

## Use of probiotics with lactobacillus to evaluate decreased motor stereotypies in children with moderate and severe autism.

## Uso de probióticos con lactobacillus para evaluar disminución de estereotipias motoras en niños con autismo moderado y severo.

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### Abstract:

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that has recently been linked to the gut microbiota based on the new biological model of the gut-brain axis. The imbalance of the microbiome has been proposed as one of the underlying causes of the disorder and that is directly related to behavior. The following research aimed to evaluate the effect of orally administered lactobacilli on motor stereotypies of early childhood children with moderate and severe autism. A quantitative study with an explanatory scope was developed based on a single case study with an A-B design in 2 stages. Independent variable: intervention with lactobacilli probiotics. Dependent variable: behavior (motor stereotypies). Data collection was carried out through interviews, diagnostic instruments of the disorder and visual inspection records of recordings. The decrease in motor stereotypies in participants was statistically significant in the treatment phase. In the evaluations, a decrease in disruptive behaviors was reported, as well as an improvement in verbal and non-verbal communication based on their individualities, as well as greater flexibility in eating routines and activities. It was concluded that the lactobacilli intervention was safe and can modify the motor stereotypies of children with moderate and severe autism.

### Keywords:

Autism, behavior, intestinal microbiota, probiotics, stereotypies.

### Resumen:

El Trastorno del Espectro Autista (TEA) es un trastorno del neurodesarrollo que se ha relacionado recientemente con la microbiota intestinal a partir del nuevo modelo biológico eje intestino cerebro. El desequilibrio del microbioma se ha planteado como una de las causas que subyacen al trastorno y que se relaciona directamente con la conducta. La siguiente investigación tuvo como objetivo evaluar el efecto de los lactobacilos de administración oral sobre las estereotipias motoras de niños en infancia temprana con autismo moderado y severo. Se desarrolló un estudio cuantitativo y alcance explicativo a partir de un estudio de Caso Único con Diseño A-B, desplegada en 2 etapas. Variable independiente: intervención con probióticos de lactobacilos. Variable dependiente: conducta (estereotipias motoras). La recogida de datos se realizó mediante entrevistas, Instrumentos de diagnóstico del trastorno y registros de inspección visual de grabaciones. La disminución de las estereotipias motoras en los participantes fue estadísticamente significativa en la fase de tratamiento. En la evaluación se reportó disminución de conductas disruptivas, así como mejoría de la comunicación verbal y no verbal a partir de sus individualidades además de mayor flexibilidad en rutinas alimentarias y de actividades. Se concluyó que la intervención con lactobacilos fue segura y puede modificar las estereotipias motoras de niños con autismo moderado y severo.

### Palabras Clave:

Autismo, conducta, microbiota intestinal, probióticos, estereotipias.

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## INTRODUCTION

Autism Spectrum Disorder (ASD) is one of the neurodevelopmental disorders whose prevalence has increased in recent decades, either due to changes in diagnostic criteria or greater recognition by the health sector. The World Health Organization (2023) reported that 1 in 100 infants are on the autism spectrum (ASD). This condition has a multifactorial etiology; however, recent empirical evidence has linked ASD and the gut microbiota based on the microbiota-gut-brain axis as a new explanatory model that integrates neuroscience and microbiology to evaluate the gut microbiome as an underlying cause of this disorder. Recent research suggests that an imbalance in the gut microbiota (dysbiosis) may be a predisposing factor in the genesis, clinical manifestations, and severity of ASD (Polykarpou, 2021; Xu, 2019).

Organisms with intestinal dysbiosis present intestinal permeability with increased propagation of lipopolysaccharides that travel via the bloodstream to areas of the brain such as the amygdala, acting as proinflammatory endotoxins and affecting related activities such as affectivity, social behavior regulation, and the fight-or-flight response generated by environmental stimuli (Mehra et al., 2022; Porges, 2020). Likewise, the gut microbiota has a regulatory function on the immune system, so a dysfunction in this system causes an alteration in the release of interleukins and tumor necrosis factors that produce changes in the brain and in neuroactive molecules such as dopamine, histamine, serotonin, and GABA (gamma-aminobutyric acid) involved in motor activity, motor inhibitory control, learning, memory, cognition, and other functions that are altered in the disorder (Mehra et al., 2022).

