REVIEW ARTICLE

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Analysis of research on the effectiveness of using probiotics for children with autism spectrum disorders, in order to reduce the core and accompanying autism symptoms. Review of randomized clinical trials

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Abstract

Purpose: The prevalence of autism spectrum disorder (ASD) has increased tenfold over the last 40 years and the World Health Organization (WHO) has placed it alongside other globally occurring common illnesses, such as cancer, cardiovascular disorders and diabetes. As there are yet no effective methods for treating ASD, the most frequently used therapeutic interventions are psychological, psychosocial, rehabilitation and developmental support, which in combination aim to support patients and their families. Early intervention improves the prognosis, but usually a cure is impossible. Patient's lives are often burdened with social difficulties in contact with their families, peers and in education, particularly when other disorders, diseases or intellectual impairment are present, leading to undesirable behaviours, including aggression or self-aggression. Aim of the study was to review the literature available, so as to determine the effectiveness of probiotics used for treating core and accompanying symptoms of autism in patients diagnosed with ASD, with a particular focus on children and adolescents.

Views: The randomised clinical trials available on the clinicaltrials.gov register (accessed on June 27th, 2021) and the PubMed database (search: probiotic + autism, probiotic + ASD, probiotic + Asperger syndrome, probiotic + pervasive developmental disorder, randomised controlled trial filter) have been analysed in the present study. All studies were included, without any operational time limit. The same PubMed search was also re-run for open-label trials. Out of the 140 papers found, five were open-trials. We also supplemented our study by additionally analysing the studies cited by the latest papers on probiotics in autism.

Conclusions: There are still no consistent outcomes in studies on the use of probiotics in children and adolescents with ASD, and the scope of existing studies is limited. Nevertheless, the authors considered it worthwhile to explore whether probiotic interventions can indeed reduce the severity of ASD-related symptoms and behaviours. Further studies are required on specific indications, duration of treatment and the effectiveness of interventions in the defined problem areas.

Key words: ASD, probiotic, microbiom, Asperger's syndrome (AS).

INTRODUCTION

Autism spectrum disorder (ASD) is a serious medical condition causing considerable suffering to patients, often leading to disability and sometimes to failure in living independently. The core symptoms of ASD are persistent disturbances in social interaction and the presence of limited, repetitive patterns of behaviour, interests or activities. Speech may also be underdeveloped. Symptoms become apparent in childhood and persist throughout the patient's lifetime. There has been a tenfold increase in the prevalence of ASD within the last 40 years [1, 2], with a global prevalence recently estimated at 0.76% [3]. The incidence ranges from 0.5-3.1% in the European Union (EU) countries for children aged 7-9 years [2]. Poland's epidemiological data is, however, incomplete. Rates of children with certified ASD disability, aged 0-15 years, were found to be 0.35% on average for those living in the Pomeranian and West-Pomeranian provinces (voivodeships) [4]. Indeed, the Supreme Audit Office report on the Polish education system shows that 0.87% of pupils were diagnosed with autism or Asperger's syndrome (Reg. No. 75/2019/P/19/073/LKI). The World Health Organization (WHO) also emphasises the need

for the coordinated management of autism spectrum disorders (WHA67/2014/REC/1).

Autism is considered a neurodevelopmental disorder where biological factors are primarily responsible for its development, without there being a single cause. Risk factors have been identified such as genetic background, perinatal burden, environmental pollution, advanced parental age, toxicity-related factors and others [5]. There is no scientific evidence linking vaccination to autism.

The core symptoms of autism include persistent abnormalities in social communication and interaction together with limited, repetitive patterns of behaviour and interests. Due to the neurodevelopmental nature of the condition, symptoms are already visible in early childhood. The ICD-11 and DSM-5 classifications enable the diagnosis of speech development levels to be refined, along with the presence of intellectual disability and the scope of the support required (in DSM-5). Problems are also posed by other disorders frequently coexisting with autism, such as gastro-intestinal symptoms, (prevalence ranging 9-70% [6]), in keeping with the main tenet of this article. Other accompanying complications include intellectual disability (17-84% [7]), sleep disorders (50-80% [6]), epilepsy in adolescence (10-20% [6]), and behavioural difficulties, such as self-harming (42% [8]) and aggressive behaviour, which are often a clinical problem. Therapeutic interventions basically consist of the psychological, psychosocial, rehabilitation and developmental support. Prognoses are improved by early intervention, however, no cure is usually found. Furthermore, no drugs can be unequivocally effective in treating the pivotal symptoms of autism. Psycho-pharmacotherapy may be used to address comorbidities or certain symptoms.

