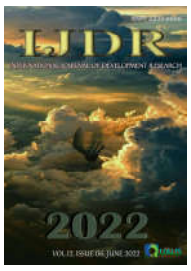
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 12, Issue, 06, pp. 56453-56459, June, 2022



RESEARCH ARTICLE OPEN ACCESS

MODULATION OF THE MICROBIOTA IN THE THERAPEUTIC APPROACH TO AUTISM SPECTRUM DISORDER: A SYSTEMATIC REVIEW

Gabriela Achete de Souza[1]; Gyovanna Sorrentino dos Santos Campanari[1]; Júlia Novaes Matias[1]; Vinícius Marinho Lima[1]; Gabriela Lopes[1]; Heron Fernando de Sousa Gonzaga[1,2]; Tereza Lais Menegucci Zutin[1,2]; Patrícia C. dos Santos Bueno[1,3]; Ricardo de Alvares Goulart[2]; Karina R. Quesada[1,4]; Raul S. J. Girio[3]; Elen Landgraf Guiguer[1,2,4]; Adriano Cressoni Araújo[1,2]; Adriana M. R. Fiorini[4]; Sandra Maria Barbalho[1,2,4*] and Marcelo Dib Bechara[1,2]

[1]School of Medicine, University of Marília (UNIMAR), Marília, São Paulo, Brazil; [2]Postgraduate Program in Structural and Functional Interactions in Rehabilitation, University of Marilia (UNIMAR), São Paulo, Brazil;[3]Department of Animal Sciences, School of Veterinary Medicine, University of Marília (UNIMAR), Avenida HiginoMuzzi Filho 1001, Marília 17525-902, São Paulo, Brazil; [4]Department of Biochemistry and Nutrition, Faculty of Food Technology of Marília, Marília, São Paulo, Brazil.

ARTICLE INFO

Article History:

Received 27th March, 2022
Received in revised form
22nd April, 2022
Accepted 20th May, 2022
Published online 22nd June, 2022

Key Words:

Autism Spectrum Disorder. Microbiota; Probiotic; Prebiotic; Gastrointestinal symptoms; Anxiety.

****Corresponding author:***
Sandra Maria Barbalho

ABSTRACT

Most children diagnosed with Autism Spectrum Disorder (ASD) have gastrointestinal manifestations. The use of probiotics and prebiotics has gained significant scientific community visibility as possible beneficial interventions in the therapeutic arsenal of ASD. Thus, the objective of this study was to conduct a systematic review to assess the effects of microbiota on ASD. MEDLINE-PubMed and EMBASE were used to perform the search and PRISMA guidelines were followed. Seven studies were selected and demonstrated significant improvement in gastrointestinal symptoms using prebiotics. A significant improvement in the severity of autism and gastrointestinal symptoms was observed in children treated with probiotics containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus,* and *Bifidobacteria longum*. The use of *Lactobacillus plantarum* PS128 was related to improvement in anxiety, externalization, hyperactivity, and impulsivity, especially in younger children. The intervention with *B. infantis* reduced the frequency of gastrointestinal, behavioral symptoms, and serum levels of inflammatory cytokines.

Citation: ***Gabriela Achete de Souza, GyovannaSorrentino dos Santos Campanari, Júlia Novaes Matias, Vinícius Marinho Lima, et al.*** "Análise de atendimentos ambulatoriais antes e durante o período de pandemia pela covid-19, no hospital veterinário joaquim felício-casu, caratinga/mg*", International Journal of Development Research*, 12, (06), 56453-56459.

INTRODUCTION

Autism Spectrum Disorder (ASD) comprises a group of neurodevelopmental disorders characterized by neurobehavioral disorders, which preferentially affect male children before three years. Among the symptoms, restricted and repetitive behaviors, impairments in social interaction and communication impact child development and the individual's life and society. The prevalence of this disorder has grown significantly in recent years, with an incidence reaching 1 in 68 children in the USA, making ASD a public health problem (Doenyas, 2018; Fattorusso *et al*., 2019; Johnson *et al*., 2020; Y. W. Liu *et al*., 2019; Mangiola *et al*., 2016; Peralta-Marzal *et al*., 2021; Ristori *et al*., 2019).

In addition to the symptoms already described, approximately half of children with ASD have a higher incidence of gastrointestinal symptoms, such as abdominal discomfort, diarrhea, constipation, and flatulence, with intensity proportional to the severity of behavioral symptoms. The cause of intestinal involvement in individuals with ASD is not fully understood, having been related to dysfunction of the intestinal microbiota, increased intestinal permeability, and changes in the immune response (Doenyas, 2018; Grimaldi *et al*., 2018; Li *et al*., 2017; Zafar & Habib, 2021). The intestinal microbiota represents the first protective barrier of the gastrointestinal system, being acquired at birth and modified by age, sex, immune status, and external factors such as breast-feeding, diet, and antibiotics use. It consists of more than a thousand different bacteria, fungi, and viruses,

