

Gut Microbiota and Autism Spectrum Disorder: Current Evidence, Mechanisms, and Therapeutic Potential

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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental condition frequently associated with gastrointestinal (GI) dysfunction and altered gut microbiota. Increasing evidence suggests that the microbiota–gut–brain (MGB) axis may contribute to ASD through interactions involving neural, immune, endocrine, and metabolic pathways. This narrative review aimed to summarize current evidence regarding gut microbiota alterations in ASD, proposed MGB mechanisms, and the therapeutic potential of microbiome-based interventions. A structured literature search was conducted using PubMed, Scopus, and Google Scholar for studies published between 2019 and 2025, and 14 core articles were selected for thematic synthesis. Current evidence indicates that individuals with ASD commonly exhibit gut dysbiosis, including altered abundance of taxa such as *Proteobacteria*, *Actinobacteria*, *Sutterella*, *Clostridium*, and *Bifidobacterium*. However, findings remain heterogeneous, and no consistent ASD-specific microbial signature has been identified. Proposed mechanisms linking dysbiosis to ASD include altered intestinal permeability, inflammatory cytokine signaling, short-chain fatty acid imbalance, and microbial metabolite dysregulation. Nevertheless, much of the mechanistic evidence derives from animal and experimental studies, while human evidence remains largely associative. Microbiome-based interventions, including probiotics, dietary modification, prebiotics, synbiotics, and fecal microbiota transplantation (FMT), show potential benefits, particularly for GI symptoms. However, current evidence is limited by small sample sizes, methodological heterogeneity, and insufficient long-term safety data. Overall, the gut microbiome appears to function as a biologically relevant modifier of ASD phenotype and symptom severity rather than a definitive causal factor. Future research should prioritize longitudinal human studies, patient stratification, multi-omics integration, and rigorous randomized controlled trials to improve mechanistic understanding and therapeutic translation.

Keywords: Autism spectrum disorder, Gut microbiota, Microbiota–gut–brain axis, Dysbiosis, Microbiome-based interventions

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by deficits in social communication and interaction, together with restricted and repetitive behaviors. In addition to core behavioral symptoms, many children with ASD experience gastrointestinal (GI) disturbances, suggesting that ASD may involve systemic as well as neurological dysfunction [1, 2]. Increasing evidence has focused on the microbiota-gut-brain axis, a bidirectional network linking the gut microbiome, intestinal barrier, immune system, metabolites, and central nervous system. Through this axis, gut dysbiosis may influence neurodevelopment, behavior, and immune regulation in ASD [3, 4]

Current literature shows that children with ASD often have gut microbiota profiles that differ from those of neurotypical children. A systematic review by Kortenien et al. (2023) found that most studies reported significant microbial differences, with the most consistent findings being higher levels of Proteobacteria, Actinobacteria, and *Sutterella* in ASD. Beyond compositional changes, dysbiosis in ASD has been linked to increased intestinal permeability, altered short-chain fatty acids, immune dysregulation, and abnormal microbial metabolites, all of which may contribute to neuroinflammatory changes and symptom severity [1, 2, 5]. Therapeutically, microbiome modulation has emerged as a promising supportive strategy. Reviews suggest that probiotics, prebiotics, dietary interventions, and fecal microbiota transplantation may improve GI symptoms and, in some cases, selected behavioral outcomes, although the evidence remains heterogeneous and preliminary [3, 6].

Taken together, these findings support the view that ASD may be better understood not only as a brain-based disorder, but also as a condition involving gut-brain interactions. Scientifically, this broadens the understanding of ASD pathophysiology; medically, it raises the possibility that microbiome-targeted therapies could serve as adjunctive approaches; and socially, it offers potential strategies to improve quality of life in a disorder with substantial long-term burden [5, 6].

Despite growing interest, important gaps remain. First, causality is still unclear: gut dysbiosis may contribute to ASD, but it may also result from ASD-related diet, medication use, or GI dysfunction. Second, study findings are inconsistent, and no single microbial signature has been universally confirmed. Third, many studies have small samples and short follow-up periods. Fourth, ASD is highly heterogeneous, yet many studies do not adequately distinguish clinical subtypes. Finally, treatment evidence is still insufficient for routine clinical application, and larger, standardized trials are needed [2, 3, 7].