In another sense, a prevalence of functional gastrointestinal symptoms in infants with the disorder of up to 69% has been described, with diarrhea, constipation, borborygmi, and more severe intestinal inflammatory processes being more common in them than in other neuropsychiatric populations, which could be related to intestinal dysbiosis and restrictive diets that generate changes in the microbiome and, therefore, these symptoms (Caycho et al., 2023; Herrera et al., 2022; Lashera et al., 2023). These intestinal conditions can cause discomfort and disruptive behaviors such as self-harm or harm to others, tantrums, etc., especially in those who find it difficult to communicate their discomfort (Madra et al., 2021).

In this regard, over the last five years, interventions have been implemented using probiotics as a therapeutic target to achieve microbiota balance and thereby bring about changes in both behavior and gastrointestinal symptoms (Ramos et al., 2022; Rodenas et al., 2021). This research has been carried out in pediatric patients diagnosed with autism, over periods ranging from 1 to 7 months, during which probiotics, generally lactobacilli, bifidobacteria, and enterococci, have been used, either in isolated strains or multi-strains, and in some cases in combination with other therapies such as oxytocin, bovine colostrum, or in conjunction with Applied Behavior Analysis. As a result, they reported positive effects on the gut microbiota and clinical expressions of autism, with a lower frequency of disruptive behavior, irritability, oppositional defiance, motor stereotypies, and adaptive improvements. They have even planned changes in the frontal lobe of the electroencephalogram for beta and gamma waves that influence attention and motor

activity. None of these studies reported serious adverse events, only mild abdominal discomfort in some cases without the need for hospitalization (Arnold et al., 2019; Kong et al., 2021; Niu et al., 2021; Li et al., 2021; Liu et al., 2021; Sánchez, 2020; Sanctuary et al., 2019).

One of the earliest core symptoms of ASD is motor stereotypies, which, when performed, focus attention on themselves and therefore interfere with social interaction and learning. These occur in more than 70% of infants and are directly proportional to the severity of the disorder, so controlling this behavior will benefit their daily activities. It has often been suggested that it is not appropriate to suppress stereotypical behaviors, as in many cases they are a form of communication or a way of coping with sensory overload. However, when they get out of control, they can have an impact on interaction and learning (De la Peña et al., 2021; Loyacono et al., 2020). These types of interventions with psychobiotics (probiotics and prebiotics) are relevant because they are simple, can be carried out by the family, and are inexpensive, which helps to reduce recurrent hospitalizations due to gastrointestinal conditions or the consequences of these conditions on behavior, which could even lead to self-harm (Marzet et al., 2022).

Lactobacilli and bifidobacteria have been extensively tested in pediatric patients and are approved for their high safety profile, placing them on the list of safe microorganisms according to the Qualified Presumption of Safety of the European Food Safety Authority (Shaaban et al., 2018), being those with the greatest recognized benefits (Sanctuary et al., 2019; Arnold et al., 2019; Liu et al., 2019; Niu et al., 2021; Cooper, 2020).

Therefore, the objective of this study was to evaluate the effect of using probiotics with lactobacilli in reducing motor stereotypies in children with moderate to severe autism.

## METHODS

A quantitative research study with an explanatory scope was conducted based on a Single Case study with an A-B Design, carried out in 2 stages, where each participant served as their own control and the variable was assessed in 2 phases: initial baseline (A) and use of probiotics with lactobacilli (B). For ethical reasons, no reversal method was used. The study period was January to December 2024 at the Autism Care Center (ATREA) in Pachuca de Soto. The sampling was non-probabilistic and intentional, with a final total of three participants who met the selection criteria and gave their consent. Infants between the ages of 2 and 6 with moderate to severe autism who were authorized to participate were included. Infants with a history of other neurodevelopmental conditions according to DSM-V criteria, a history of food allergies or intestinal pathologies, or those who had used antibiotics or probiotics in the 6 months prior to the study were excluded. The exclusion criteria were any type of morbidity during the intervention that made it impossible to continue, the decision not to continue with the study, or discontinuation of lactobacillus use for 30 consecutive days. Motor stereotypies were evaluated as a dependent variable throughout the two phases.