with a predominance of the phyla Firmicutes and Bacteriodetes, relating dynamically and symbiotically to the organism. The spectrum of functions of the intestinal microbiota is broad, some of which is the maintenance of the epithelial barrier, maturation of the gastrointestinal and systemic immune system, protection against infections by pathogenic organisms, production of short-chain fatty acids, nutrients, hormones, and vitamins necessary for the metabolism, as well as pathogenic role depending on its composition(Balan *et al*., 2021; Doenyas, 2018; Fattorusso *et al*., 2019; Johnson *et al*., 2020; Li *et al*., 2017; Mangiola *et al*., 2016). Due to its great importance in systemic hemostasis, qualitative and quantitative changes in the microbiota, such as dysbiosis, have been associated with several gastrointestinal and extra-intestinal pathologies, such as neuropsychiatric disorders including dementia, depression, schizophrenia, and ASD, thus constituting a bidirectional axis known as microbiota-gut-brain (Chernikova *et al*., 2021). Therefore, therapeutic measures such as the use of antibiotics, probiotics, prebiotics, and transplantation of fecal microbiota have gained importance, as they act in the control of the intestinal microbiota, being a promising therapy still little explored in ASD (Chernikova *et al*., 2021; Fattorusso *et al*., 2019; Grimaldi *et al*., 2018; Johnson *et al*., 2020; Li *et al*., 2017; Y. W. Liu *et al*., 2019; Mangiola *et al*., 2016). For these reasons, this systematic review aims to investigate the effects of prebiotics and probiotics on ASD.

METHODS

Focal question: This review was built to answer the following focal question: Can the microbiota modulation through the use of probiotics and prebiotics have beneficial effects on the therapeutic approach of patients with ASD?

Language: Only studies in English were selected.

Database: This review included studies available on the MEDLINE – PubMed and EMBASE platforms. The Mesh terms used were "Probiotics" or "Prebiotics" and "Autism Spectrum Disorder." The use of these descriptors favored the identification of studies related to prebiotics and probiotics and their beneficial effects in the treatment of ASD. The guidelines of PRISMA (Preferred Reporting Items for a Systematic Review and Meta-Analysis) were followed.

Selection of the studies: Studies published between June 2015 and December 2021 that aimed to report the effects of probiotics and prebiotics on the modulation of the microbiota of patients with ASD were included. Inclusion criteria were trials performed in humans, including randomized controlled trials and open studies. Literature reviews, articles not written in English, editorials, essay protocols, and poster presentations were excluded.

Eligible criteria: This study's eligible criteria followed the *PICO* (Population, Intervention, Comparison, and Outcomes) format, and the studies involving ASD patients who were treated with probiotics and prebiotics were included.

Data extraction: Two independent judges, independently performed the search to identify the databases' RCTs. The MESH terms were Autism Spectrum Disorder, prebiotics, and probiotics. The abstracts of the studies were evaluated, and full-text articles were also evaluated to support the decision-making process. Disagreements between the two judges were evaluated and decided by a third reviewer.

Quality Assessment: The evaluation of the risk of bias concerning the selection, detection, and reporting bias of each Randomized Clinical Trial was evaluated according to the Cochrane Handbook for Systematic Reviews of Interventions(Higgins *et al*., 2019). Other risks of bias in the classification of interventions, selection of patients, missing data, and bias in the measurement of outcomes were also performed.

RESULTS

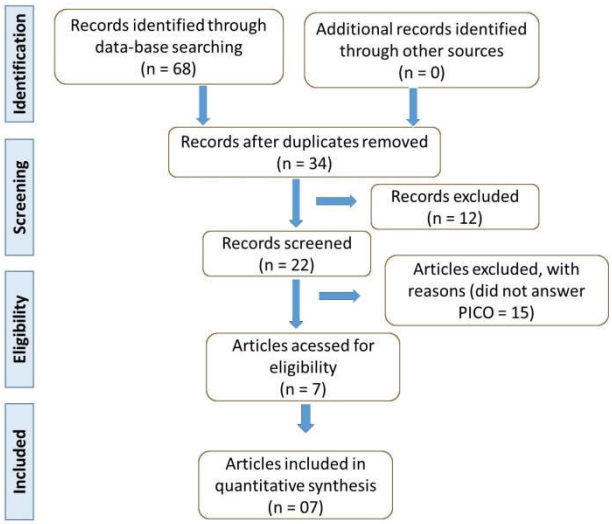


Figure 1. Flowchart showing the study selection.

The flowchart (Figure 1) shows the selection of articles. Six randomized clinical trials and one open study (open-label) were included. In all, 170 individuals composed the selected articles, aged between 2 and 20 years, 122 were male, and 21 were female. One study did not provide clear information about the participants' genders. Table 1 shows the studies that were included in this review and Table 2 shows the potential biases of the studies.

DISCUSSION

Autism Spectrum Disorder: ASD is a pathological condition with complex and little-known neurobiology that compromises cognitive development, sensory processing, and social communication and generates repetitive behavior and interest patterns. Other comorbidities have been included in ASD, such as Autistic Disorder, Asperger's Syndrome, Childhood Disintegrative Disorder, and Generalized Developmental Disorder. All of these conditions manifest symptoms since childhood through neural reorganization and altered brain development. Although the etiology of ASD is not entirely known, it can be related to genetic and environmental factors, including epigenetic mechanisms (Johnson *et al*., 2020; Lord *et al*., 2018; Robertson & Baron-Cohen, 2017; Sanchack & Thomas, 2016).
In order to confirm the diagnosis of ASD, there must be past or present difficulties in each of the communication subdomains, which are deficits in development, maintenance, and understanding, deficits in socioemotional reciprocity, and deficits in non-verbal communicative behaviors. Subjects must also have or have had difficulties with two of the four different types of restricted and repetitive sensory-motor behavior, such as stereotyped or repetitive motor movements, use of objects and speech (aligning toys or throwing objects and simple motor stereotypes), inflexibility to ritualized routines or patterns of verbal and non-verbal behavior, highly restricted and abnormal fixed interests in intensity or focus (seen in attachment or concerns about unusual objects) and hyper-reactivity or hyper-reactivity to sensory input or unusual interests in sensory aspects of the environment (Lord *et al*., 2018). There is no specific treatment for ASD. The existing treatment consists of managing associated symptoms, such as hyperactivity, sleep disorders, psychomotor aggression, and anxiety. The medications used are those used to treat other psychiatric disorders. However, critical adverse effects are found, and there is no standardized treatment(Persico *et al*., 2019).

