The Role of Gut Microbiome in Autism Spectrum Disorder (ASD) and Emerging Treatment Strategies

Abstract:

Autism spectrum disorder (ASD) is a neurodevelopmental disorder. It is distinguished by repetitive behaviors, trouble in social interaction, and difficulty in communication. Recent research has demonstrated a strong association between gut microbiota and ASD. The purpose of this review is to highlight the relationship between the gut microbiome and autism spectrum disorder and how variations in the gut microbiome lead to severe psychological and autistic symptoms. We conducted a systematic review following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines, reviewing experimental and epidemiological studies from the last decade (2013-2023). Google Scholar and PubMed were used for data collection. This review includes a total of 37 studies from the past decade. Our findings revealed that individuals with ASD often had variations in their gut microbiome composition, and these distinct compositions are associated with behavioral symptoms and many gastrointestinal (GI) problems. The connection between the gut and the brain has demonstrated promising potential. These connections help scientists to come up with new interventions and potential treatments. By using microbiota transfer therapy (MTT), probiotics, and manipulating the gut microbiome through exclusive diets, there is a possibility that we can positively impact ASD patients' social and GI problems. In conclusion, further studies are essential to explore specific gut microbiota and their mechanisms linking the gut and brain. Such research could lead to better treatment opportunities and improve the quality of life for people with autism spectrum disorder.

Keywords: Autism Spectrum Disorder; Gut Microbiota; Gut-Brain Axis; Microbiota Transfer Therapy; Probiotics; Gastrointestinal Symptoms; Microbiome Manipulation

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Introduction:

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by repetitive behaviors, deficits in social interactions, and communication impairments. In addition to these behavioral symptoms, individuals with ASD often experience metabolic abnormalities, seizures, anxiety, and sleep disturbances (Qinrui Li, Han, Dy, & Hagerman, 2017). Recent data

suggests that approximately 1 in every 59 children is diagnosed with ASD, with a global prevalence rate of about 1%, though there are some regional variations, particularly in developing countries (Ho et al., 2020). The exact etiology of ASD remains unclear, but both environmental and genetic factors are believed to play significant roles. Genetic risk factors, including single nucleotide polymorphisms and de novo mutations, account for about 50% of cases (Vuong & Hsiao, 2017).

Gastrointestinal (GI) abnormalities, such as constipation, diarrhea, bloating, and abdominal pain, are commonly reported in ASD patients (Xu, Xu, Li, & Li, 2019). Studies have also shown that individuals with ASD may have structural brain differences, including an increased number of neurons in the cortex, greater brain weight, and abnormal cortical patterns (Vuong & Hsiao, 2017). Over 100 genes have been implicated in ASD, with subtypes such as Asperger's syndrome and pervasive developmental disorder being classified under the broader ASD umbrella (De Angelis, Francavilla, Piccolo, De Giacomo, &Gobbetti, 2015).

A growing body of evidence highlights the role of the gut microbiota in ASD. The human gastrointestinal tract contains approximately 1 kilogram of microorganisms, collectively referred to as the gut microbiota, which consists of an estimated 9.9 million microbial genes (Qinrui Li et al., 2017). These microorganisms are crucial in regulating host metabolism, digestion, and synthesizing essential vitamins, such as B-vitamins, folates, and thiamine (Vuong & Hsiao, 2017). Diet plays a pivotal role in maintaining the balance of gut microbiota, with the main phyla in humans being Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia (Q Li & Zhou, 2016).

An imbalance in gut microbiota, known as dysbiosis, has been associated with the disruption of the mucosal barrier, leading to the production of inflammatory cytokines that can impact neural, endocrine, and immunological pathways (Fattorusso, Di Genova, Dell'Isola, Mencaroni, & Esposito, 2019). The gut microbiota communicates with the brain through the gut-brain axis, a bidirectional interaction involving the enteric nervous system, which connects the gastrointestinal tract with the central nervous system (Bundgaard-Nielsen et al., 2020). This signaling pathway plays a significant role in the pathophysiology of ASD (Fattorusso et al., 2019). The brain, in turn, can influence gut microbiota by regulating intestinal permeability, motility, and secretions (Zhang et al., 2021).