COUNTING THE HEALTH COSTS OF AUTISM SPECTRUM DISORDERS

The search for newer and more effective means of treating ASD has become very important to healthcare systems, patients and their families due to current shortcomings in this field. The Supreme Polish Audit Office (Reg. No. 75/2019/P/19/073/LKI), reported that the number of students with ASD and disabilities, including autism were 27,794 students with ASD and 10,264 students with multiple disabilities in 2016/2017, whereas in 2017/2018 the equivalent figures were respectively 34,437 and 11,742 and in 2018/2019, they were 40,884 and 13,520. The numbers are quite clearly constantly rising. An autistic child is subsidised according to the Educational A Standard in Poland multiplied by the P7 factor = 9.5. This meant that over 50,000 PLN was allocated annually to support one pupil over these years. The overall calculation is that the Polish educational spent PLN 2,878,046,876 on children with ASD in 2019, obtained through multiplying the base amount of the educational standard A by the aforementioned P7 factor and the number of children. This amount, however, only includes the cost of education, but there is also the financial burden to consider incurred by the public health and social care systems related directly to the patient's family. The Supreme Audit Office report also emphasises a lack of continuity in supporting adolescents with ASDs and preparing poorly for independent life. Some patients with ASD may be able to cope with living independently after receiving an appropriate treatment, but otherwise, the public finance system bears an additional burden. A study on Australian families estimated that families with autistic children annually incurred AUD 35,100 in 2014; equivalent to around PLN 100,000. Most of this amount was due to a loss of income because of the parent's inability to take up full-time employment [9]. A UK study by Buescher *et al.* [10], of the same year estimated that respectively 2.2 and 1.3 million USD is spent on supporting patients with ASD over their lifespan, both with or without intellectual disabilities. The corresponding amounts for the USA were found to be USD 2.44 million and USD 1.43 million respectively, which indicates a markedly higher cost of supporting autistic persons and also patients suffering also from a comorbid intellectual decline. The emotional burden on the mental health of caregivers is another public health-related problem [11]. Furthermore, the cost-effectiveness of the methods used so far, has not quite been confirmed in few studies available to date [12].

STUDIES ON THE MICROBIOME

There is no effective treatment for ASD at present. As mentioned, current therapy consists of rehabilitation, revalidation and psychological interventions. These are extremely important, but nevertheless their effectiveness remains limited. Psychopharmacology should be used to treat comorbidities or to reduce symptoms like sleep disturbances or irritability. However, any chronic use of antipsychotic medications runs the risk of metabolic complications and premature death [13]. Furthermore, there is no approved drug at present for treating the pivotal symptoms of autism. This has led to a multidisciplinary approach to finding an effective treatment. A new direction has been indicated by studies focused on investigating the links between intestinal microbiota status and mental disorders. The microbiota consists of a community of commensal, symbiotic and pathogenic microorganisms that share our body. The microbiome are the genes of the microbiota, where the number of bacterial genes, (making up the vast majority of microbiota), actually exceeds the number of human genes by 150 times [14]. The human microbiota is involved in many key activities: digestion, nutrition, detoxification, co-constructing

the protective barrier of the immune system and the development and modulation of certain disease states.

It is important to take note that the brain-gut-microbiota axis is a two-way communication system that includes the vagus nerve, the intestinal nervous system (containing 100 to 500 million neurons responding to intestinal microbiota metabolites), the gut-associated lymphoid-tissue (GALT) [15], the hypothalamic-pituitary-adrenocortical axis (HPA), neurotransmitters and neuromodulators produced by the microbiota such as GABA, serotonin, dopamine, noradrenaline, acetylcholine and short-chain fatty acids (SCFAs).

