authors, and then the final data extraction sheet was prepared.

Risk of Bias Assessment

Assessment of the risk of bias within the individual trials was independently done by two authors using the Cochrane Collaboration tool.[17] Discrepancies in the allotting level of bias in the individual trials were sorted after arriving at a consensus between the authors. Publication bias was analyzed by the funnel plot method. A funnel plot is a scatter plot of the effect size measures of individual trials plotted along the horizontal axis against the effect size measure of the study (meta-analysis) along the vertical axis. An asymmetrical funnel plot implies the possibility of publication bias or systematic difference between larger and smaller trials.

Summary Measures

The primary outcome measure analyzed was the mean change in the total score of any clinical parameters/scales used to assess ASD severity. The mean change in individual clinical parameters or scales used to assess the severity of ASD were the secondary outcome measures analyzed. The other secondary outcome measures analyzed were the mean change in individual ABC (Aberrant Behavior Checklist) sub-scores and the gastrointestinal Symptoms Severity Index (GSI).

Subgroup Analysis

Subgroup meta-analysis is conducted by including identical trials; identical based

on either type of probiotic used or baseline demographic or clinical features was planned. This was done to ascertain that the meta-analysis results that included all trials did not differ significantly and thus are not sensitive or vary significantly with variation in intervention, baseline demographic, or clinical features of patients.

Synthesis of Results and Statistical Methods
Various clinical parameters/scales were used to assess the effect of probiotics on the severity of ASD in our included trials. Hence, we estimated the efficacy of probiotics in reducing ASD severity by calculating the Standardized Mean Difference (SMD) values of these parameters/scales. An efficacy analysis by including only those trials publishing identical clinical parameter/scale was also done by estimating risk difference (RD) values. The mantel-Haenszel method and both fixed and random effect models were used in the analysis by Revman 5.4.1 software.

Apart from subgroup analysis, the sensitivity of the results was analyzed by comparing the results of the fixed effect model and the random effect model. The lack of significant variation in the results analyzed by the fixed effect model and the random effect model indicated that the effect size measured is robust. Heterogeneity between the included trials was analyzed using the Cochrane Q test for heterogeneity and the I^2 test. A chi-square test with a P value of 0.10 and an I^2 test value of > 50% was considered an indicator of significant heterogeneity.

Results and Discussion
Five randomized controlled trials were eligible and included in the quantitative synthesis of the meta-analysis.[9–13] However, there was a lack of uniformity in the clinical parameters/ scales used to assess the severity of ASD and the benefits of probiotics on ASD symptoms. Therefore, we preferred and included ABC or SRS (Social Responsiveness Scale) or ADOS (Autism Diagnostic Observation Schedule) scores for estimating standardized mean difference values to assess the efficacy of all probiotics used in all included trials in reducing ASD severity. (Figure 1)

Table 1 shows the baseline demographic, clinical features, and characteristics of individual trials included in the analysis. Of the five included trials, probiotic *L.Plantarum* was used in two trials, and a combination of eight propbiotic preparation was used in the other two trials. The remaining trials varied significantly regarding the use of Bovine Clostrum Product (BCP) in combination with the probiotic preparation *B.Infantis*.

The forest plot in Figure 2 shows the results of SMD analyzing the overall efficacy of all probiotics using total scores of any clinical parameters or scores used to assess the severity of ASD. The reduction in the severity of total ASD score was small (SMD: -0.14) and insignificant (95% CI -0.45 to 0.17). Quantities of reduction in total scores of individual clinical parameters/ scores considered under secondary outcomes measures were also insignificant and as follows: total ABC score (RD:2.64, 95% CI: -8.19 to 13.47, N=114, n=3), total SRS score (RD:-3.65, 95% CI: -8.36 to 1.05, N=118, n=3) and total CBCL score (RD:1.51, 95% CI: -6.25 to 9.28, N=134, n=2).

The efficacy of probiotics on individual ABC scale sub-scores were also insignificant and as follows: ABC-Irritability (RD:0.33, 95% CI: -4.63 to 5.3, N=63, n=3), ABC-Stereotype (RD:0.84, 95% CI: -1.39 to 3.08, N=63, n=3), ABC-Lethargy (RD:1.69, 95% CI: -1.46 to 4.84, N=63, n=3).