Objective of this review

Therefore, this review aims to evaluate whether microbiome-based interventions, such as probiotics and dietary modification, can alleviate ASD symptoms and whether these approaches are ready for clinical application or remain experimental. Specifically, this review will examine: (1) gut microbiota alterations in ASD, (2) mechanisms of the microbiota-gut-brain axis, and (3) current evidence from microbiome-modulating interventions. By synthesizing these areas, this review seeks to clarify the current state of the field and identify priorities for future research [1, 3].

Study Methods

This study was conducted as a narrative review to synthesize current evidence regarding the relationship between gut microbiota and autism spectrum disorder (ASD). The review was guided by three predefined objectives: (1) to examine gut microbiota alterations in individuals with ASD, (2) to explain the mechanisms of the microbiota-gut-brain axis, and (3) to evaluate current evidence from microbiome-modulating interventions. A narrative review design was considered appropriate because the topic encompasses diverse forms of evidence, including systematic reviews, observational studies, mechanistic studies, and pilot clinical trials, making interpretive thematic synthesis more suitable than quantitative pooling.

To improve methodological transparency and reproducibility, a structured literature search was conducted between January and March 2026 using multiple electronic databases, including PubMed, Scopus, and Google Scholar. Additional manual searches of reference lists from relevant review articles were also performed to identify potentially eligible studies. The search strategy combined keywords and Medical Subject Headings (MeSH)-related terms associated with ASD, gut microbiota, and microbiome-based interventions. Example search terms included “autism spectrum disorder” AND “gut microbiota,” “microbiota–gut–brain axis” AND autism, “gut dysbiosis” AND ASD, “probiotics” AND autism, “fecal microbiota transplantation” AND ASD, and “short-chain fatty acids” AND autism. Searches focused primarily on literature published between 2019 and 2025 to emphasize recent developments in microbiome research, although several earlier foundational studies were also considered when directly relevant to mechanistic interpretation or therapeutic context.

Studies were selected on the basis of their direct relevance to the objectives of the review. Eligible literature included systematic reviews, narrative reviews, observational human studies, mechanistic studies, and pilot intervention trials addressing one or more of the following domains: gut microbiota composition in ASD, microbiota–gut–brain axis mechanisms, intestinal barrier dysfunction and immune signaling, microbial metabolites associated with ASD, and microbiome-based interventions such as probiotics, prebiotics, dietary modification, and fecal microbiota transplantation (FMT) or microbiota transfer therapy (MTT). Only English-language articles were included. Conference abstracts, editorials, duplicate reports, and studies unrelated to ASD or the gut microbiome were excluded. Greater emphasis was placed on recent systematic reviews, controlled human studies, and papers with strong mechanistic or clinical relevance to the review objectives. Because this study was designed as a focused narrative review rather than a systematic review, the intention was not to identify all available publications in the field, but rather to synthesize representative and high-relevance evidence across major mechanistic and therapeutic domains. After screening the literature

for thematic relevance and methodological contribution, 14 core papers were selected for detailed synthesis in this manuscript.

Data were extracted manually from each included study and organized according to the predefined objectives of the review. For each article, the following information was considered: author, year, study design, study population or model, principal focus, main findings, and relevance to ASD pathophysiology or microbiome-based interventions. The extracted evidence was then synthesized thematically under the major sections of the manuscript, including Gut Microbiota Alterations in ASD, Microbiota–Gut–Brain Axis Mechanisms, Intestinal Barrier, Immune Signaling, and Microbial Metabolites, Therapeutic Potential of Microbiome-Based Interventions, and Knowledge Gaps and Future Directions. Greater interpretive weight was given to systematic reviews, controlled human studies, and clinically relevant evidence, whereas mechanistic findings derived primarily from animal or experimental models were interpreted more cautiously and distinguished from direct human evidence where appropriate.

Because this study was conducted as a narrative review, no formal systematic review protocol, risk-of-bias assessment, or meta-analysis was performed. Nevertheless, efforts were made to improve methodological transparency through structured database searching, predefined thematic objectives, and explicit eligibility criteria. This approach was intended to provide a focused and critical synthesis of current evidence while acknowledging the methodological heterogeneity and evolving nature of microbiome research in ASD. The characteristics and principal findings of the studies included in this narrative review are summarized in Table 1.