Relationship between intestinal microbiota and ASD: microbiota-gut-brain axis: Recent studies have demonstrated a connection between the microbiota, gut, and brain, which advocate bidirectional

communication through these components. The brain influences the intestine through peptides and neurotransmitters sent by the peripheral nervous system and acting through the adrenal pituitary hypothalamus axis, controlling cortisol secretion, which in turn regulates intestinal motricity and integrity (Ding *et al*., 2017; Mangiola *et al*., 2016). In turn, the microbiota influences the nervous system through neural, endocrine, immunological, and metabolic mechanisms, and it is therefore suggested that modifications in the composition of the microbiota in children with ASD may contribute to both behavioral and gastrointestinal symptoms in genetically predisposed individuals (Fattorusso *et al*., 2019; Hughes *et al*., 2018; Ristori *et al*., 2019). In studies of the intestinal microbiota of patients with ASD, a higher incidence of dysbiosis was demonstrated compared to healthy individuals, with a decrease in the number of Bifidobacteria and Firmicutes and an increase in the number of Bacteroidetes, *Clostridium spp*, *Desulfovibriospp, Sutterellaspp, Veillonellaceae, Faecalibacterium* and *Candida* (Ding *et al*., 2017; Li *et al*., 2017; Mangiola *et al*., 2016; Strati *et al*., 2017; Xu *et al*., 2019). Also, patients with autism had a history of extensive antibiotic use before three years of age and selective eating habits, factors that directly alter the microbiota and lead to more significant colonization by pathogenic microorganisms that produce neurotoxins that contribute to ASD symptoms(Grimaldi *et al*., 2018; Li *et al*., 2017; Mangiola *et al*., 2016; Ristori *et al*., 2019). Nevertheless, the ASD children's diet tend to be more restricted, with inadequate fiber intake, which negatively modulate gut bacterial genera and its metabolites(Martin & Mayer, 2017).

Dysbiosis causes an increase in intestinal permeability, which leads to the transport of bacteria and endotoxins into the bloodstream, such as lipopolysaccharide (LPS). This is also known as "leaky gut theory" (White, 2003).Besides, there is a high production of pro-inflammatory cytokines, such as Interleukin (IL) -1, IL-6, Interferon (IFN)-ɣ and Tumor Necrosis Factor (TNF)-α, and of metabolites, including short-chain fatty acids as acetic acid, propionic acid, butyrate, isobutyric acid, valeric and isovaleric acid stand out, along with ammonia, phenol compounds and glutamate(Ding *et al*., 2017; Fattorusso *et al*., 2019; Hughes *et al*., 2018; Li *et al*., 2017; Ristori *et al*., 2019). Short-chain fatty acids are produced mainly by *Clostridia*, *Bacteroidetes,* and *Desulfovibrio*, the most found species in the microbiota of patients with ASD (Ding *et al*., 2017; Li *et al*., 2017) (Figure 2).

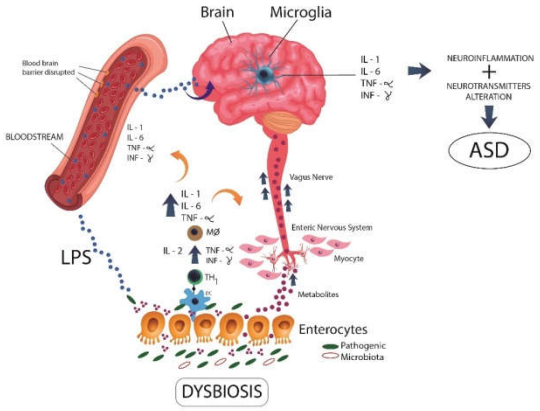


Figure 2. The association of dysbiosis, neuroinflammation, and alteration in neurotransmitters and Autism Spectrum Disorder.

These compounds exert an effect on the nervous system retrograde, through the enteric nervous system and the vagus nerve, and through the bloodstream, where they alter the blood-brain barrier, thus contributing to ASD symptoms (Ding *et al*., 2017; Hughes *et al*., 2018; Li *et al*., 2017). Some studies have shown that specific bacteria genera are capable of producing neurotransmitters, such as GABA (γ-aminobutyric acid), which directly interacts with the vagus nerve (Peralta-Marzal *et al*., 2021). Another mechanism proposed for communication between the intestine and the brain is through the oropharynx, which indicates the influence of the oral microbiota on the pathogenesis of ASD(Johnson *et al*., 2020).