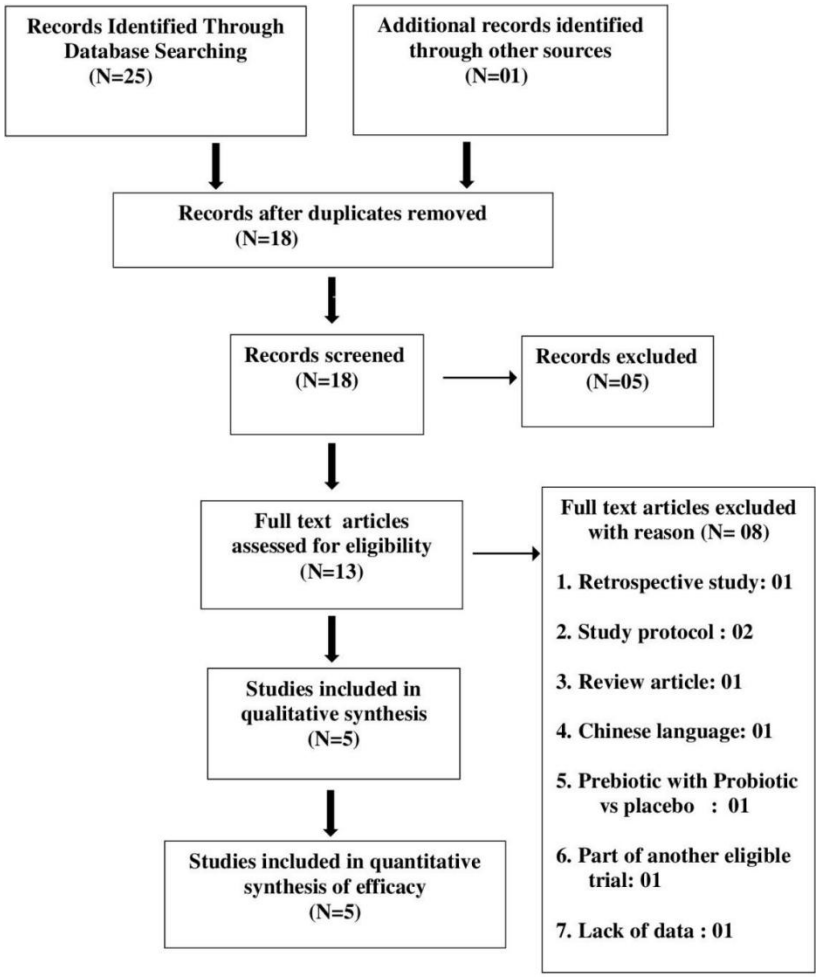

Figure 1. Showing Literature Search Results and Study Attrition Diagram

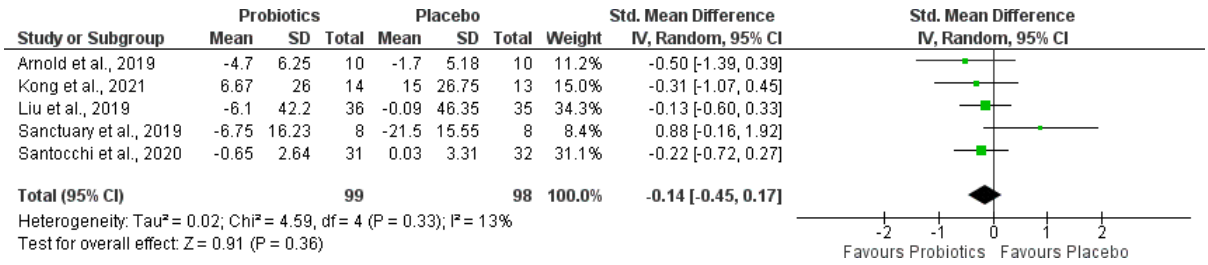

Figure 2. Forest Plot Showing SMD of Overall Benefits of Probiotics

ABC-Hyper activity (RD:1.65, 95% CI: -2.51 to 5.81, N=63, n=3), ABC-Inappropriate speech (RD:-0.60, 95% CI: -2.35 to 1.15, N=47, n=2). Individual estimation of efficacy about two other parameters, total ADOS score and total Vineland Adaptive Behavior Scales-II (VABS II) score, was not possible due to a lack of sufficient data. Additional analysis on the benefits of probiotics in reducing gastrointestinal symptom severity Index (6-GSI) was also not possible for the same reason.

