

Microorganisms can Change Behavior on Autism Patients Incidence of Fungi Bacteria and Parasites

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Received: 📅 2025 Aug 28

Accepted: 📅 2025 Sep 08

Published: 📅 2025 Sep 30

Abstract

Objectives

It is estimated that 1.0% of the global population suffers from Autism Spectrum Disorder (ASD), its pathogenesis remains unclear. According to the World Health Organization, approximately 1/160 children is affected with a notable increase over the past 50 years. Our aim is to emphasize the importance in knowing the etiologies of the microbiota, focusing on the presence of bacteria, fungi, and/or parasites, as several studies have shown.

Methods

Different etiologies were investigated in feces; parasites, fungi, and bacteria such as *Yersinia*, *Klebsiella*, *Morganella*, *Salmonella*, *Proteus*, *Pseudomonas*, *Shigella*, and *Serratia*. The samples were processed within one to two hours after collection. Feces were cultured on MacConkey agar, EMB (Eosin Methylene Blue) agar, CLED (Cystine-Lactose-Electrolyte Deficient) medium with Andrade Indicator and selective media for *Campylobacter*. Fungal cultures were grown on Sabouraud Dextrose Agar or Potato Dextrose Agar. All cultures were incubated at 22°C for fungi and 37°C for bacteria.

Results

A total of 143 cases were analyzed, with 19% female and 81% male participants, between the ages of 2 and 11. The most frequent parasites identified were: 51.9% *Entamoeba histolytica*, 30.7% *Strongyloides stercoralis*, and 23.2% *Ascaris lumbricoides*. Fungi identified, included 62.4% *Candida*. The most prevalent bacterial species were: 65.1% *Campylobacter jejuni*, 28.4% *Escherichia coli* and 19.2% *Yersinia enterocolitica*, among others.

Conclusions

This study highlights the importance of early detection of dysbiosis in ASD. Our study showed multiple etiologies and supports an association between the dysbiosis and their symptoms and behavior. In a follow-up survey conducted with parents after treatment, 89% reported significant improvements.

Keywords: Autism Spectrum Disorder, Gut Microbiome, Gut Etiologies, Autistic Disorders

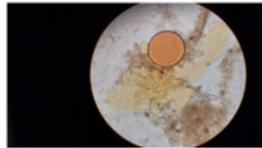
1. Introduction

Autism Spectrum Disorder (ASD) is a complicated neurodevelopmental disorder usually has a decreased or none verbal and social interactions, repetitive behaviors, limited interests and activities, plus they have clinical gastrointestinal disturbances (up to 70%), motor deficits (79%), sleep problems (50-80%), and intellectual disability (45%) [1-5].

It is estimated that 1.0% of the global population suffers from Autism Spectrum Disorder (ASD), and its pathogenesis remains unclear. According to the World Health Organization, approximately one in 160 children is affected by ASD, with a notable increase over the past 50 years [5]. Our aim is to emphasize the importance of studying the microbiota of individuals with ASD, focusing on the presence of bacteria, fungi, and/or parasites, as several studies have shown associations between these microorganisms and ASD [2,4].

OBJECTIVES AND STUDY

- Autism Spectrum Disorder (ASD)
 - 1% children suffer
 - Unclear its etiology
 - WHO → 1 of 160 (last 50 years)
- Our aim is to show the association of Bacteria, Parasites and Fungi with ASD:



2. Methods

Different etiologies were investigated in fecal samples, including parasites, fungi, and bacteria such as: *Yersinia*, *Klebsiella*, *Morganella*, *Salmonella*, *Proteus*, *Pseudomonas*, *Shigella*, and *Serratia*. The samples were collected and processed within one to two hours after collection or in the new Transport Tube Media [6]. Feces were cultured on MacConkey agar, EMB (Eosin Methylene Blue) agar, CLED (Cystine-Lactose-Electrolyte Deficient) medium with Andrade Indicator, Mueller Hinton agar and selective media for *Campylobacter*. Fungal cultures were grown on Sabouraud Dextrose Agar or Potato Dextrose Agar. All cultures were incubated at 22°C for fungi and 37°C for bacteria. The selective media for *Campylobacter* was incubated in a microaerophilic atmosphere with 5-6% of oxygen, 10% of carbon dioxide and 84-85% of nitrogen for 24 to 48 hours at 37°C. The Antimicrobial Susceptibility Test (AST) was done with all the enterobacteria or the predominant bacteria grown in the coprocultures. Parasites were analyzed in a

microscope with saline solution and parasitological lugol, they were observed in 40X and Gram stain in 100X [6-10]. (In our study there was not necessary an institutional review board or ethics committee approval in our methods).