The increase in LPS in patients with ASD has been demonstrated in some studies, and its levels are associated with the severity of symptoms, corroborating the hypothesis of the existence of a microbiota-intestine-brain axis in the genesis of this disorder. LPS and pro-inflammatory cytokines, formed by the exacerbated activation of the immune system, alter the blood-brain barrier, reaching the central nervous system in regions responsible for emotional control, such as the amygdala, leading to neuroinflammation and alteration of neurological physiology(Ding *et al*., 2017; Li *et al*., 2017; Mangiola *et al*., 2016). In fact, in *post-mortem* studies with the cerebrospinal fluid of ASD patients, a neuroinflammatory response involving excessive microglia activation has been demonstrated, with high levels of pro-inflammatory cytokines(Johnson *et al*., 2020; Ristori *et al*., 2019). Changes in the maternal vaginal microbiota due to stress, infection, and a high-fat diet during pregnancy were also related to an increased incidence of ASD in animal models, as it influences the composition of the fetus' intestinal microbiota at birth(Li *et al*., 2017)(Buffington *et al*., 2016) (Figure 3). Bacterial vaginosis during pregnancy is related to prematurity, and higher ASD rates are also found in premature newborns(Johnson *et al*., 2020). It was also demonstrated in maternal separation models that early life stress can be one of the causes of dysbiosis and influence the immune response, contributing to the pathogenesis of the disorder(Hughes *et al*., 2018). Moreover, the microbiota affects the levels of melatonin, serotonin, histamine, and acetylcholine, important mediators for brain maturation(Ristori *et al*., 2019).

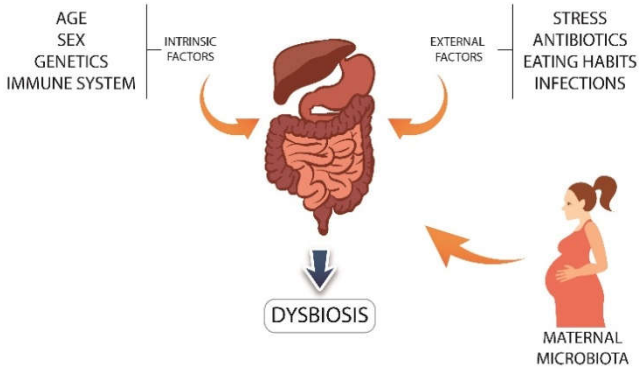


Figure 3. Intrinsic and External factors related to dysbiosis.

Prebiotics and Probiotics: main aspects: According to several factors, the human diet has varied, including social, economic, regional, and temporal. The development of society is accompanied by a higher presence of industrialized foods and xenobiotics. Given these changes in modernity in eating habits, science has turned to the study of probiotics and prebiotics, living components and their substrates, known to medicine for over a century (Azad *et al*., 2018; Floch, 2018; Neri-Numa & Pastore, 2020; Suez *et al*., 2019). Probiotics are living microorganisms, mostly bacteria, of a non-pathogenic nature to humans, which, from their metabolism, can produce or provide the production of substances capable of promoting regulation in the number and quality microscopic populations make up the intestinal biota. In this way, probiotics control the growth and development of potentially pathogenic bacteria, viruses, and fungi that inhabit the gastrointestinal tract (Gasbarrini *et al*., 2016; Islam, 2016). The most well-known and used probiotics are bacteria Lactobacillus and Bifidobacterium, and the fungus *Saccharomyces boulardii*. These organisms regulate the growth of other potentially pathogenic ones through competition for substrates and produce substances that alter the intestinal microenvironment's biochemistry, such as changes in pH, which hinder the proliferation of harmful agents. Moreover, probiotics stimulate the development of low-grade inflammatory immune responses and promote the production of anti-inflammatory cytokines (Lee *et al*., 2018; Lopez-Santamarina *et al*., 2020; Sanders *et al*., 2019; Tanner *et al*., 2018; Tsai *et al*., 2019). Prebiotics are components of food, non-digestible fibers, which serve as substrates for the growth of certain non-pathogenic microorganisms, naturally present in the gastrointestinal tract.

Table 1. Clinical studies showing the effects of prebiotics or probiotics in patients with Autism Syndrome Disorder (ASD).