Subgroup analysis was conducted by including those trials using identical probiotic agents. This analysis was done for the outcome measures: total ABC score and SRS score. We estimated the efficacy of two probiotic preparations, *L. plantarum* (PS128) and VISBIOME (a probiotic preparation containing eight probiotic species, mostly *Lactobacillus* and *Bifidobacterium*), in two subgroup analyses. There was no statistically significant change in SMD values of total ABC score after including VISBIOME preparation (SMD: -0.29, 95% CI: -0.72 to 0.14, n = 2, N =83). To assess the efficacy of L.Plantarum (PS128), we calculated RD values rather than SMD value. There was no significant reduction in ASD severity when L.Plantarum (PS128) was used as a probiotic preparation in total ABC score (RD: -7.21, 95% CI: -21.54 to 7.12, n = 2, N = 98) as well as total SRS score (RD: -8.22, 95% CI: -21.5 to 5.07, n = 2, N = 98).

Subgroup analysis after excluding the trial by Sanctuary et al., which included both prebiotic and probiotic preparations in the control group, was conducted. The result of this subgroup analysis on the total ABC score was also insignificant (SMD: -0.23, 95% CI: -0.52 to 0.06, n = 4, N = 181). Similarly, the result of subgroup analysis, including long-duration treatment (> 2 months) trials on total ABC score, also remained insignificant (SMD: -0.25, 95% CI: -0.66 to 0.17, n = 2, N = 90). Due to a lack of data, subgroup analysis based on patient age (less than and more than 7 years of age) and the presence or absence of gastrointestinal symptoms was not possible. Nevertheless, study results appear robust since there was no significant variation in effect measures analyzed by random and fixed effect models.

There was no evidence of publication bias in any of the outcome measures analyzed. There was evidence of heterogeneity between the trials only in two secondary outcome measures: ABC-Irritability and ABC-hyperactivity.

Results of our study suggest that probiotics are ineffective in reducing gastrointestinal and ASD symptom severity, irrespective of the type of probiotic preparations used and their duration of treatment. There was no significant reduction in any of the major sub-scores or symptoms of ASD. An interesting observation is their insignificant effectiveness in relieving gastrointestinal symptoms. Consequently, it is unfair to ascertain the ineffectiveness of probiotics in ASD for the heterogeneous demographic and clinical factors of patients included in our study.

There are significant results between patients' age range, varying treatment duration, type of probiotic tested, and inclusion of ASD patients with and without gastrointestinal symptoms. The influence of the duration of treatment and type of probiotic tested was insignificant in subgroup analysis. Patients' age and gastrointestinal symptoms significantly influence probiotic efficacy and appear to be strong confounding factors.[10,12]

Table 1. Baseline Demographic and Clinical Features of Included Trials (1)

Study	Arnold et al., 2019 (Cross over trial)		Liu et al., 2019		Sanctuary et al., 2019 (Cross over trial)
	Placebo (N=4)	Probiotics (N=6)	Placebo (N=35)	Probiotics (N=36)	(N = 8)
Age (Yrs):	8.76±1.18	8.83±2.80	9.91±2.33	10.11±2.34	6.8 ± 2.4
Male (%):	83.3	25	100	100	87.5
Baseline					
ABC:	NA	NA	17±9.31*	15.81±8.39*	NA
SRS:	87.0±4.76	82.17 ±7.85	135.8±26.04	138.8±24.19	NA
ADOS:	NA	NA	NA	NA	NA
VABS II:	NA	NA	NA	NA	NA
CBCL:	NA	NA	50.6±25.91	49.63±25.4	NA
GSI:	NA	NA	NA	NA	NA
Design:	R, UB, CO, PC, Pilot		R, DB, PG, PC		R, DB, CO, PC
Duration:	8 weeks		4 weeks		5 weeks
Country:	USA		Taiwan		USA
Sites:	Single		Single		Single
Probiotics:	VISBIOME		*L.Plantarum* (PS128)		*B.Infantis* ± BCP
Bias risk					
RSG:	UR		LR		LR
AC:	UR		LR		LR
BPP:	UR		LR		LR
BOA:	UR		UR		UR
IOD:	LR		LR		LR