The vagus nerve transmits 90% of signals from the gut to the brain, but only 10% from the brain to the gut. Studies have shown that the many beneficial effects of gut flora transplantation were ended when the vagus nerve was severed [16]. Direct connections with neurons are made by enteroendocrine cells in the intestinal wall (being part of the intestinal nervous system), enabling information to be rapidly transmitted about the intestinal contents via the inferior ganglion of the vagus nerve to the solitary nucleus of the human brainstem. Gut bacteria influence the development of GALT. After birth there are no bacteria present in the digestive system, however, during the first months of the baby's life, the immune system has to learn to distinguish pathogens from harmless entities and between threatening and safe antigens. The first 1000 days of life is also the time when the intestinal microbiota evolve; later on their composition remains relatively stable. The intestinal surface area is 200 m^2 (100 times more than the surface of the skin), and GALT is separated from the intestinal lumen by only one layer of the epithelium, where additionally it has dendritic cells whose projections penetrate the intestinal lumen. The human immune system contacts the external environment mainly in the intestine. It is stimulated by gut bacteria through toll-like-receptors (TRLs), which respond to specific structural elements on the surface of the bacteria called pathogen-associated molecular patterns (PAMPs).

Maternal milk contains spore-oligosaccharides, especially at the beginning of the infant's life, which are of no nutritional value to the infant, but they are a breeding ground for intestinal bacteria and additionally block lectin receptors on pathogenic bacteria, thus preventing them from attaching to the intestinal epithelium. The intestinal microbiota also stimulates the immune system to produce antibodies resulting in high concentrations in the intestinal mucus which constitute a first line of defence. The mucus itself and the commensal bacteria closely adhering to the intestinal epithelium also have a protective function of serving as a barrier which, if efficient and tight, protects intestinal cells against contact with pathogens. Studies have linked macrobiotic programming to the development of allergies, autoimmune diseases as well as obesity. SCFAs produced by the intestinal microbiota induce intestinal epithelial cells to synthesise claudin and occluding proteins that seal both the intestinal barrier and the blood-brain barrier, thereby modulating the immune system response.

The HPA axis is also related to the intestinal microbiome, where the commensal microflora affect the HPA's normal development. Sterile animals (i.e., those microbiota-deficient) have an over-reactive stress axis and excessively excrete corticosterone and ACTH upon being stressed, however this effect is reversible, but only when the intestinal flora is transplanted during an initial short period of 6 weeks [17]. Chronic stress mediated by the HPA axis is significantly reflected, also by the composition of the intestinal microbiota. Recent studies demonstrate that the gut microbiota can not only affect the brain function, but also the structure through regulating the myelination process in the prefrontal cortex [18]. The two-way brain-intestinal-microbiota axis can be additionally modulated by environmental factors such as stress, drugs, (especially antibiotics), and eating habits, whilst dysbiosis leads to an increased intestinal permeability and activation of the pro-inflammatory immune response. A number of mental disorders have also been associated with intestinal dysbiosis, which is considered to be a risk factor and/or a factor limiting an effective treatment of a disease [19].

Faecal microbiota transplantation (FMT) has been a recognised method for treating recurrent *Clostridium difficile* infections since 2013, and has been proven effective in over 90% cases, whereas vancomycin is approximately 40% effective. The effectiveness of vancomycin has been reported in individual studies on improving the health status of the children that significantly regress during autism, where strains belonging to 9 species of the *Clostridium* genus were isolated and bred from the stools of children with autism, however these were not found in control group samples from healthy children [20]. There are numerous studies in progress on the effect of FMT on other diseases, including the neurological and psychiatric, that show great promise [21]. These concern faecal transplantation from depressed people to animals reared in sterile conditions with no intestinal bacterial flora, where these animals acquire depression-resembling symptoms [22]. Similar findings were observed when microbiota were transferred from patients with Parkinson's disease to transgenic animals that caused symptoms of locomotory disorders to intensify, coupled with increased expression of alpha-synuclein in the brain [23]. An open-label study found that autistic subjects significantly improved their symptoms when undergoing FMT from healthy persons, where this effect continued for 2 years after the therapy had been completed [24, 25]. Transplanting gut microbiota from either ASD patients or healthy human controls into sterile-reared mice subjects demonstrated that

the former microbiota caused mice to exhibit repetitive and socially distant behaviour [26].