In phase A, medical records were reviewed based on selection criteria, semi-structured interviews were developed, and Claudia Ocampo, as the study's support network, administered

the Questionnaire for the Diagnosis of Autism Spectrum Disorders (CRIDI\_TEA) and the Autism Spectrum Assessment Instrument for Mexican Children (VEANME), as well as weekly video recordings for 5 weeks for the baseline of behavior and 12 weeks in the treatment phase, with a duration of 40 minutes in the play and demand condition. The video recordings were visually inspected by three trained observers, and the data were analyzed by Observer Agreement, as this is the most common indicator used in applied behavior analysis, with an accepted index of over 80% (Lekue, 2020).

The lactobacilli administered in phase B were of the rhamnosus and acidophilus strains, in chewable tablet form due to the characteristics of these children, which made other forms of administration difficult. These contained 10<sup>(11)</sup> colony-forming units (CFU) per tablet, which was a previously used therapeutic dose that also considered possible loss during chewing (Bautista et al., 2022). The administration was daily for 3 months, during which time the recordings were kept. The type of data analysis was through visual inspection of the recordings, evaluating the frequency of the behavior under study as a descriptive measurable unit, and they were represented in line or split diagrams to analyze them using the Split Middle Technique to determine the significance of the intervention (Rodas, 2024).

The research was submitted to the Ethics Committee of the Institute of Health Sciences at the Autonomous University of the State of Hidalgo with approval letter 248/2024 (Appendix 10). The corresponding provisions of the Health Law Regulation on health research of the Ministry of Health were applied, according to Title II on the ethical aspects of research in human beings, Chapter I, Articles 16 and 17 (Category II or minimal risk) (De La Madrid, 2014).

## RESULTS

The data obtained from the interviews and medical records are presented below. The three participants in the study were male, which is consistent with the prevalence of autism in males (Andreo et al., 2019). They were between the ages of 4 and 6 and came from nuclear, functional families. In all three cases, they were diagnosed with ASD before the age of 3, so early therapies were started and they already had a well-defined ASD profile, which is positive because early intervention using different proven methods positively exploits their potential and functionality.

The results of the neuropsychological assessment scores for CRIDI and VEANME in each infant, before and after the use of lactobacilli, are presented below. Table 1 shows the results of the application of the VEANME instrument for the three participants, pre- and post-use of lactobacilli.

**Table 1**

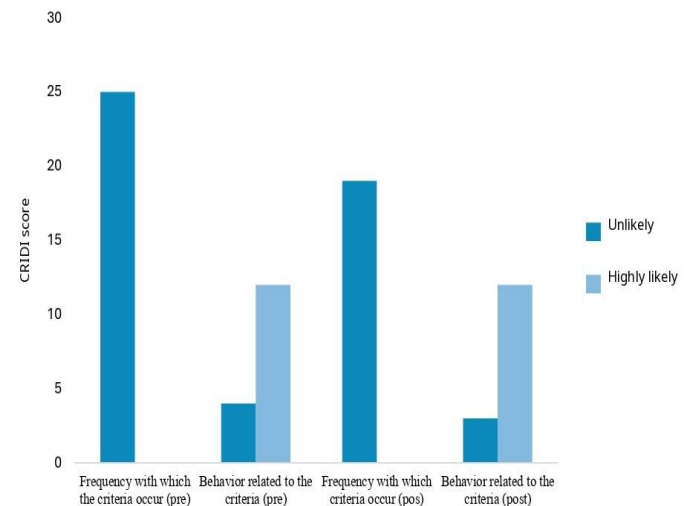
*Assessment results of the autism spectrum in Mexican children (VEANME) in pre- and post-intervention phases*

Cases	Pre Intervention	Post Intervention	Cut-off score
Case 1	22	20	17
Case 2	28	27	17
Case 3	33	31	17

Case 1 showed a decrease in scores on the CRIDI\_TEA instrument in the post-test, both for behaviors related to the criterion and for the frequency with which they occur, as well as a decrease of 2 points on the VEANME re-test instrument (Figure 1 and Table 1).