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Moreover, gut microbiota can affect levels of neurotrophins and monoamine neurotransmitters, potentially altering brain development and plasticity, particularly under extreme conditions (De Angelis et al., 2015). Studies have found that ASD patients often exhibit an increased concentration of pathogenic bacteria, such as Clostridium, and a decreased presence of beneficial bacteria, such as Bifidobacterium (Cao et al., 2021; Dargenio et al., 2023; Korteniemiet al., 2023).These microbiota shifts may activate immune pathways like Th1 and Th2, contributing to the behavioral and physiological symptoms observed in ASD (Carissimi et al., 2019).

Aim and Objectives

This review aims to explore the relationship between gut microbiota and autism spectrum disorder, with a focus on understanding how variations in gut microbiota composition contribute to the development of ASD symptoms. Additionally, the review will examine potential treatment strategies, such as microbiota transfer therapy (MTT) and probiotics, that target the gut-brain axis as therapeutic interventions for ASD. The key objectives are to:

1. Identify specific gut microbiota associated with ASD.
2. Investigate the mechanisms linking gut microbiota dysbiosis with ASD pathophysiology.
3. Evaluate current therapeutic approaches aimed at restoring gut microbiota balance in ASD patients.
4. Provide recommendations for future research directions to better understand and treat ASD.

Methodology:

We systematically reviewed experimental and epidemiological studies from the last 10 years (2013–2023) to investigate the role of gut microbiota in ASD and explore potential treatment strategies. Databases such as Google Scholar and PubMed were used for data collection. The keywords included “autism spectrum disorder/ASD,” “gut microbiota,” “gut microbiota transplantation,” “microbiota transfer therapy,” and “ASD potential therapies” to identify relevant studies. After careful screening, 1,500 duplicates were excluded. Articles published outside the specified timeline were disregarded. Additionally, 90 articles were excluded due to

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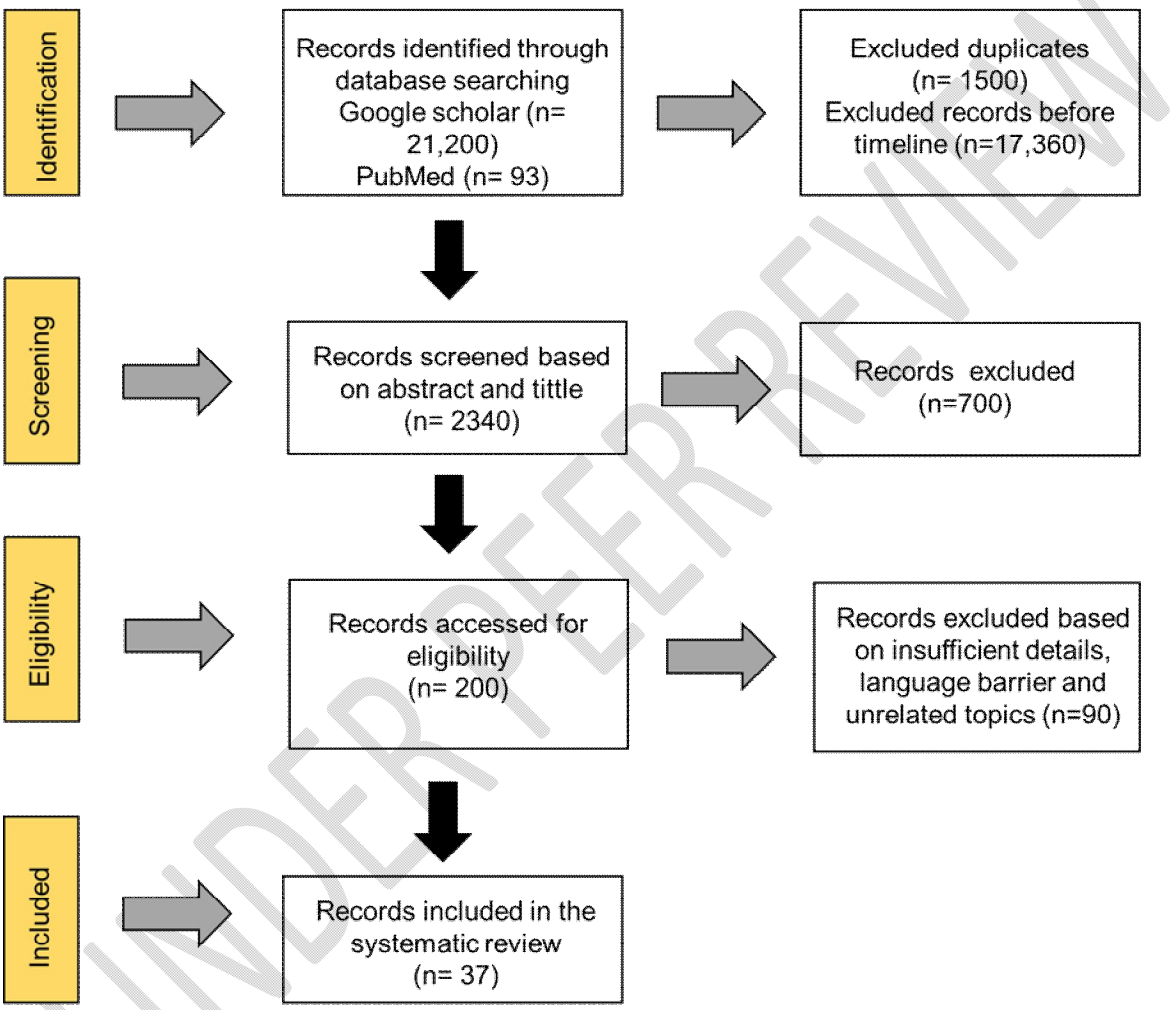
insufficient details, non-English language, lack of original research, or insufficient evidence. Studies with both positive and negative findings were included. Ultimately, 37 articles were reviewed in accordance with the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Fig 1: Meta-Analysis Protocols (PRISMA).