Table 1. Characteristics and Principal Findings of Studies Included in the Narrative Review

No.	Author (Year)	Study Type	Main Focus	Population / Model	Principal Findings	Key Limitations	Relevance to Review
[1]	Alharthi et al. (2022)	Narrative review	Gut microbiome and ASD pathophysiology	Human and mechanistic literature	Dysbiosis may influence neurodevelopment through immune, metabolic, and inflammatory pathways	Primarily review-based synthesis; limited primary clinical data	Overview of microbiota alterations and mechanistic pathways
[2]	Dargenio et al. (2023)	Review article	Intestinal barrier dysfunction and microbiota-gut-brain axis	Human and experimental evidence	Increased intestinal permeability and immune activation may contribute to ASD symptom expression	Mechanistic conclusions rely partly on animal studies	Intestinal barrier and immune signaling mechanisms
[3]	Petropoulos et al. (2025)	Review article	Mechanisms of microbiota-gut-brain axis	Human and preclinical evidence	Neural, endocrine, immune, and metabolic pathways mediate gut-brain communication in ASD	Limited direct mechanistic human evidence	Mechanistic framework of the microbiota-gut-brain axis
[4]	Taniya et al. (2022)	Review article	Therapeutic regulation of gut microbiome	Human and experimental studies	Gut microbiota modulation may improve GI and selected behavioral symptoms	Intervention studies heterogeneous in design and outcomes	Therapeutic potential of microbiome modulation
[5]	Mamun et al. (2025)	Review article	Gut microbiome signaling pathways and targeted therapies	Mechanistic and translational evidence	Dysbiosis-associated signaling pathways may represent therapeutic targets in ASD	Heavy reliance on experimental and preclinical findings	Mechanisms and therapeutic implications
[6]	Młynarska et al. (2025)	Review article	Gut-brain-microbiota connection	Human and experimental literature	Dysbiosis, immune activation, and microbial metabolites are linked to ASD-related abnormalities	Narrative synthesis without standardized quantitative analysis	Integrative overview of gut-brain interactions
[7]	Korteniemi et al. (2023)	Systematic review	Gut microbiota composition in ASD	Human studies	Increased Proteobacteria, Actinobacteria, and <i>Sutterella</i> commonly reported in ASD	Significant heterogeneity across included studies	Evidence for recurrent microbiota alterations
[8]	Tan et al. (2021)	Systematic review	Probiotics, prebiotics, synbiotics, and FMT in ASD	Human intervention studies	Evidence for behavioral benefit remains inconsistent; GI improvement more reproducible	Small sample sizes and variable intervention protocols	Evaluation of microbiome-based interventions
[9]	Chang et al. (2024)	Original research study	Gut microbiome and amino acid metabolomics	Children with ASD	Altered gut microbiota and serum amino acid metabolome identified in ASD	Cross-sectional design limits causal interpretation	Metabolic and biomarker relevance
[10]	Hrnciarova et al. (2024)	Randomized placebo-controlled pilot study	Gut microbiome modulation in ASD	Children with ASD	Supplementation altered microbiota composition and improved selected symptoms	Pilot-scale study with limited sample size and follow-up	Controlled intervention evidence
[11]	Sanctuary et al. (2019)	Pilot clinical study	Probiotic/colost rum supplementation	Children with ASD and GI symptoms	Improvement in GI function observed following supplementation	Small cohort and absence of long-term evaluation	GI-focused microbiome intervention
[12]	Pietruszka et al. (2023)	Systematic review	Gut microbiota in ASD	Human studies	Reported heterogeneous but recurrent dysbiosis patterns in ASD	Lack of universally reproducible microbial signature	Confirmation of microbiota heterogeneity
[13]	Liu et al. (2022)	Review article	Gut microbiota alteration as treatment strategy	Human and mechanistic evidence	Microbiota modulation may represent a future supportive therapeutic strategy	Clinical applicability remains preliminary	Therapeutic and mechanistic synthesis
[14]	Mehra et al. (2023)	Review article	Pathogenesis and therapeutic perspectives	Human and experimental evidence	Gut dysbiosis may contribute to neuroinflammation and behavioral abnormalities	Mechanistic interpretations partly inferential	Pathophysiology and treatment perspectives