Reference	Country	Type of the study	Population	Intervention and Comparison	Results
[47]	USA	Randomized, double-blind, placebo-controlled study.	35 individuals 3-20 y, 26 ♂ and 9 ♀, with a previous diagnosis of ASD and using stable medications for at least 4 weeks.	*Lactobacillus plantarum* (PS128) 2x/d (6×10^{10} CFUs) was administered in probiotics group (n=18), while the placebo group (n=17) received oral placebo for 16w. After, intranasal oxytocin spray (Syntocinon®) was added in both groups, in increasing doses of 4UI-32UI/d,totaling28w.	Improvements in ASD scores were observedin combination group (PS128 + oxytocin) compared to the control group. No significant resultswas observed inPS128 or oxytocin alone groups. Besides, a significant improvement in the microbiome was found in the intervention groups, mainly in the combination group. No difference was found in serum inflammatory markers.
(Sanctuary et al., 2019)	USA	Randomized, double-blind, crossover clinical trial.	8 children 2-11y, 7 ♂ and 1 ♀, with previous diagnosis of ASD and gastrointestinal comorbidities.	*B. infantis*4 x 10^9 UFC 2x/d + PCB 0.15 g/kg/dia/5 w, and PCB alone for more 5 w, with 2 weeks apart. The results were evaluated through questionnaires, blood, feces, and urine analysis. No comparison with a placebo group was reported.	There was a significant reduction in the frequency of gastrointestinal, behavioral symptoms, and serum levels of inflammatory cytokines in both groups, with better rates in the group, treated with PCB alone. No changes were seen in the fecal and urinary analysis.
(Y. W. Liu et al., 2019)	China	Randomized, double-blind, controlled, parallel.	71 children (♂) diagnosed with ASD, 7-15 years old, divided into PS128 (n = 36) and placebo (n = 35) groups.	Probiotic *L. plantarum* PS128 was used in capsules (25mg) / 4 weeks. ABC-T, SRS, CBCL questionnaires, and age improvement analysis (7-12 years and 13-15 years) were performed.	The group treated with the probiotic showed improvement in anxiety, externalization of hyperactivity and impulsivity, and disobeyed the imposed rules less. These results were not seen in the placebo group.
(Arnold et al., 2019)	United States of America	Randomized, crossover, controlled, pilot trial.	10 children, 3-12 years old, 6 ♂ and 4 ♀, diagnosed with ASD, anxiety, and gastrointestinal symptoms.	Participants were divided into two crossover groups: probiotic then placebo (n=4) and placebo then probiotic (n=6). VISBIOME was administered orally in half or a full powder packet (900 billion bacteria) 2xd/8w, with 3 w apart between the crossover.	There was a greater improvement in gastrointestinal and anxiety scales in probiotic group, but with no statistical significance. The fecal analysis showed a relative increase in *Lactobacillus* proportionally to the improvement of gastrointestinal symptoms, but there was no difference in microbiota composition/diversity.
(Grimaldi et al., 2018)	United Kingdom	Randomized, double-blind, controlled, parallel.	26 children previously diagnosed with ASD, 4-11 years old, divided into a group (A) without diet restriction (n = 14) and another (B) with diet restriction (n = 12).	In groups A and B, patients were randomized into two subgroups to receive 1.8g of GOS / day / 6 weeks. The placebo group received maltodextrin.	Patients in group B treated with placebo did not significantly improve gastrointestinal symptoms and behavior questionnaires compared to the group with diet restriction and treatment with the prebiotic. There was a change in the composition of organisms in the feces and a decrease in amino acids in the group treated with GOS.
(Shaaban et al., 2018)	Egypt	Open-labelprospectivestudy.	30 children with ASD (19 ♂ and 11 ♀), and 30 controls, 5-9 years.	Powdered probiotic composed of dried carrots and three strains: *L. acidophilus, L. rhamnosus* and *Bifidobacteria longum*, (5g)/3 months. Fecal samples were collected before and after the intervention in both groups.	There was an increase in colonization by *Bifidobacteria* and *Lactobacilli* in the treated group and a reduction in weight, and a significant improvement in the severity of autism and gastrointestinal symptoms.
(Pärtty et al., 2015)	Finland	Randomized, double-blind, placebo-controlled prospective study	75 pregnant women were divided in probiotic (n=40) and placebo (n=35). The intervention was developed from 4 weeks before expected delivery until the next six months after giving birth.	Probiotic group received 1x10¹□ colony-forming units of *Lactobacillus rhamnosus GG,* and the placebo received microcrystalline cellulose daily/4 w before expected delivery. The capsule contents were given continuously to the children or to the mothers (breast-feeding) for 6 m. The children were analyzed clinically and behaviorally up to 13 years old.	Early administration of *Lactobacillus rhamnosus GG* may reduce the risk of ADHD and AS. *Lactobacillus rhamnosus GG* has been shown to stabilize the gut permeability barrier by effects on tight junctions, mucin production, and antigen-specific immunoglobulin A production.

ABC-T: Autism Behavior Checklist-Taiwan version; ADHD: Attention-deficit hyperactivity disorder; AS: Asperger syndrome; ASD: Autism Syndrome Disorder; CBCL: Child Behavior Checklist; GOS: Galactooligossacarídeo; PCB: Produto de ColostroBovino; SRS: Social Responsiveness Scale.

Table 2. Descriptive Table of the Biases of the Included Randomized Clinical Trials

Study	Question focus	Appropriate randomization	Allocation blinding	Double-blind	Losses (<20%)	Prognostics or demographic characteristics	Outcomes	Sample calculation	Adequate follow-up
[47]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
(Sanctuary et al., 2019)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
(Y. W. Liu et al., 2019)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
(Arnold et al., 2019)	Yes	No	Yes	Yes	No	Yes	No	NR	Yes
(Grimaldi et al., 2018)	Yes	Yes	Yes	Yes	No	No	Yes	NR	Yes
(Shaaban et al., 2018)	Yes	No	No	No	NR	Yes	Yes	Yes	Yes
(Pärtty et al., 2015)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NR	Yes

NR: Notreported.

Consequently, these species develop and exert benefits for the host, mainly controlling the growth of potentially pathogenic microorganisms (Holscher, 2017; Hutkins *et al*., 2016). Prebiotics are used alone or in combination with probiotics. However, a challenge in the use of these substances is because they can be familiar with the development of both a species of commensal organisms beneficial to man and a pathogenic one. Thus, it is important to carefully identify, know, and select the best substrate for each organism. The most well-known and used prebiotics are fructooligosaccharide (FOS) carbohydrates present in the fibers of fruits and vegetables, germinate barley foodstuff (GBF), and galactooligosaccharides (GOS), present in milk formulas. These substances act as a substrate for the metabolism and development of certain species of bacteria, enabling the formation of substances such as butyrate and short-chain fatty acids, which affect inflammatory processes (Enam & Mansell, 2019; Guarino *et al*., 2020; Hijová *et al*., 2019; R. T. Liu *et al*., 2019; Valcheva & Dieleman, 2016). Currently, studies have pointed out several possible mechanisms by which these substances and organisms interact with the host and promote better results in treating diseases not only restricted to the gastrointestinal tract but also skin, kidney, psychiatric and neurological disorders(Kao *et al*., 2016; Kim *et al*., 2018; Paiva *et al*., 2020; Pugliese *et al*., 2020).