Nevertheless, studies have shown that the composition of microbiota is inconsistent in healthy and autistic children [27], and it is difficult to compare data or draw conclusions from studies where subjects are heterogeneous in terms of age, symptoms, diet, pharmacological treatment, gastrointestinal problems and family burden. ASD is a heterogeneous group of neurodevelopmental disorders with a strong genetic background. Meta-analyses on monozygotic twins estimate that ASDs coexist at between 96-99% cases, and 53% in fraternal twins [28]. There is poor correlation between genetic test outcomes with family burdens, cognitive, communication abilities and phenotypic characteristics. The emergence of post-genomic concepts in biology has made it worthwhile to consider studying various relationships between the brain-gut-microbiota axis and epigenetic reprogramming. It is difficult to establish a causal relationship between the CNS and the gut microbiota because of the nature of its bi-directional interactions, however the gut microbiota appear to affect behaviour (possibly via gene expression) by producing neuroactive metabolites, suggesting that the brain-gut-microbiota axis may contribute to the pathophysiology of ASD. Treating dysbiosis includes administering vancomycin, transplanting faecal microbiota and giving pre- and pro-biotics. An intervention trial demonstrated improvements in autism when vancomycin was used on a small group of children but without involving a control group [29]. An open-label study has also suggested the efficacy of using faecal microbiota transfer (e.g., [24, 25]).

Current state microbiota data may be useful for planning patient-tailored treatment in autistic children. For example, a study by Pärtty *et al.* [30] randomised 75 infants into receiving *Lactobacillus rhamnosus* or being the placebo group, where at the age of 13 a significant difference was found in the prevalence of neurodevelopmental disorders; ADHD or Asperger's syndrome was diagnosed in 6 (17%) children receiving the placebo in infancy but none from the active test group.

Another option is to use probiotics. The term 'probiotics' can be defined as 'live microorganisms which, when administered in appropriate amounts, exert a beneficial health effect according to the International Scientific Society for Probiotics and Prebiotics (ISAPP). In fact, there are 4 basic criteria that must be met in order for a microorganism to be qualified as a probiotic, as follows:

1) identifying and characterising not only the genus and species, but also the strain;
2) safety of use for the intended target group as confirmed by evidence based studies;
3) documented evidence base on beneficial effect, i.e. at least one study on human subjects;
4) having an appropriate population of microorganisms in a commercially available product at the end of its shelf life.

Current 2020 guidelines set out by the American Gastroenterological Association (AGA) are based on systematic reviews and meta-analyses of randomized trials (287 studies qualified), targeting the USA and Canadian population. This consisted of analysing the effectiveness of probiotics in preventing or treating 8 diseases of the gastro-intestinal tract in children and adults.

A Grading of Recommendations Assessment Development and Evaluation (GRADE) was used to assess the robustness of recommendations and data quality. There are however no clear-cut guidelines for all probiotics on dosing according to the available literature. The duration of treatment as well as the dosage depend on the strain used in any given probiotic. Guidelines for using probiotics assume that a probiotic capsule is administered orally during or 30 minutes after a meal. If swallowing is not possible (e.g., infants), the contents of a capsule can be mixed in with food or cool water.

The presented study aims to review randomised clinical trials on the use of probiotics to evaluate their suitability in reducing the symptoms of ASDs.

METHODS

Randomised clinical trials were accessed from clinicaltrials.gov (on June 27, 2021) and PubMed databases (search: probiotic + autism, probiotic + ASD, probiotic + Asperger syndrome, probiotic + pervasive developmental disorder using the randomised controlled trial filter). Studies were excluded with endpoints not including ASD symptoms. All the relevant studies so found, had been chosen without defining any timeframe. The same PubMed search was then repeated, but for open-label trials. In all, out of the 140 papers found, 5 were open-trials. The search was further supplemented by manually analysing the studies cited by the latest publications on probiotics in autism.