Gut Microbiota Alterations in ASD

Current evidence indicates that gut microbiota alterations are a recurring feature of autism spectrum disorder (ASD), although these changes are better interpreted as heterogeneous dysbiosis rather than as a single disease-specific microbial signature. Across multiple studies, individuals with ASD consistently demonstrate differences in microbial composition, abundance, and diversity compared with neurotypical controls [1, 4, 7, 12]. However, no universally reproducible “autism microbiome” has been identified, suggesting that microbiota alterations in ASD are biologically relevant but strongly influenced by clinical and environmental variability.

At the phylum level, recurrent findings include altered relative abundance of Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria [1]. Increased Proteobacteria and Actinobacteria are among the most consistent observations, whereas some studies also report a reduced Bacteroidetes/Firmicutes ratio [7, 12]. These shifts are mechanistically relevant because these microbial groups contribute to epithelial homeostasis, immune modulation, fermentation, and production of neuroactive metabolites [3]. Nevertheless, phylum-level findings remain inconsistent across cohorts and should therefore be interpreted cautiously rather than as definitive disease markers.

At the genus and family levels, altered abundance of *Sutterella*, *Clostridium*, *Desulfovibrio*, *Bifidobacterium*, *Prevotella*, *Faecalibacterium*, and Lachnospiraceae has been reported repeatedly, although the direction and magnitude of these changes vary considerably between studies [7, 12, 13]. In general, taxa associated with intestinal homeostasis and anti-inflammatory activity, including *Bifidobacterium*, *Prevotella*, and *Faecalibacterium*, are often reduced in ASD, whereas taxa linked to inflammatory signaling or altered sulfur metabolism, such as *Sutterella*, *Clostridium*, and *Desulfovibrio*, are frequently increased [1, 14]. These patterns support the hypothesis that dysbiosis in ASD may influence epithelial barrier integrity, immune activation, and microbial metabolite production rather than simply reflecting taxonomic variation alone [2, 6].

Altered microbial diversity has also been reported in ASD, although findings remain inconsistent. Some studies describe reduced alpha diversity, particularly among children with coexisting gastrointestinal (GI) symptoms, whereas others report no significant difference or even increased richness [1, 7]. This variability suggests that microbial diversity alone is unlikely to function as a reliable biomarker for ASD. Instead, diversity measures appear highly sensitive to confounding factors including diet, medication exposure, age, stool consistency, GI phenotype, and methodological differences in sequencing and analysis [12].

Dietary selectivity represents a particularly important confounder in ASD microbiome research. Restricted eating behaviors and limited dietary diversity are common among children with ASD and may independently alter microbial composition and metabolic activity [4, 6]. Consequently, some observed microbiota alterations may reflect secondary effects of dietary behavior rather than primary ASD-specific pathology. This issue remains a major challenge when attempting to determine causal relationships between dysbiosis and ASD symptomatology.

Several studies further suggest that microbiota alterations may correlate with symptom severity and GI burden rather than diagnosis alone [7]. Higher abundance of Lachnospiraceae and Erysipelotrichaceae, together with reduced *Faecalibacterium*, has been associated with more severe clinical phenotypes and GI symptoms [9]. These findings raise the possibility that the gut microbiome may act as a modifier of ASD phenotype, particularly in subgroups characterized by gastrointestinal dysfunction or inflammatory abnormalities. However, most available evidence remains cross-sectional, limiting interpretation of temporal or causal relationships.

Importantly, the heterogeneity across studies should not be interpreted solely as a methodological weakness. Differences in age, dietary exposure, antibiotic use, GI comorbidity, sequencing platforms, and ASD subgroup classification likely contribute substantially to variation in microbial findings [1, 7]. The absence of a single reproducible microbial signature therefore does not necessarily undermine

the relevance of the microbiome in ASD; rather, it suggests that ASD-related dysbiosis is embedded within a broader network of developmental, metabolic, environmental, and behavioral influences [3, 6].