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Results and discussion

1. Autism Spectrum Disorder and Microbiota Transfer Treatment

Out of 37 studies, 3 focused on ASD and microbiota transfer treatments. One experimental study using a mouse model compared the effectiveness of gut microbiota transplantation (GMT) with both original and in vitro cultured microbiota. The results indicated significant improvements in

anxiety levels and repetitive behaviors in ASD mice, along with normalization of certain chemokine levels. The study concluded that in vitro cultured microbiota were as effective as the original microbiota in treating ASD. Two epidemiological studies examined metabolite changes in ASD children following Microbiota Transfer Therapy (MTT), which involves bowel cleansing, antibiotics, and fecal microbiota transplantation (Chen et al., 2020). One study found that ASD patients exhibited a different metabolic profile compared to typically developing children, with MTT leading to significant plasma metabolite changes, though no major differences were observed in fecal metabolites. The reduction of p-cresol sulfate levels after MTT was notable, offering evidence of MTT as a potential treatment and underscoring the role of metabolites in the gut-brain connection (D.-W. Kang et al., 2020). Another study highlighted that MTT resulted in a reduction of gastrointestinal symptoms, along with an improvement in behavioral symptoms in ASD patients (D.-W. Kang et al., 2017).

2. Probiotic, Prebiotic, and Symbiotic Treatments on the Gut Microbiota in Autism Spectrum Disorder

Six studies investigated the impact of probiotic, prebiotic, and symbiotic treatments on ASD patients. Of these, three were epidemiological. One study demonstrated that a combination of *L. reuteri* and *B. longum* prebiotics showed high resistance to the digestive system, with probiotics increasing *Lactobacillus* and prebiotics increasing *Bifidobacterium*, while reducing *Lachnoclostridium*. These treatments boosted beneficial SCFA levels and lowered ammonium levels, suggesting microbiota-based strategies may help manage ASD (Duque et al., 2021; Niu et al., 2019). Another epidemiological study revealed that the ASD group had lower concentrations of *Bacteroidetes*, *Bacteroides*, *Bifidobacterium*, *Ruminococcus*, *Roseburia*, and *Blautia* compared to neurotypical children. A four-week combination of applied behavior analysis (ABA) and microbiota treatment led to improvements in Autism Treatment Evaluation Checklist (ATEC) and gastrointestinal scores, outperforming ABA training alone. A third epidemiological study showed that probiotic treatment improved anti-social behavior in ASD patients, with a reduction in abdominal pain and bowel problems reported in those on an exclusive diet (Grimaldi et al., 2018). One experimental study modeled the gut microbiota-ASD relationship and found that probiotics could enhance beneficial *Bifidobacterium* and slow the growth of harmful microbes like *Clostridia*, suggesting probiotics as a valuable therapeutic option (Weston, Fogal,

Cook, &Dhurjati, 2015). Two experimental studies on rodents supported the link between probiotic treatments and ASD. One study on Shank3 knockout mice, a model for ASD, found that treatment with *Lactobacillus reuteri* decreased unsocial behavior and repetitive actions, suggesting probiotics as a promising intervention (Tabouy et al., 2018). Another rodent study showed that antibiotic-induced microbiome disruption led to ASD-like symptoms, which were alleviated by probiotic treatment, indicating the potential for probiotics to mitigate ASD symptoms (Mintál et al., 2022).