Use of Probiotics e Prebiotics on ADS: In the study conducted byKong *et al*.(Kong *et al*., 2021), the administration of probiotics combined with ocytocin, known to modulate behavior and emotions, was performed. In the study, 35 individuals with ASD were randomized to receive *Lactobacillus plantarum* (PS128) twice a day or placebo for 16 weeks. After that, intranasal oxytocin spray (Syntocinon®) was added in both groups, accounting 28 weeks of treatment. The treatment was well-tolerated and the only side effect reported was minor nose bleeding. To access the results, questionnaires such as Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS) and GI severity index (GSI) were Applied, besides serum biomarker and stool samples analysis. The results showed a significant improvement in ASD symptoms and microbiome network of individuals in combined group (probiotics + oxytocine), compared to control and probiotics or oxytocine groups alone. No difference was verified in serum inflammatory markers between all groups. The biases of the study include the follow-up losses and the small sample at the end of the intervention period.

In the study performed by Sanctuary *et al*.(Sanctuary *et al*., 2019), the authors evaluated the tolerability and benefits of treatment with the probiotic *Bifidobacterium infantis* associated with a prebiotic (bovine colostrum product - PCB) in 8 children diagnosed with ASD and with gastrointestinal comorbidities (according to the *Questionnaire on Pediatric Gastrointestinal Symptoms-Rome III Version (QPGS-RIII)*, including diarrhea, constipation and/or irritable bowel syndrome. One group underwent treatment with powdered formulations of *Bifidobacterium infantis* associated with PCB or just PCB. After two weeks, the groups were inverted so that all children received both treatments for five weeks. Both treatments were well-tolerated among participants, and the reported adverse effects were flatulence, stomach pain, and weight gain. In the results, a general improvement in gastrointestinal symptoms was observed in both groups. Still, there was a significant reduction in aberrant behaviors such as irritability, stereotype, hyperactivity, and lethargy during treatment with PCB compared to the combined one. The results were attributed to a decrease in the levels of IL-13 and TNF-α in some participants. There was no difference in the study of feces and urine. A possible bias in this study was the reduced number of patients, and the lack of a control group and another group treated only with probiotics. Liu *et al*.(Y. W. Liu *et al*., 2019)evaluated whether the use of *L. plantarum* PS128 would be able to reduce clinical symptoms of boys diagnosed with ASD and found that patients who used the probiotic showed improvement in scores in the Autism Behavior Checklist-Taiwan version (ABC-T) and Social Responsiveness Scale (SRS), with no adverse effects. This study proved to be promising regarding the use of probiotics to treat ASD, demonstrating a decrease in the intensity of symptoms, allowing a better quality of life for the patient. However, the reduced number of patients may be a factor that interferes with the interpretation of results. Arnold *et al*. (Arnold *et al*., 2019) studied the effects of the probiotic VISBIOME (*L. casei, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus delbrueckii subsp. bulgaricus, B. longum, Bifidobacterium infantis, Bifidobacterium breve* and *S. thermophiles*) versus placebo in anxiety and gastrointestinal symptoms of 10 children diagnosed with ASD, anxiety and gastrointestinal symptoms (abdominal pain, constipation, diarrhea and/or vomiting). The results were assessed by scales such as Gastrointestinal Module of the PedsQL (Pediatric Quality of Life Inventory) and PRAS-ASD (Parent-Rated Anxiety Scale for ASD), in addition to fecal examination. Adverse effects were well tolerated and more observed in the placebo group, with higher flatulence rates, nasopharyngitis, and other upper respiratory tract infections in the probiotic group. Unfortunately, the number of participants in this study was small and, besides, there were food intake variation, probiotic dose variation, and concomitant medication intake during the study, which may have interfered with the results. Grimaldi *et al*. (Grimaldi *et al*., 2018) conducted a randomized clinical trial whose objective was to assess whether dietary restriction has a positive impact on the treatment of gastrointestinal and behavioral symptoms in patients diagnosed with ASD compared to non-restrictive diets. The researchers also assessed whether the introduction of prebiotics in both diets would positively impact these populations (Table 1). The adverse effect observed in patients undergoing non-restrictive diets was a deficiency in vitamin D intake, and, in both types of diet, well-tolerated gastrointestinal symptoms, such as constipation and discomfort, were observed. Despite the promising results, the number of patients included in the study is small. The study by Shaaban *et al*.

(Shaaban *et al*., 2018) showed that the use of *L. acidophilus, L. rhamnosus,* and *Bifidobacteria longum*, results in good effects on the intestinal manifestations inherent to ASD, as well as a reduction in the severity of the autistic condition, showing a possible adjuvant therapeutic alternative in the treatment of these patients. The observed adverse effects were mild and transient, including diarrhea, bloating, abdominal cramps, and a case of rash. However, it is substantial to note that this is a single-center study with a small number of participants. Pärtty *et al*. (Pärtty *et al*., 2015) conducted a randomized clinical trial with the objective of assessing the relationship between a previous *Lactobacillus rhamnosus GG* administration and neuropsychiatric disorders. The administration was made from four weeks before de expected delivery until six months after the birth, with *Lactobacillus rhamnosus* GG or placebo to the children or the breast-feeding mother. The children were analyzed clinically up to 13 years old and behaviorally by their parents and by anchild psychiatrist or neurologist not involved in the study or follow-up and blinded with randomization. Attention-deficit hyperactivity disorder (ADHD) or Asperger syndrome (AS) was diagnosed in 6/35 (17.1%) of the children in the placebo and zero in the probiotic group by the age of 13 y (P = 0.008). As noted in the studies above, there is a need for more randomized clinical trials with larger populations to outline the real effects that these supplements can promote on patients with ASD. Table 2 gives the bias risk assessment for the included studies.