**Figure 1**

*Total scores for criterion Social Communication and Repetitive Behaviors and Restricted Interests for Case 1, before and after the use of lactobacilli*



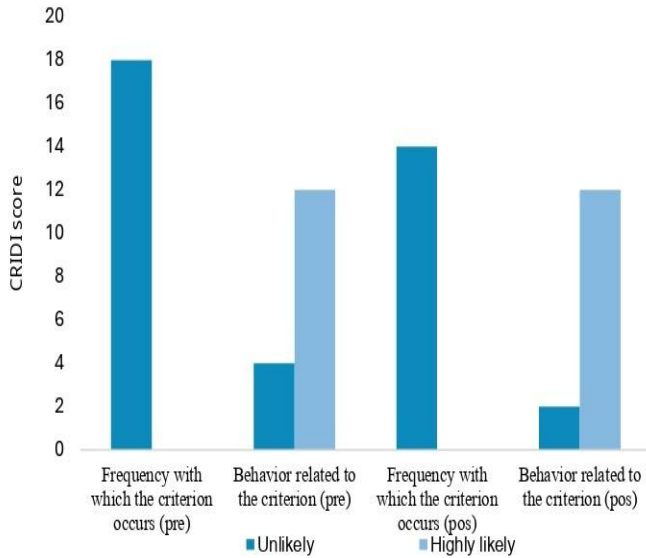
The qualitative description of the post-intervention instruments showed an increase in role-playing and the integration of other people into these roles, so that during these activities, the participant was observed to share emotions with others and to be tolerant of sharing with others. Recognition of expressions of sadness was reported, and in turn, the participant was interested or concerned, this being a new adaptive behavior for the participant. In relation to language and communication, he made proto-imperative gestures, and although he did not achieve proto-declarative communication during the study, the family reported that he was in the process of doing so, as he pointed to objects, although he did not seek to communicate with others. In terms of repetitive behaviors and restricted interests, there was more noticeable flexibility in changes in routines and

Acceptance of touching new objects. Infrequent motor stereotypies remained but were reported to be shorter in duration. In sensory exploration, he tolerates noises, crunchy and viscous foods more easily, incorporating nopal, soups, cereals, broccoli, and others.

In case 2, there was a quantitative decrease in the post-use phase of lactobacilli, in the scores of both instruments for the frequency of presentation of the criteria and related behaviors. In the qualitative description, it incorporated proto-imperative signalling, flexibility to routines if warned, improved mood, tolerance to frustration, and parents reported that he understood short commands more easily and, although he maintained motor stereotypies, they were less frequent. In the sensory sphere, there were changes that the family reported as significant in the incorporation of different foods into the diet, flexibility to textures and smells, adding avocado, legumes, cold cuts, roasted and ground meat to his diet (Figure 2 and Table 1).

**Figure 2**

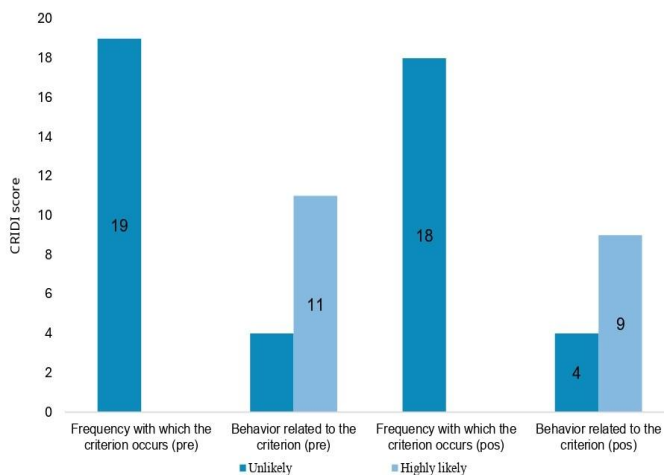
Total scores for criterion Social Communication and Repetitive Behaviors and Restricted Interests for Case 2, pre- and post-use of lactobacilli



In case 3, there was a quantitative decrease for both instruments, although less than for the other participants. The family reported that it was easier for others to interpret his gestures and that he showed changes in relation to objects and their use, as well as in their manipulation. Less motor activity was reported, although stereotypical behaviors persisted and greater flexibility was observed in routines and eating habits, incorporating corn tortillas, pork rinds, mixiotes, and textures that were previously not tolerated, with a considerable reduction in the pica behaviors that had been present since diagnosis (Figure 3 and Table 1).