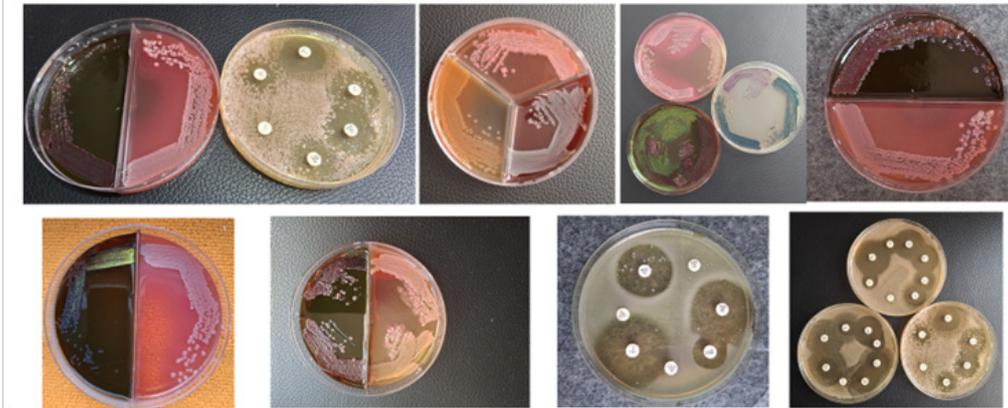
2.1. Methods: Feces Samples

- **Samples:** They were collected and processed within 1 to 2 hours after collection. If the samples are collected outside the city, the samples were taken in a Transport Media Tube.
- **Bacterias:** They were cultured on MacConkey agar, EMB (Eosin Methylene Blue) agar, CLED (Cystine-Lactose-Electrolyte Deficient) medium with Andrade Indicator and selective media for *Campylobacter*, Mueller Hinton agar to do the AST (Antibiotic Susceptibility Test)
- **Fungi:** Cultured on Sabouraud Dextrose Agar or Potato Dextrose Agar and incubated at 22°C
- **Parasites:** In a microscope with Saline Solution and Parasitological Lugol, observed in 40X and Gram 100x



BACTERIA CULTURES

For: *Escherichia*, *Yersinia*, *Klebsiella*, *Proteus*, *Serratia*, *Campylobacter*, *Morganella*, *Salmonella*, *Pseudomonas*, *Shigella*.



3. Results

A total of 143 cases between the ages of 2 and 11 were analyzed, with 19% female and 81% male participants, this difference has been shown in other studies [11]. The most frequent parasites identified were: 51.9% *Entamoeba histolytica*, 30.7% *Strongyloides stercoralis*, and 23.2% *Ascaris lumbricoides*. Fungi identified, included 62.4% *Candida* and 9.8% of *Rhodotorula* sp. The most prevalent bacterial species were: 65.1% *Campylobacter jejuni*, 28.4% *Escherichia coli*, 19.2% *Yersinia enterocolitica*, 14.5% *Shigella*, 11.8% *Klebsiella oxytoca* or *pneumoniae*, 8.3% *Morganella morganii*, less than 5%: *Proteus*, *Edwardsiella tarda*, *Pseudomonas aeruginosa*, among others. None of the ASD cases had 0 etiologies. Parasites and bacteria had 22.5%, parasites with bacteria and fungi 67.0 %, only had bacteria 6.5 %. In a follow-up survey conducted with parents an 89% reported significant improvements after treatment with the known etiology.

4. Discussion

Our results have shown that giving the accurate treatment in the gut microbiota etiologies, as enteropathogens found, parasites and/ or fungi, the children showed an improve in their behavior and had no abdominal pain, diarrhea or many other symptoms that they had before the laboratories tests. This study can help ASD patients, because it is much cheaper and easier than a manipulation of the gut microbiota by fecal microbiota transplantation (FMT) that have shown [11]. Their research FMT found a large difference in baseline characteristics of behavior, gastrointestinal symptoms, and gut microbiota between children with ASD and typically developing control children. In our study 67.0% of the cases had three kinds of etiologies: bacterias, parasites and fungi at the same time. This shows that these microorganisms can change the behavior and maybe can affect the brain development in the ASD patients. In Latin America there is an incidence of 1 in 163 of ASD. In 2016 in the United States was 1 in 54. In Asia Pacific region 1 in 65 people consider the need to study more on the “numerous medical comorbidities associated with ASD, gastrointestinal

issues have a significant impact on quality of life for these patients” [12-14]. suggest also to be aware of the kind of food, allergies of food, intoxication caused by heavy metals and the incidence of *Candida* in our study was 62.4% [4]. has shown in their research that gut microbiota dysbiosis is frequently observed in ASD children with the modulation of brain function and social behavior [13]. Also, have identify associations between gut microbial tryptophan metabolites, ASD symptoms, and brain activity in humans, specially with brain regions with interceptive processing. In Spain, has said that the use of probiotics and prebiotics has helped mental disorders, especially in autism. In Poland showed the gut-brain-microbiota connection and its role in ASD (2025) [15-17].