3. Advanced Sequencing Techniques and Autism Spectrum Disorder

Five computational studies used advanced sequencing techniques to explore the relationship between gut microbiota and ASD. A pilot study comparing ASD patients with their first-degree relatives found significant differences in microbial communities, with the development of gut microbiome markers emerging as a promising tool for monitoring gut health in ASD patients (Kong et al., 2019). Another two-staged study used fecal sample analysis, shotgun metagenomic sequencing, and liquid chromatography-mass spectrometry to reveal altered glutamate metabolism in the ASD group, marked by decreased 2-keto-glutaramic acid and microbial changes. The findings suggest 2-keto-glutaramic acid as a potential biomarker for ASD (M. Wang et al., 2019). A study investigating the impact of different primers on microbiota analysis in ASD patients revealed that different primers detected varying bacterial phyla, with the 27f/1492r primer set showing a lower Firmicutes/Bacteroidetes ratio (Palkova et al., 2021). Another study explored the relationship between single nucleotide variations (SNVs) and gut microbiota in ASD, identifying SNVs involved in innate immune responses and protein glycosylation processes, which are relevant to microbiota functioning (Z. Liu et al., 2021). Lastly, an experimental study integrating brain and gut metabolism suggested that high-fiber diets, coupled with probiotics like *Lactobacillus acidophilus* and *Bifidobacterium longum*, restored gut balance and reduced oxidative stress, offering a potential ASD treatment (Mohammad et al., 2022).

4. Short-Chain Fatty Acids (SCFAs), Gastrointestinal Issues (GI), and Autism Spectrum Disorder

Four studies examined the link between ASD, gastrointestinal symptoms, and gut microbiota. Two epidemiological studies highlighted significant differences in SCFA levels between ASD and neurotypical children. One study involving 90 children, including 45 with ASD, found that while GI problems were prevalent among ASD patients, they had a minor impact on core ASD symptoms. Metabolic changes related to SCFAs were connected to these GI issues (Deng et al., 2022). Another study conducted in Korea found that the ASD group had an abundance of *Bacteroidetes* and *Actinobacteria* phyla, along with elevated SCFA concentrations, compared to controls (Ha et al., 2021). One rodent study demonstrated that treatment with SCFAs like butyric acid and propanoic acid (PPA) led to changes in behavior and brain function in ASD models. Notably, PPA triggered genes involved in catecholamine production, which are linked to mood control, indicating that SCFAs contribute to ASD through epigenetic mechanisms (Nankova, Agarwal, MacFabe, & La Gamma, 2014). An experimental study further confirmed that differences in SCFA levels, particularly lower butyrate and higher valeric acid levels, were associated with GI symptoms in ASD patients, suggesting that modulating SCFA-producing bacteria could be a viable treatment strategy (S. Liu et al., 2019).

5. Variation of Gut Microbiome Between ASD Patients and Controls

Sixteen studies compared the gut microbiome in ASD patients and controls. One epidemiological study assessed the impact of *Aureobasidium pullulans* (AFO-202 strain) beta-glucan, also known as Nichi Glucan, in 18 children, showing that treatment led to a reduction in harmful *Enterobacteriaceae* and an increase in beneficial bacteria like *Faecalibacteriumprausnitzii* and *Prevotellacopri*, suggesting it could be a beneficial adjunct to conventional ASD treatments.Figure 2 represents the schematic diagram of the above results.