ANALYSIS OF STUDIES

Randomised trials

Studies are summarised in Table 1, where appropriately acquired methodological data on the effectiveness of probiotic intervention in autism are scarce. The following 6 studies were so found:

1) A randomised control trial on 41 children aged 3-6 years diagnosed with ASD [31]. All subjects benefited from therapeutic interventions in the form of Applied Behaviour Analysis (ABA). The study randomly assigned 21 children to the probiotic group (*Bifidobacterium longum*, *Lactobacillus acidophilus*, *Enterococcus faecalis*) and 20 children as controls. Symptom severity was assessed by a parents' questionnaire based on the Autism Treatment Evaluation Checklist (ATEC)

before the study and at after 3 months. The faecal flora was also examined at both time points. Initially, the severity of autistic symptoms did not vary between groups and a statistically significant improvement was observed in both groups after 3 months. Nonetheless, the severity of symptoms (ATEC score) was significantly lower at the endpoint for the test group.

2) NCT02903030 – *Probiotics for quality of life in autism spectrum disorders* [32]. The authors hypothesised that altered host microbial signals, including altered faecal GABA levels (i.e., the neurotransmitter: gamma-aminobutyric acid), are implicated in anxiety and sensory overactivity in ASD. The study proposed a cross-test to assess the relationships between microbiome-mental/physical function in ASD, gastrointestinal dysfunction and anxiety. Subjects were 10/13 children in a randomised trial who managed to complete the entire study cycle. No serious adverse events (SAEs) were found. Over the course of the 19-week study, each score improved from baseline, and the PedsQL (Pediatric Quality of Life Inventory) correlated significantly with *Lactobacillus* abundance with no discernible change in microbiome composition/diversity. Although the probiotic-given group showed improved PedsQL and PRAS-ASD (Parent-Rated Anxiety Scale for ASD) scores compared to placebo, the difference was statistically insignificant; indeed, the authors expected this because of the small sample size. The probiotic with the VISBIOME formula (*Lactobacillus* sp. and *Bifidobacterium* sp.) was found to be safe and showed health benefits in children with ASD symptoms and gastro-intestinal complaints.
3) ACTRN12616001002471 – *Effects of Lactobacillus plantarum PS128 on children with autism spectrum disorder in Taiwan: a randomized, double-blind, placebo-controlled trial* [33]. This was a 4-week, randomised, double-blind, placebo-controlled study assessing the effect of *Lactobacillus plantarum* PS128 on boys with ASDs aged 7-15 years in Taiwan [34]. All subjects qualified for a ASD DSM-V diagnosis and a structured diagnostic interview of autism (ADI-R). Improvements were assessed by several scales: ABC-T (Autism Behaviour Checklist-Taiwan version), SRS (Social Responsiveness Scale), CBCL (Child Behavior Checklist), SNAP-IV (Swanson, Nolan, and Pelham Rating Scale, designed to assess ADHD symptoms and oppositional-defiant behaviour) and the commonly used CGI-S and CGI-I in psychiatry (General Clinical Impression Scale, Clinical Global Impression Scale – Severity and Clinical Global Impression Scale – Improvement). There were no significant differences at baseline nor endpoint between test subjects (n = 36) and controls (n = 35, placebo group) in any of the test scales. Nevertheless, further analyses demonstrated some significant differences between starting and endpoints within the test or control groups. The subscale of CBCL externalising behaviours (one of the 10 identified in this tool), found such differences to be significant in the placebo, whereas the PS128 test group showed significant differences in one of the five ABC-T subscales (body and object utilization) as well as the SRS total score, but none in any of the four subscales. Likewise, changes between baseline and end point were also statistically significant for two of the ten CBCL subscales as well as for the total score and one of the three subscales of the SNAP-IV tool. The effectiveness of the PS128 intervention appeared to be age dependent, with better effects seen in young than in older children. It was concluded that *Lactobacillus plantarum* PS128 can alleviate some of the symptoms of autism, mainly those related to destructive-type behaviour, breaking rules and hyperactivity/impulsiveness. Despite multiple comparisons, the authors did not, however, apply the Bonferroni correction. It should also be noted that there is a discrepancy between the abstract conclusion, in which the authors reported the presence of differences between the placebo and active interventions, and the numerical data presented in the text.
4) Another trial was the NCT02708901 entitled *Effects of probiotic supplementation on gastrointestinal, sensory and core symptoms in autism spectrum disorders: a randomized controlled trial* [35]. Subjects were 85 children aged 1.5 to 6 years and the test probiotic, included *Streptococcus thermophilus*, *Lactobacillus* sp. and *Bifidobacterium* sp.; a preparation readily available on the market.