ABC:Aberrant Behavior Checklist, SRS: Social Responsiveness Scale,ADOS:Autism Diagnostic Observation Schedule, VABS II: Vineland Adaptive Behavior Scales-II, CBCL:Child Behavior Checklist, GSI: gastrointestinal severity index, R: Randomized, UB: Un-blinded, CO: Cross Over, PC: Placebo-controlled, PG: Parallel Group, VISBIOME: (Probiotic preparation containing eight probiotic species, mostly Lactobacillus and Bifidobacterium), *ABC-Taiwan version, BCP: Bovine Colostrum Product, RSG: Random Sequence Generation, AC: Allocation Concealment, BPP: Blinding of Participants and Personnel, BOA: Blinding of Outcome Assessment, IOD: Incomplete Outcome Data, SR: Selective Reporting, UR: Unclear Risk, HR: High Risk, LR: Low Risk, N/A: Not Available, All values are in mean±SD

Table 1. Baseline Demographic and Clinical Features of Included Trials (2)

Study	Santocchi et al., 2020		Kong et al., 2021	
	Placebo (N=32)	Probiotics (N=31)	Placebo (N=17)	Probiotics (N=18)
Age (Yrs):	4.09±0.97	4.29±1.22	10.7±4.76	9.85±4.91
Male (%):	84.4	77.4	64.7	83.3
Baseline	NA	NA	278±34.8	272± 0.2
ABC:	NA	NA	83.0±12.1	82.3±11.5
SRS:	6.97±1.91	6.84±1.39	NA	NA
ADOS:	57±16.7	63.87±22.1	NA	NA
VABS II:	62.8±10.9	60.9±9.94	NA	NA
CBCL:	1.38±1.45	2.06±2.14	NA	NA
6-GSI:	NA	NA	NA	NA
Design:	R, DB, PG, PC		R, DB, PG, PC	
Duration:	6 months		16 weeks	
Country:	Italy		USA	
Sites:	Single		Single	
Probiotics:	VISBIOME		*L.Plantarum* (PS128)	
Bias risk:				
RSG	LR		LR	
AC	LR		LR	
BP	LR		LR	
BOA	LR		LR	
IOD	LR		LR	

ABC: Aberrant Behavior Checklist, SRS: Social Responsiveness Scale, ADOS: Autism Diagnostic Observation Schedule, VABS II: Vineland Adaptive Behavior Scales-II, CBCL:Child Behavior Checklist, GSI: gastrointestinal severity index, R: Randomized, UB: Un-blinded, PC: Placebo-controlled, PG: Parallel Group, VISBIOME: (Probiotic preparation containing eight probiotic species, mostly Lactobacillus and Bifidobacterium), RSG: Random Sequence Generation, AC: Allocation Concealment, BPP: Blinding of Participants and Personnel, BOA: Blinding of Outcome Assessment, IOD: Incomplete Outcome Data, SR: Selec- tive Reporting, UR: Unclear Risk, HR: High Risk, LR: Low Risk, N/A: Not Available, All values are in mean±SD

Due to a lack of sufficient data, the influence of these factors could not be assessed in subgroup analysis. It is imperative to rule out these two factors' influence to ascertain probiotics' ineffectiveness in ASD. Observed insignificant clinical benefits of probiotics in ASD patients could be due to the presence of gastrointestinal symptoms as a co-morbidity. Based on a clinical trial results, we believe that probiotics could be effective in a subgroup of ASD patients without gastrointestinal symptoms.[12]