CONCLUSION

The human diet has been varying intensely according to changes in daily life. The choice for fast, industrialized foods, associated with lifestyle and genetic factors can cause changes in the intestinal microbiota and consequent physiological changes. Recent studies have made an association between dysbiosis and ASD. Such discoveries allow the emergence of new therapeutic possibilities. Studies reveal that prebiotics and probiotics can be beneficial to patients diagnosed with ASD, leading to an improvement in the severity of the condition and impulsivity, anxiety, and hyperactivity resulting in an improvement of the quality of life of patients. Despite promising results and the possibility of few adverse effects, studies in the literature differ regarding the type of prebiotic or probiotic and the doses and pharmaceutical forms used. Therefore, there is a necessity for more standardized clinical trials to prescribe these supplements in children with ASD.

AUTHOR'S CONTRIBUTION

Conceptualization: GAS, GSSC, JNM, GL, VML, and SMB; Literature search: RAG and HFSG; Writing: SMB, TLMZ, RSJG and PCSB; Review and editing: SMB, RAG, GAS, GSSC, HFSG, and VML.

CONFLICT OF INTEREST: The authors declare no conflict of interest and no funding support.

REFERENCES

Doenyas C: Gut Microbiota, Inflammation, and Probiotics on Neural Development in Autism Spectrum Disorder. *Neuroscience.* 2018;374:271-286.

Fattorusso A, Di Genova L, Dell'Isola GB, Mencaroni E, Esposito S: Autism Spectrum Disorders and the Gut Microbiota. *Nutrients.* 2019;11(3).

Johnson D, Letchumanan V, Thurairajasingam S, Lee LH: A Revolutionizing Approach to Autism Spectrum Disorder Using the Microbiome. *Nutrients.* 2020;12(7).

Liu YW, Liong MT, Chung YE, *et al*.: Effects of Lactobacillus plantarum PS128 on Children with Autism Spectrum Disorder in Taiwan: A Randomized, Double-Blind, Placebo-Controlled Trial. *Nutrients.* 2019;11(4).

Mangiola F, Ianiro G, Franceschi F, Fagiuoli S, Gasbarrini G, Gasbarrini A: Gut microbiota in autism and mood disorders. *World J Gastroenterol.* 2016;22(1):361-368.

Ristori MV, Quagliariello A, Reddel S, *et al*.: Autism, Gastrointestinal Symptoms and Modulation of Gut Microbiota by Nutritional Interventions. *Nutrients.* 2019;11(11).

Peralta-Marzal LN, Prince N, Bajic D, *et al*.: The Impact of Gut Microbiota-Derived Metabolites in Autism Spectrum Disorders. *Int J Mol Sci.* 2021;22(18).

Grimaldi R, Gibson GR, Vulevic J, *et al*.: A prebiotic intervention study in children with autism spectrum disorders (ASDs). *Microbiome.* 2018;6(1):133.

Li Q, Han Y, Dy ABC, Hagerman RJ: The Gut Microbiota and Autism Spectrum Disorders. *Front Cell Neurosci.* 2017;11:120.

Zafar U, Habib H: The Link Between Autism Spectrum Disorder And Gastrointestinal Microbiota. *J Ayub Med Coll Abbottabad.* 2021;33(3):513-518.

Balan Y, Gaur A, Sakthivadivel V, Kamble B, Sundaramurthy R: Is the Gut Microbiota a Neglected Aspect of Gut and Brain Disorders? *Cureus*. 2021;13(11):e19740.

Chernikova MA, Flores GD, Kilroy E, Labus JS, Mayer EA, Aziz-Zadeh L: The Brain-Gut-Microbiome System: Pathways and Implications for Autism Spectrum Disorder. *Nutrients.* 2021;13(12).

Higgins JP, Thomas J, Chandler J, *et al*.: *Cochrane handbook for systematic reviews of interventions*: John Wiley & Sons; 2019.

Lord C, Elsabbagh M, Baird G, Veenstra-Vanderweele J: Autism spectrum disorder. *Lancet.* 2018;392(10146):508-520.

Robertson CE, Baron-Cohen S: Sensory perception in autism. *Nat Rev Neurosci.* 2017;18(11):671-684.

Sanchack KE, Thomas CA: Autism Spectrum Disorder: Primary Care Principles. *Am Fam Physician.* 2016;94(12):972-979.

Persico AM, Ricciardello A, Cucinotta F: The psychopharmacology of autism spectrum disorder and Rett syndrome. *Handb Clin Neurol.* 2019;165:391-414.

Ding HT, Taur Y, Walkup JT: Gut Microbiota and Autism: Key Concepts and Findings. *J Autism Dev Disord.* 2017;47(2):480-489.

Hughes HK, Rose D, Ashwood P: The Gut Microbiota and Dysbiosis in Autism Spectrum Disorders. *Curr Neurol Neurosci Rep.* 2018;18(11):81.