**Figure 3**

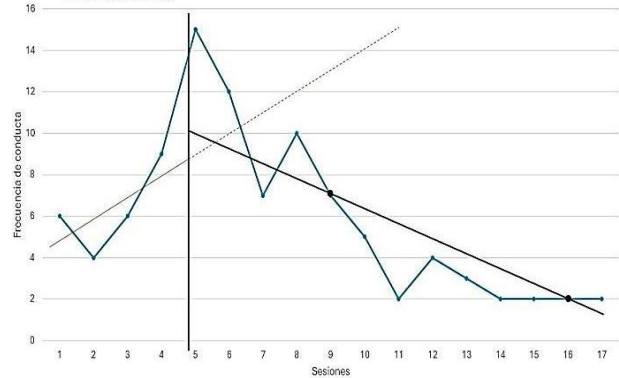
Total scores for criterion Social Communication and Repetitive Behaviours and Restricted Interests for Case 3, pre- and post-use



The figures shown below display the graphical representation of the behavior under study (motor stereotypes) for each participant.

**Figure 4**

Graphical record of stimming behavior in Case 1

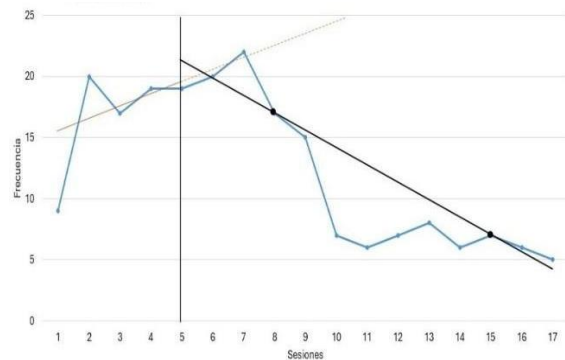


Level change: 7.5 LB slope: 0.5 Treatment slope: 3.5 Slope change: 7

An initial 20-minute recording was used to define which behavior was most frequent in each of them, so that for Case 1 (Figure 4), stimming behavior was evaluated, for Case 2 ((Figure 5 and 6), stimming and finger movements were evaluated, and for Case 3 (Figure 7), hand agitation behavior was evaluated.

**Figure 5**

Graphical record of stimming behavior in Case 2.



Level change: 3.8 LB slope: 0.9

Treatment slope: 1.08 Slope change: 1.2

Table 2 below shows the analysis of the level and slope of the data record obtained from the video recordings, as well as their changes in both the baseline and treatment phases with respect to motor stereotypes. It was processed using the Split Middle Technique for significance.

**Table 2**

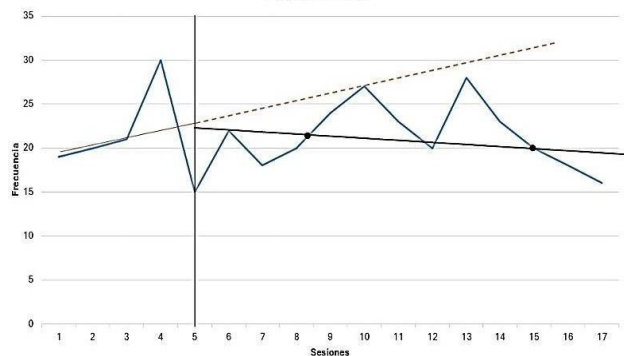
Analysis of motor stereotypy behavior indicator data for each participant at Baseline (LB) and intervention (Tx).

Cases	Behaviors	Level (Baseline)	Level Tx	Change level	Slope (Baseline)	Slope Tx	Change slopes
Case 1	Stimming	15	4	3.75	0.5	2	4
Case 2	Stimming	19	5	3.8	0.9	1.08	1.2
	Fingers movement	15	16	1.07	0.8	1.1	1.3
Case 3	Stimming	33	25	1.32	1.07	1.12	1.04

In Case 1, a 50% gain was observed in the intervention phase, with a mean frequency that decreased from 8 to 4 in the baseline and intervention phases, respectively. Changes in slope and level were obtained as shown in Table 2, with a significance of  $p=0.001$ , which is statistically significant.

**Figure 6**

Graphical record of the finger movement behavior of Case 2



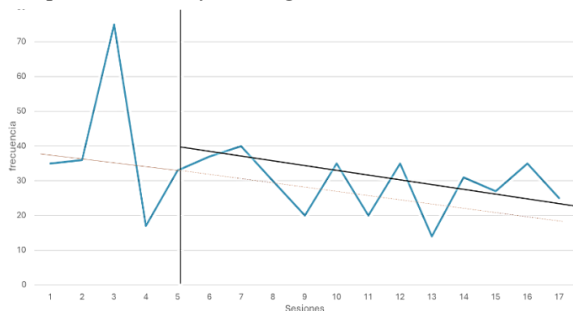
Level change: 0.9 LB slope: 0.8

Pending Treatment: 1.1 Change in slope: 1.3

In Case 2, the mean frequency of the stimming behavior was 15.8 to 11.6 in phases A and B, respectively, for a gain of 26.6%, with significance at baseline of  $p=0.001$  and changes in level and slope.