Dysbiosis, or altered gastrointestinal microbial flora, is attributed to consequent leaky gut epithelium, inflammation, and altered neurotransmitter and biochemical levels apart from gastrointestinal symptoms.[6,20] Apart from its significant impact on brain function, dysbiosis is framed as the pathological basis for various systemic, psychological, and neurological disorders.[18-21] Restoration of favorable gastrointestinal microbial flora has proven clinically beneficial in these disorders.[19] A large proportion of ASD patients present with dysbiosis or gastrointestinal symptoms.[6,20] However, restoration of gastrointestinal microbial flora by probiotics failed to reduce ASD symptoms, especially in the subgroup of patients with gastrointestinal symptoms. The reasons for this failure and whether the association between ASD and dysbiosis is a coincidence or comorbidity is quite intriguing.

ASD aetiology and pathogenesis is a complex interplay of genetic and environmental factors, and dysbiosis is unlikely to be the single most important contributor. In addition, dysbiosis is not a co-feature in all ASD patients. Gastrointestinal symptoms in ASD patients do not correlate with the type of microbial flora colonizing their gut.[12]

Since restoring balanced microbial flora with probiotics was ineffective in reducing gastrointestinal symptoms in all ASD patients, the association of ASD appears to be not with dysbiosis but with gastrointestinal symptoms. "gastrointestinal symptoms" are a frequent co-morbidity in ASD patients.[19-21] It has been correlated with increased severity of ASD symptoms, especially irritability and social skill impairment.[20] Consequently, the ineffectiveness of probiotics in a subgroup of ASD patients with gastrointestinal symptoms is anticipatory and exploratory. There is also a lacuna in understanding the reasons for the co-morbid presentation of gastrointestinal symptoms in ASD.

We hypothesize that intolerance to dietary components is responsible for gastrointestinal symptoms and inflammation. Clinical symptoms and pathological changes of leaky gut epithelium, inflammation, and altered gastrointestinal microbial flora in ASD patients with gastrointestinal symptoms are identical to gluten intolerance.[20,22] Significant clinical benefits observed with interventions preventing exposure to intolerant dietary components or alleviating gut inflammation strengthen our hypothesis.

These clinical benefits were evident from nascent clinical trials employing four intervention strategies:

1. Prebiotics (Bimuno-galactooligosaccharide, B-GOS) plus gluten/casein exclusion diet
2. Prebiotics plus immune factors such as BCP (bovine colostrum product, an immune factor and prebiotic preparation)
3. Probiotic plus prebiotic preparation
4. Synbiotic 2000 (anti-inflammatory fibres and probiotic preparation).[7,12,23,24]

The success of the first strategy could be due to prevention of exposure to intolerant

dietary components (gluten/casein). While the gastrointestinal inflammation is reduced in the other three strategies.[7,12] In a trial analyzing the benefits of BCP, despite no change in the gastrointestinal microbial flora, there was a significant reduction in inflammation with a reduction in ASD and gastrointestinal symptoms.[12] It is unclear whether BCP preparation has direct anti-inflammatory properties and is responsible for gastrointestinal anti-inflammatory action. However, there was a significant reduction in inflammatory biomarkers in patients receiving BCP.

In the third strategy, adopting anti-inflammatory fibers and probiotics (Synbiotic 2000), apart from clinical benefits, favorable gastrointestinal microbial flora was restored along with a significant reduction in inflammation.[23] Nevertheless, the results of these trials demonstrate the significance of preventing or reducing gastrointestinal inflammation in alleviating gastrointestinal and, thus, ASD symptoms severity. There appears to be no significance in restoration of normal or good gastrointestinal microbial flora in ASD patients with gastrointestinal symptoms.