Strati F, Cavalieri D, Albanese D, *et al*.: New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome.* 2017;5(1):24.

Xu M, Xu X, Li J, Li F: Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Front Psychiatry.* 2019;10:473.

Martin CR, Mayer EA: Gut-Brain Axis and Behavior. *Nestle Nutrition Institute workshop series.* 2017;88:45-53.

White JF: Intestinal pathophysiology in autism. *Experimental biology and medicine (Maywood, N.J.).* 2003;228(6):639-649.

Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M: Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell.* 2016;165(7):1762-1775.

Azad MAK, Sarker M, Wan D: Immunomodulatory Effects of Probiotics on Cytokine Profiles. *Biomed Res Int.* 2018;2018:8063647.

Floch MH: The Role of Prebiotics and Probiotics in Gastrointestinal Disease. *Gastroenterol Clin North Am.* 2018;47(1):179-191.

Neri-Numa IA, Pastore GM: Novel insights into prebiotic properties on human health: A review. *Food Res Int.* 2020;131:108973.

Suez J, Zmora N, Segal E, Elinav E: The pros, cons, and many unknowns of probiotics. *Nat Med.* 2019;25(5):716-729.

Gasbarrini G, Bonvicini F, Gramenzi A: Probiotics History. *J Clin Gastroenterol.* 2016;50 Suppl 2, Proceedings from the 8th Probiotics, Prebiotics & New Foods for Microbiota and Human Health meeting held in Rome, Italy on September 13-15, 2015:S116-s119.

Islam SU: Clinical Uses of Probiotics. *Medicine (Baltimore).* 2016;95(5):e2658.

Lee ES, Song EJ, Nam YD, Lee SY: Probiotics in human health and disease: from nutribiotics to pharmabiotics. *J Microbiol.* 2018;56(11):773-782.

Lopez-Santamarina A, Miranda JM, Mondragon ADC, *et al*.: Potential Use of Marine Seaweeds as Prebiotics: A Review. *Molecules.* 2020;25(4).

Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA: Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nat Rev Gastroenterol Hepatol.* 2019;16(10):605-616.

Tanner G, Matthews K, Roeder H, Konopasek M, Bussard A, Gregory T: Current and future uses of probiotics. *Jaapa.* 2018;31(5):29-33.

Tsai YL, Lin TL, Chang CJ, *et al*.: Probiotics, prebiotics and amelioration of diseases. *J Biomed Sci.* 2019;26(1):3.

Holscher HD: Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes.* 2017;8(2):172-184.

Hutkins RW, Krumbeck JA, Bindels LB, *et al*.: Prebiotics: why definitions matter. *Curr Opin Biotechnol.* 2016;37:1-7.

Enam F, Mansell TJ: Prebiotics: tools to manipulate the gut microbiome and metabolome. *J Ind Microbiol Biotechnol.* 2019;46(9-10):1445-1459.

Guarino MPL, Altomare A, Emerenziani S, *et al*.: Mechanisms of Action of Prebiotics and Their Effects on Gastro-Intestinal Disorders in Adults. *Nutrients.* 2020;12(4).

Hijová E, Bertková I, Štofilová J: Dietary fibre as prebiotics in nutrition. *Cent Eur J Public Health.* 2019;27(3):251-255.

Liu RT, Walsh RFL, Sheehan AE: Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. *Neurosci Biobehav Rev.* 2019;102:13-23.

Valcheva R, Dieleman LA: Prebiotics: Definition and protective mechanisms. *Best Pract Res Clin Gastroenterol.* 2016;30(1):27-37.

Kao AC, Harty S, Burnet PW: The Influence of Prebiotics on Neurobiology and Behavior. *Int Rev Neurobiol.* 2016;131:21-48.

Kim N, Yun M, Oh YJ, Choi HJ: Mind-altering with the gut: Modulation of the gut-brain axis with probiotics. *J Microbiol.* 2018;56(3):172-182.

Paiva IHR, Duarte-Silva E, Peixoto CA: The role of prebiotics in cognition, anxiety, and depression. *Eur Neuropsychopharmacol.* 2020;34:1-18.

Pugliese D, Acampora A, Porreca A, Schips L, Cindolo L: Effectiveness of a novel oral combination of D-Mannose, pomegranate extract, prebiotics and probiotics in the treatment of acute cystitis in women. *Arch Ital Urol Androl.* 2020;92(1):34-38.

Kong XJ, Liu J, Liu K, *et al*.: Probiotic and Oxytocin Combination Therapy in Patients with Autism Spectrum Disorder: A Randomized, Double-Blinded, Placebo-Controlled Pilot Trial. *Nutrients.* 2021;13(5).

Sanctuary MR, Kain JN, Chen SY, *et al*.: Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms. *PLoS One.* 2019;14(1):e0210064.

Arnold LE, Luna RA, Williams K, *et al*.: Probiotics for Gastrointestinal Symptoms and Quality of Life in Autism: A Placebo-Controlled Pilot Trial. *J Child Adolesc Psychopharmacol.* 2019;29(9):659-669.

Shaaban SY, El Gendy YG, Mehanna NS, *et al*.: The role of probiotics in children with autism spectrum disorder: A prospective, open-label study. *Nutr Neurosci.* 2018;21(9):676-681.

Pärtty A, Kalliomäki M, Wacklin P, Salminen S, Isolauri E: A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. *Pediatric research.* 2015;77(6):823-828.

****