5. Conclusions

This study highlights the importance of early detection of dysbiosis in children with ASD [4]. Our findings indicate that multiple etiologies may be involved, and the study supports the relationship between the gut microbiome and their symptoms and behavior as has hown with the FMT [11]. It is very important to do the tests as soon as they see their son or daughter has a problem, in order to give an accurate treatment for the etiology found in the laboratory’s tests. Our 89% reported significant improvements shows it is worthy to do all this research in the gut microbiota. Plus, it can reduce the autism cost in their families and in Public Health of many countries. The great thing about this study: improves patients health.

Acknowledgments

I will like to thanks the physicians that trust in our results: Dra Lyda Perez, Dayana Galvis, Javier Galvis, and my staff that works in the laboratory, specially to Cristina Olarte, Teresa Pinzón and Valeria Ascendra.

References

1. Gyawali, S., & Patra, B. N. (2019). Trends in concept and nosology of autism spectrum disorder: A review. *Asian journal of psychiatry*, 40, 92-99.

2. Lasheras, I., Seral, P., Latorre, E., Barroso, E., Gracia-García, P., & Santabárbara, J. (2020). Microbiota and gut-brain axis dysfunction in autism spectrum disorder: Evidence for functional gastrointestinal disorders. *Asian journal of psychiatry*, 47, 101874.
3. Rylaarsdam, L., & Guemez-Gamboa, A. (2019). Genetic causes and modifiers of autism spectrum disorder. *Frontiers in cellular neuroscience*, 13, 470832.
4. González, L.G. (2005). Manifestaciones gastrointestinales en trastornos del espectro autista. *Colombia Médica*, 36(2. Supl. 1), 36-38.
5. Elsabbagh, M., Divan, G., Koh, Y. J., Kim, Y. S., Kauchali, S., Marcín, C., ... & Fombonne, E. (2012). Global prevalence of autism and other pervasive developmental disorders. *Autism research*, 5(3), 160-179.
6. Ordonez-Smith, M. E. (2024). A-295 New Transport Media to Preserve Viable Parasites, Bacteria and Fungus. *Clinical Chemistry*, 70(Supplement_1), hvae106-292.
7. Smith, M. O. (2023). *Clinical Microbiology Procedures Handbook 5th edition* ASM Press (American Society for Microbiology). Author of a Chapter: Rapid Disk, Spot Tests, and Other Rapid or Primary Methods for the Identification of Anaerobes en el libro CMPH5 (Clinical Microbiology Procedures Handbook 5th edition) ISBN (Print):9781683673989ISBN.
8. Smith, M. O. (2024). *Microbes of Medical Importance. IIP Series_V3*. Author of a Chapter Unit I: Bacteria Campylobacter, pages 96 a 112. 2024. ISBN: 978-93-6252-342-6.
9. Ordonez-Smith M. Antimicrobial Resistance Detection and Rapid Susceptibility Testing a Chapter in the IIP Book Series. *Novel Diagnostic Methods in Clinical Microbiology*. Vol 5. DOI iip2025C9A09A7F25E2114. ISBN/ISSN
10. Ash, L. R, Orihel, T.C. (2013). *Atlas of Human Parasitology*. 5th Ed. 2nd Reprint. Buenos Aires: Médica Panamericana 556. ISBN 978-950-06-0128-3
11. Li, N., Chen, H., Cheng, Y., Xu, F., Ruan, G., Ying, S., ... & Wei, Y. (2021). Fecal microbiota transplantation relieves gastrointestinal and autism symptoms by improving the gut microbiota in an open-label study. *Frontiers in cellular and infection microbiology*, 11, 759435.
12. Heasley, S. (2025). Autism affects more than 60 million worldwide, study finds. *Disabilityscope*.
13. Alharthi, A., Alhazmi, S., Alburae, N., & Bahieldin, A. (2022). The human gut microbiome as a potential factor in autism spectrum disorder. *International journal of molecular sciences*, 23(3), 1363.
14. Saurman, V., Margolis, K. G., & Luna, R. A. (2020). Autism spectrum disorder as a brain-gut-microbiome axis disorder. *Digestive diseases and sciences*, 65(3), 818-828.
15. Aziz-Zadeh, L., Ringold, S. M., Jayashankar, A., Kilroy, E., Butera, C., Jacobs, J. P., ... & Mayer, E. A. (2025). Relationships between brain activity, tryptophan-related gut metabolites, and autism symptomatology. *Nature Communications*, 16(1), 3465.
16. Álvarez, G. (2020). Microbiota, probióticos y trastornos del espectro autista (TEA).
17. Młynarska, E., Barszcz, E., Budny, E., Gajewska, A., Kopeć, K., Wasiak, J., ... & Franczyk, B. (2025). The Gut-Brain-Microbiota Connection and Its Role in Autism Spectrum Disorders. *Nutrients*, 17(7), 1135.