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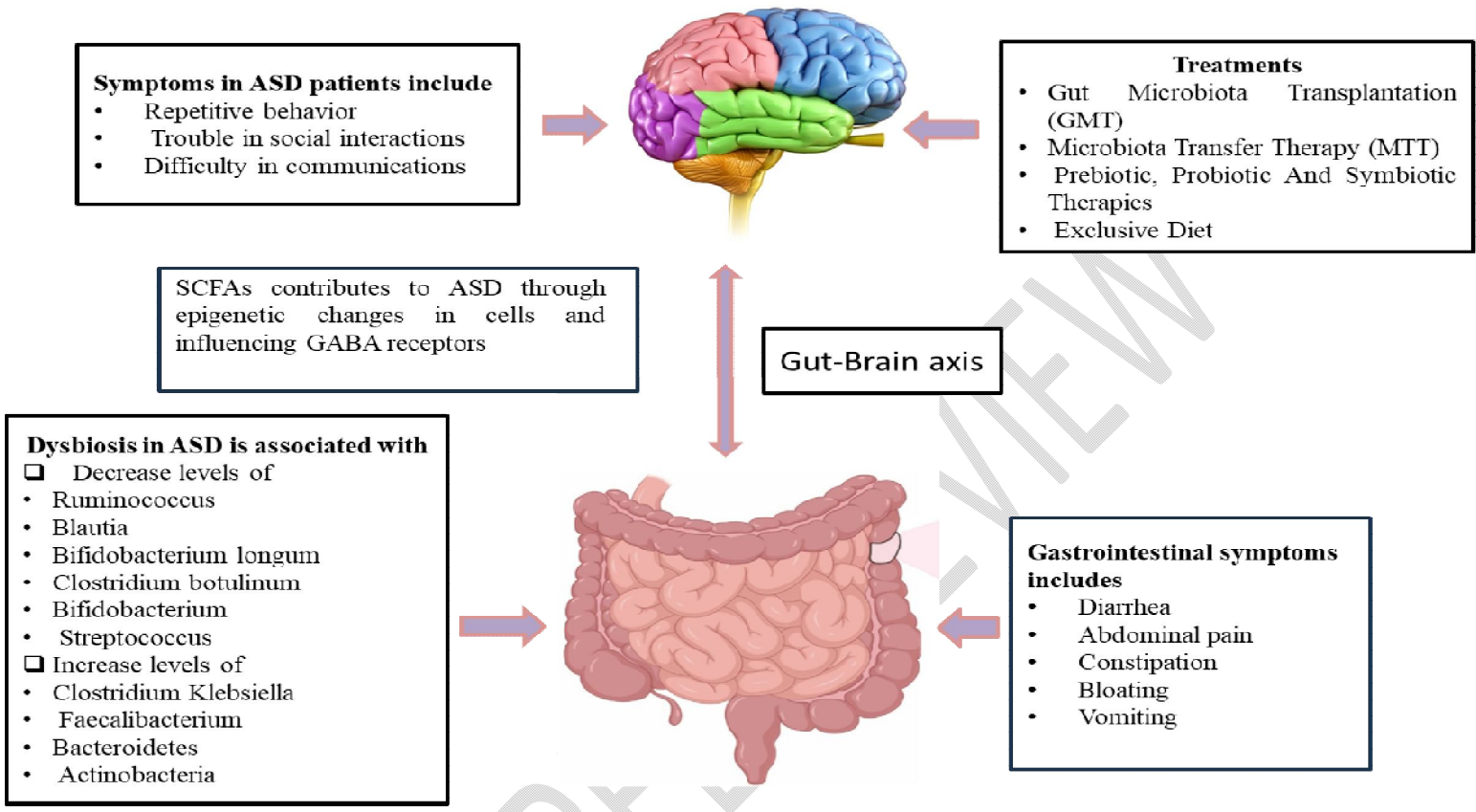

Figure 2: ASD association with gut microbiota and potential treatments

Conclusion:

Although there are inconsistencies among studies, the strong link between gut microbiota dysbiosis and severe ASD symptoms suggests that variations in gut microbiota are closely correlated with the worsening of psychological and behavioral symptoms in ASD patients. Different therapeutic approaches are needed to effectively treat individuals with ASD. This review highlights several promising treatment strategies, such as MTT, which involves the transfer of beneficial bacteria into ASD patients. MTT has been shown not only to alleviate gut-related symptoms but also to reduce behavioral issues in ASD patients. Probiotic treatments, as reported by several studies, also improve gut health, and exclusive diets may further benefit these patients. However, more research is necessary to develop additional microbiota-based therapies and offer a brighter, more effective future for individuals with ASD.

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