Changes in the severity of autism symptoms were assessed by a battery of tests, including ADOS-2 (Observation protocol for diagnosing autism spectrum disorders ADOS-2) on the Calibrated Severity Scores (CSS) scale. There were no differences found between the test group (n = 42) and placebo (n = 43). Further analyses however revealed that when the groups were alternatively divided into 4 arms, based on the presence or absence of gastrointestinal complaints, then the latter demonstrated that probiotics had a significantly greater improvement in the subscales for receptive, home, and coping skills of the Vinelandian Adaptive Behaviour Scale (VABS-II). Significantly more children taking the probiotic also achieved normal scores in the Multisensory Processing subscale of the Sensory Profile questionnaire. In contrast, those without gastrointestinal symptoms, had decreased intensities of symptoms assessed by ADOS-CSS decreased in subjects receiving the probiotic but were increased in the placebo group. This also applied to the Social Affect subscale of this test.

Table 1. Randomised control studies on using probiotics in autism based on the clinicaltirals.gov registry (accessed June 27, 2021) and the PubMed database

Study code	Test treatment	Study group	Publication progress	Number of subjects	Outcomes
Not applicable	ABA + probiotic (*Bifidobacterium longum, Lactobacillus acidophilus, Enterococcus faecalis)* vs. ABA + placebo	Children with ASD aged 3-6 years	Completed and published (Liu *et al.*, 2021)	41	Both groups improved; test group endpoint had lower symptom severity on the ATEC scale; details in the text
NCT03337035	Phase 1: *Lactobacillus planarum* vs. placebo Phase 2: Additional oxytocin in both groups	Subjects with ASD aged 3-25 years	Completed and published as a pilot study by Kong *et al.*, 2021	35	The changes between initial and study endpoints (according to some of the defined parameters) of Phase 2; details in the text
NCT02086110	Phase 1: Bovine colostrum oligosaccharides vs. bovine colostrum oligosaccharides + *Bifidobacterium infantis* for 5 weeks; following a 2-week break, interventions were switched	Children aged 2-11 years diagnosed with ASD and gastro-intestinal symptoms	Completed and published (Sanctuary *et al.*, 2019)	11; subjects, of which 9 completed and 8 were analysed	Study aimed to assess treatment tolerance according to a number of biological parameters; significantly improved Aberrant Behavior Checklist range observed when taking the prebiotic without a probiotic; details in the text.
NCT03514784	*Lactobacillus rhamnosus* and *Bifidobacterium* sp.	Children aged 4-16 years exhibiting gastro-intestinal symptoms	Ongoing recruitment	Planned at 70	Study in progress
NCT04939974	*Lactobacillus* sp. and *Bifidobacterium* sp.	Children with ASD aged 2-18 years	Study not yet started	Planned at 100	Study not yet started
NCT02903030	*Lactobacillus* sp. and *Bifidobacterium* sp.	Children with ASD aged 3-12 years exhibiting gastro-intestinal symptoms and anxiety	Completed and published (Arnold *et al.*, 2019)	13	Advantageous safety profile; details in the text
NCT02708901	*Streptococcus thermophilus*, *Lactobacillus* sp. and *Bifidobacterium* sp.	Children with ADS aged 1.5-6 years	Completed and published (Santocchi *et al.*, 2020)	85	No differences between intervention group and placebo; significant differences in subgroups found; children with or without gastrointestinal symptoms showed improvement in some indicators; details in the text
NCT03369431	*Streptococcus thermophilus*, *Lactobacillus* sp. and *Bifidobacterium* sp.	Children with ADS aged 3-16 years exhibiting gastro-intestinal symptoms	Completed	69	Results are unavailable
NCT04655326	Individually selected based on microbiota testing	Sun-Genomics customers aged 2.5-75 years with ASD	Recruitment in progress	Planned at 100	Study in progress
NCT03982290	*Lactobacillus planarum*	Children with ASD aged 2.5-7 years	Information unavailable	Planned at 250	Information unavailable