Quite interesting is the inclusion of probiotics and or prebiotics in all four strategies. Both probiotics and prebiotics have direct anti-inflammatory effects and other indirect beneficial effects that reduce gastrointestinal inflammation.[25,26] This could be the mechanism behind significant clinical benefits observed in the fourth strategy adapting supplementation of combined probiotic and prebiotic preparations.[24] Perhaps this combination is synergistic and significantly enhances their anti-inflammatory efficacy, sufficient to reduce gut inflammation. Additional evidence from clinical and animal studies supports the anti-inflammatory action of this combination to be beneficial in relieving chronic gut inflammation and ASD symptoms, respectively.[26,27]

Variable reductions in the quality and quantity of gastrointestinal or ASD symptoms were evident in these four strategies. Excluding intolerant dietary components (gluten, casein) has extrapolated to improved gastrointestinal symptoms but not ASD symptoms. Adding a prebiotic preparation to it has significantly improved the social behaviour domain of ASD. Combining prebiotics (FOS) with probiotics improved the language and speech domains of ASD symptoms.

The Synbiotic 2000 preparation significantly decreased the severity of stereotypical behaviors. Among the four interventions, BCP improved the most in the ASD domains of stereotypical behavior, irritability, and hyperactivity. Quite interestingly, benefits were the opposite in the group receiving a combination of probiotics plus BCP, which had a significant reduction only in the social behaviour domain. Hence, a strategy to combine these interventions to gain maximum benefits may not be beneficial. Probiotics alone have improved the social interaction domain, but only in a subgroup of ASD patients without gastrointestinal symptoms.[12] Hence, probiotics like prebiotics could also be beneficial in patients with gastrointestinal symptoms when combined with additional specific anti-inflammatory action agent. Overall, the reduction in core symptoms of ASD by these interventions further strengthens the significance of the gut-brain axis in ASD.

The involvement of hundreds of genes and multiple genetic deficits in patients of ASD support a strong genetic basis of its etiopathogenesis.[1,2] There is a complex interplay of genetic and environmental factors behind the etiology and pathogenesis of ASD.[1,2] Higher incidence in twins and male

gender, variation in clinical manifestations and its concurrence with other genetic disorders strengthens the genetic basis of its etiopathogenesis. Intolerance to dietary components could be one such strong environmental factor.

The presence of gastrointestinal symptoms enhances the severity of ASD symptoms.[20] Hence, relieving gastrointestinal symptoms' severity extrapolates into a reduction in the actual severity of ASD needs to be clarified. Heterogeneity in etiology and pathogenesis, variation in clinical manifestation, and coincidence of other co-morbid illnesses with ASD have led to inter-individual variations in response and inconsistency in the efficacy of both non-pharmacological and pharmacological interventions employed for ASD treatment.[2–5] Perhaps this could be another factor responsible for the insignificant effects of probiotics in patients with gastrointestinal symptoms.

The need to assess the efficacy of any intervention used in ASD based on the patients' individual genetic and phenotypic traits is also relevant for probiotics.[4] In addition, the effects of other non-genetic traits which are predictors of response to pharmacological interventions, especially age and gender, also need to be identified. Future trials analyzing the efficacy of any intervention need to be stratified based on these confounding factors. We didn't have sufficient data to conduct a network meta-analysis to compare the efficacy of prebiotic and probiotic-based interventions indirectly. Hence, the inclusion of a single standard clinical parameter to assess the efficacy and severity of ASD will be beneficial in comparing them.

The major drawback of our study is the exclusion of two randomized clinical trials, one due to publication in Chinese and the other due to the need for more sufficient data.[8,28] The influence of excluding them on our results could be altogether different. In addition, the inclusion of few trials and a small patient population in overall and subgroup analysis limits the strength of our evidence. However, the inclusion of more trials and conducting subgroup analysis are our major strengths compared to a previously published meta-analysis. We also highlighted the benefits of probiotics and other interventions in ASD patients with and without gastrointestinal symptoms.

Conclusion

The observed insignificant clinical benefits of probiotics in ASD patients could be due to the presence of gastrointestinal symptoms as a co-morbidity. We hypothesize that intolerance to dietary components is responsible for gastrointestinal symptoms and inflammation. Perhaps probiotics are still beneficial in ASD patients without gastrointestinal symptoms, while their combination with prebiotics is effective in patients with gastrointestinal symptoms. Prebiotic or probiotic-based combination intervention strategies aimed at preventing or reducing gut inflammation appear to be beneficial in ASD patients with gastrointestinal symptoms.

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Conflict of interest

None

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