**Figure 6**

Graphical record of hand agitation behavior in Case 3.



Level change: 1.32 LB slope: 1.07  
Treatment slope: 1.12 Slope change: 1.04

On the other hand, the change in finger movement behavior was smaller, with similar frequency in both phases, but results below the baseline projection line, a change in slope, and significance  $p = 0.0002$  in the intervention phase, showing statistically significant changes.

In case 3, a level change of 1.32, a slope change of 1.04, and significance in the intervention phase of  $p=0.0006$  were observed, with a gain in the intervention phase for the frequency of hand-wringing behavior of 31%.

In addition, indices were calculated to evaluate the magnitude of the therapeutic change or the effect size of the most frequently used treatment, such as PEM (percentage of data exceeding the median) and NAP (non-overlap of all pairs) (Sanz and García, 2015), as shown in Table 3.

**Table 3.**  
Case Comparison

Participant	PEM (%)	Effectiveness	NAP (%)	Effect
Case 1	76.0	Moderately effective treatment	74.2	Medium effect
Case 2	Fingers torsion (58.3) stimming (83.3)	Questionable or ineffective treatment Moderately effective treatment	Fingers torsion (41.8) stimming (78.7)	Weak effect Medium effect
Case 3	58.0	Questionable or ineffective treatment	64.2	Weak effect

**DISCUSSION**

ASD is a multidimensional condition linked to numerous risk factors for its onset, but its root cause remains unknown. However, it is understood to be heterogeneous in nature, and the term "spectrum" refers precisely to the individuality of each person within the disorder. Considering this, it has been approached from different scientific disciplines, most frequently cognitive-behavioral therapies, pharmacological

treatments for the control of depression, psychosis, hyperactivity, and other disorders associated with it, occupational therapies, applied behavior analysis programs, and others with proven results of varying intensity. Therefore, in the search for other therapeutic avenues, the new explanatory model of the gut-brain axis and the impact of the gut microbiome on human behavior and on autism as a neurodevelopmental disorder is beginning to be analyzed. Different multidisciplinary fields then began to propose symbiotics (probiotics and prebiotics) as an alternative treatment, modulating the gut microbiota and behavior in ASD, as a correlation was established between the high prevalence of gastrointestinal symptoms in ASD and its microbiota and the severity of the disorder (Bustos et al., 2022; Moreno et al., 2020; Nova et al., 2022; Sanctuary et al., 2019).

Considering this evidence, the present study was conducted at an Autism Care Center (ATREA) in the town of Pachuca, Hidalgo, with the aim of evaluating the effectiveness of the use of probiotics with *Lactobacillus rhamnosus GG* and *acidophilus*, in a composition of 10<sup>11</sup> or ten trillion CFU in a chewable tablet form to assess whether there was a decrease in motor stereotypies in children, which are highly prevalent and interfere with their daily lives when excessive.

ASD has a high prevalence of unpleasant and frequent digestive symptoms that sometimes require medication to control. Recent research has linked both their rigid eating patterns and their altered gut microbiota and secondary aberrant immune function (Marzet et al., 2022; Sanctuary et al., 2019; Wong et al., 2024). The present study coincides with the findings of these investigations, as it was observed that the symptoms of abdominal distension, diarrhea, and constipation predominated in the population evaluated, with a reported hyperselectivity towards foods such as sweets and flour. However, after the use of lactobacilli, flexibility was achieved in terms of the textures, smells, and flavors of foods incorporated into the diet that were previously rejected. As a result, changes in stool habits and consistency began to occur, coinciding with other studies (Arnold et al., 2019; Niu et al., 2019; Sanctuary et al., 2019). Although it was not the objective of the research, changes in diet were reported by families as a fundamental change.