Table 1. Cont.

Study code	Test treatment	Study group	Publication progress	Number of subjects	Outcomes
NCT04293783	*Lactobacillus reuteri*	Children with ASD aged 1.5-8 years	Recruitment in progress	Planned at 80	Study in progress
Registered on Australia and New Zealand research database ACTRN12616001002471	*Lactobacillus planarum*	Boys with ASD aged 7-15 years	Completed and published (Liu *et al.*, 2019)	71	No differences between test intervention group vs. the placebo; significant changes in test group seen between starting and endpoints according to some assessed indicators; details in the text
Registered on the Chinese Clinical Trials Registry no. ChiCTR1900023609	All patients under therapeutic care (ABA); in addition 6 strains of bacteria vs. placebo for 4 weeks 9	Children with ASD aged 3-8 years	Completed and published (Niu *et al.*, 2019)	65	Reduced ATEC questionnaire-assessed symptoms in probiotic group; details in the text
No registration information Non-indexed publication in PubMed	*Lactobacillus plantarum* Cross-over study; 3 weeks intervention (test intervention vs. placebo), 3 weeks off, 3 weeks on second intervention	Children with ASD aged 3-16 years	Completed and published (Parracho *et al.*, 2010)	62 subjects started but only 17 completed	Improvement in the Development Behaviour Checklist scale on overall score, both with probiotic and placebo, and also in subscales when using a probiotic

ABA – applied behaviour analysis, ASDs – autism spectrum disorders, ATEC – Autism Treatment Evaluation Checklist

5) The NCT02086110 study *Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms* [36] evaluated a combination of a prebiotic + probiotic vs. the prebiotic alone on 8 child patients in a cross-over study randomised according to the above treatments and the order that they were given. Subjects were aged 2-11 years old, diagnosed with ASD and gastrointestinal symptoms. The severity of autism symptoms was assessed using the Aberrant Behaviour Checklist (ABC), the Repetitive Behaviour Scale-Revised (RBS-R) and the Adaptive Behaviour Assessment System-Second Edition (ABAS-II). Significant improvements during the treatment were observed for the ABC scale when the prebiotic was used alone.
6) A study from 2010 [37], but not indexed in the PubMed database, assessed 17 children and cross-overed each subject in taking the test intervention (*Lactobacillus plantarum*) and the placebo. The severity of autism symptoms was assessed using the Development Behaviour Checklist. The overall score improved with both probiotic and placebo treatment, as shown in the subscale results during when the probiotic was administered.

Open trial studies

In 2012, a Kałużna-Czaplińska *et al.* [38] study was performed on 22 children aged 4 to 10 who had received a probiotic (*Lactobacillus acidophilus*) for 2 months. The study aimed to measure D-arabinitol concentrations and the ratio of D- to L-arabinitol in the urine. The authors also showed, however, that some children improved in the following areas: the ability to concentrate, maintain eye contact, follow requests and understand other people's emotions. Nevertheless, there are no details on how these features were assessed, thereby making any interpretation impossible.

In 2018, Shaaban *et al.* [39], presented an open-label study on 30 children aged 5 to 9, diagnosed with ASD, who received a probiotic (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacteria longum*) for 3 months. The severity of symptoms was assessed by the ATEC scale in the study period, which significantly improved in terms of the total score and the subscales of the questionnaire. Beneficial changes observed, also included gastrointestinal symptoms, the faecal microbiome and body weight.

A study by Mensi *et al.* [40] on autistic children aged 7.2 ± 3.5 years assessed 105 patients taking *Lactobacillus planarum* (LP) and 26 others on various probiotics (OP). The CGI-S (Clinical Global Impression-Severity) scale was used to assess function at baseline (before taking probiotics) whilst the CGI-S and CGI-I (Clinical Global Impression – Improvement) were used after 6 months. Other forms of therapy were additionally used according to standard practice. At baseline, the test groups differed in the severity of symptoms according to the CGI

scale, where they were less severe in the LP group and also in terms of gastrointestinal symptoms. Children from both groups showed an improved health condition. The LP group had less severe symptoms at the end point of the study according to the CGI scale, which is hardly surprising, but there were greater improvements according to CGI-I. Side effects were more frequent in the OP group, $p = 0.059$.

The Study No. ChiCTR1900023609 entitled *Characterization of intestinal microbiota and probiotics treatment in children with autism spectrum disorders in China* enrolled 114 ASD children aged 3 to 8 years, of whom 37 received ABA + probiotic intervention, whilst 28 only received therapeutic ABA intervention. Improvements were observed in all subscales of the probiotic group and in the total ATEC score, however there was no effect in the ABA-only subgroup. Nonetheless, the paper does not describe how the randomisation and blinding were performed. A study by Ray *et al.* [41] is not indexed in PubMed but has been cited by other papers and was conducted on children aged 4-15 with autism and immunodeficiency (without details given on participants inclusion criteria). Participants were administered a preparation of probiotics via lysed cell walls. The severity of autism symptoms was assessed using the ATEC scale. After 3 weeks of treatment, a statistically significant improvement was observed in the total ATEC score as well as in the subscales of this questionnaire. When the preparation was discontinued, a recurrence of previous symptoms was reported after 2 weeks; however, there was no data shown.

CONCLUSIONS

Studies on the use of probiotics in children and adolescents diagnosed with autism are still few and far between. Their interpretation is hindered by the small size of the study groups and methodological flaws (including not properly dealing with the statistical problems posed by multiple comparisons). An undoubted advantage of using probiotics is the favourable tolerance profile. Despite their imperfections, these studies indicate reduced symptoms of autism during probiotic treatment. Additional research is still needed on specific indications, duration of treatment and the effectiveness of interventions in the problem areas of relevance.

Conflict of interest

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Clinical trials

1. ClinicalTrials.gov Identifier: NCT02903030 – *Probiotics for quality of life in autism spectrum disorders.* L. Eugene Arnold, 2019.
2. ClinicalTrials.gov Identifier: NCT02708901 – *Effects of probiotic supplementation on gastrointestinal, sensory and core symptoms in autism spectrum disorders: a randomized controlled trial.* E. Santocchi, 2020.
3. ClinicalTrials.gov Identifier: NCT02086110 – *Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms.* E. Sanctuary, 2019.
4. ClinicalTrials.gov Identifier: NCT03514784 – *Combination probiotic: BB-12 with LGG (different doses) in treating children with autism spectrum disorder*. J. Marc Rhoads, The University of Texas Health Science Center, Houston (in process).
5. ClinicalTrials.gov Identifier: NCT04939974 – *Probiotic in autism*. S. Gulati, All India Institute of Medical Sciences, New Delhi (in process).
6. ClinicalTrials.gov Identifier: NCT03369431 – *Efficacy of Vivomixx on Behaviour and gut function in autism spectrum disorder (VIVO-ASD)*. University College, London (no data).
7. ClinicalTrials.gov Identifier: NCT04655326 – *Probiotic therapy for children and adults with autism spectrum disorder*. J.B. Adams, Arizona State University (in process).
8. ClinicalTrials.gov Identifier: NCT03982290 – *Psychophysiological effects of Lactobacillus plantarum PS128 in preschool children with autism spectrum disorder.* H.J. Chen, Mackay Memorial Hospital (in process).
9. ClinicalTrials.gov Identifier: NCT04293783 – *Randomized double-blind clinical trial with L. reuteri supplementation in children with autism spectrum disorder*. L. Mazzone, University of Rome Tor Vergata (in process).