Changes in their diet could be justified by the probable change generated in the intestinal microbiome with the use of lactobacilli and the balance that probiotics can cause at this level, since restrictive diets can cause intestinal dysbiosis and permeability with the release of endotoxins. However, the influence of probiotics on the intestinal inflammatory process has an effect on the cerebral amygdala, creating positive effects on behaviors related to it, such as salience, motor activity, tactile and gustatory sensitive areas, salience, and other effects caused by the controlled activity of neuroactive metabolites in terms of emotions, communication, mood, and other functions that influence behavior, diet, and intestinal inflammation, constipation, diarrhea, and other symptoms present in infants with ASD, coinciding with a 2019 study that found that the positive effect of probiotics on gastrointestinal symptoms in autistic individuals improved their scores on instruments that assessed quality of life in this population (Arnold et al., 2019; Mehra et al., 2022).

Considering the evidence, this intervention evaluated the behavior of motor stereotypies in children after the use of

probiotics with the aim of reducing them based on what was reported in the evidence. The bacilli were consumed for 12 weeks, during which visual inference of behavior was maintained, and a 5-week behavioral baseline was recorded prior to treatment. The behavioral indicator of motor stereotypies was evaluated because they have a prevalence of 70% in ASD and their impact on children's learning and attention (De la Peña et al., 2021; Loyacono et al., 2020).

In the behavioral profile at the end of the treatment phase, a statistically significant decrease in motor stereotypies was observed in the video recordings, although not with the same intensity across the entire sample. In the evaluation of the VEANME instrument and the CRIDI TEA questionnaire, at the end of treatment, there was a reported decrease in motor activity in stimming, spinning objects, and spinning around, as well as other behaviors considered stereotypical in the given context, and a decrease in the duration of these behaviors. This coincides with research that found decreased motor activity after modification of the gut microbiota in ASD (Mc. Carty, 2021; Li et al., 2021; Sanctuary et al., 2019).

Although several studies reported improvements in the severity of the disorder, in the present study no variations in the classification were observed in the neuropsychological interview (Cooper, 2020; Liu et al., 2019; Niu et al., 2019; Sánchez, 2020).

Stereotypies are one of the central symptoms of the disorder and have different reasons for occurring depending on the context. They can be emotional regulators, a means of self-stimulation, and even communication, depending on the severity of the ASD, to which they are directly proportional. Considering this, they should not always be suppressed, as they could be an expression of sensory overstimulation without leading to a crisis, or of under-stimulation, and could be a way of feeling, so that sometimes stereotypies are a means of balance, communication, pain, and others. However, when these behaviors are excessive, they can interfere with learning, interaction with the environment, or be an inappropriate way of stimulating oneself that could trigger self-injury if they are maintained and of high intensity (De la Peña, 2021; Schmitt et al., 2023).

Previous studies have reported better results in this population in terms of oppositional and defiant behaviors, disruptive behaviors, and hyperactivity in the instruments they applied. In line with these results, even though the present study evaluated stereotypical behavior in children with autism, parents perceived more intense changes in disruptive behaviors such as tantrums, throwing objects, and flexibility in routines and eating, incorporating foods that they previously did not accept (Liu et al., 2019; Niu et al., 2019; Sanctuary et al., 2019).

In another sense, other studies evaluated these probiotics from the perspective of multisensory processing and adaptive functioning in terms of communication, activities of daily living, and social skills, where improvements were reported after treatment. These results coincide with the present study, as positive results were obtained in language and communication in the participants according to their own characteristics, with gains in proto-imperative and declarative pointing, longer sentences, better understanding by the family of what was expressed by the verbal autistic infant, as well as the onset of

pointing, babbling, and better understanding of commands given to nonverbal autistic individuals. One of them even performed self-care activities independently, which had not been done previously. These findings are considered positive for the research, as communication and social skill deficiencies have an effect on their overall adaptive functioning and can generate stereotypes as a form of communication and coping with social situations (Santoncchi et al., 2020; Schmitt et al., 2023).

The use of these lactobacilli was safe, as tolerance was very good, with no complicated adverse events reported, only mild symptoms of diarrhea and gas in one case, which did not require medical treatment, supporting the findings of previous research on the safety of their use (Niu et al., 2019; Sanctuary et al., 2019).

### CONCLUSION

We can state that the use of orally administered lactobacilli was effective in reducing motor stereotypes in children with moderate to severe autism in early childhood and that its use was safe. In addition, neuropsychological assessments and reports from family members revealed positive changes in adaptive functions and disruptive behaviours, as well as improvements in eating habits, with tolerance to new foods and flexibility in routines.

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