Overall, current evidence supports the interpretation that gut microbiota alterations in ASD are common, biologically relevant, and clinically heterogeneous [1, 4, 7]. Rather than representing a definitive diagnostic biomarker, dysbiosis is more plausibly interpreted as a contributor to gastrointestinal dysfunction, immune dysregulation, metabolite imbalance, and variability in clinical presentation. However, because much of the evidence remains associative and methodologically heterogeneous, further longitudinal and mechanistic human studies are required before microbiota alterations can be translated into reliable biomarkers or therapeutic targets.

Microbiota-Gut-Brain Axis Mechanisms

The microbiota-gut-brain (MGB) axis has emerged as a major framework for explaining how gut dysbiosis may influence autism spectrum disorder (ASD). Rather than functioning as a single linear pathway, the MGB axis represents a multidirectional communication network linking the gut microbiota, intestinal barrier, immune system, endocrine pathways, enteric nervous system (ENS), and central nervous system (CNS) [2, 3]. Current evidence suggests that gut-brain communication occurs primarily through interconnected neural, neuroendocrine, immune, and metabolic pathways, providing a biologically plausible mechanism through which gastrointestinal dysfunction may influence neurodevelopment and behavior in ASD.

Neural communication occurs mainly through the vagus nerve, ENS, and autonomic pathways. The ENS regulates gastrointestinal function while maintaining continuous bidirectional signaling with the CNS, allowing gut-derived signals to influence neural activity and central outputs to regulate intestinal motility, secretion, permeability, and microbial ecology [3]. Experimental studies further suggest that microbiota manipulation may alter neurotransmitter release, stress responsiveness, and social or anxiety-

related behaviors through vagal signaling pathways [2]. However, most evidence supporting direct vagus-mediated microbiota-brain communication derives from animal models rather than definitive human studies. Consequently, although neural signaling pathways are biologically plausible, their precise contribution to ASD pathophysiology in humans remains uncertain.

The neuroendocrine pathway, particularly through the hypothalamic-pituitary-adrenal (HPA) axis, provides another important mechanism linking gut and brain function. Stress-related mediators such as corticotrophins-releasing hormone and cortisol may alter intestinal permeability, immune activity, and microbial composition, whereas microbial metabolites may influence enteroendocrine signaling and stress responses [2, 6]. This reciprocal relationship suggests that dysbiosis and stress physiology may reinforce one another, potentially contributing to epithelial dysfunction and inflammatory activation. However, the extent to which HPA-axis dysregulation directly mediates ASD symptomatology remains incompletely defined.

Among the proposed mechanisms, immune signaling currently provides one of the strongest links between gastrointestinal abnormalities and neurobehavioral manifestations in ASD. Under physiological conditions, the gut microbiota and mucosal immune system cooperate to maintain epithelial integrity and immune tolerance. Dysbiosis may disrupt this balance, increasing production of cytokines such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β), and interferon gamma (IFN- γ), together with greater exposure to bacterial products such as lipopolysaccharide (LPS) [1, 2]. These inflammatory mediators may influence blood-brain barrier permeability and microglial activation, thereby contributing to neuroinflammatory processes implicated in ASD [3, 6]. Nevertheless, much of the evidence connecting gut-derived inflammation directly to behavioral abnormalities remains inferential and heavily dependent on experimental or animal-based research. Human studies demonstrating direct mechanistic causality remain limited.

The metabolic pathway further supports the mechanistic relevance of the MGB axis. Gut microbes produce numerous bioactive compounds, including short-chain fatty acids (SCFAs), tryptophan metabolites, indoles, and neurotransmitter-related molecules that may influence epithelial integrity, immune regulation, mitochondrial function, neurotransmitter synthesis, and blood-brain barrier function [4]. However, the effects of these metabolites appear context-dependent rather than uniformly beneficial or harmful. Butyrate is generally considered protective because of its role in maintaining epithelial integrity and anti-inflammatory signaling, whereas excess propionate has been associated in animal models with mitochondrial dysfunction, oxidative stress, neuroinflammation, repetitive behaviors, and impaired social interaction resembling ASD-like phenotypes [3, 5]. Importantly, these findings are derived primarily from experimental models and therefore should not be interpreted as definitive evidence of causality in human ASD. Even so, they support the broader hypothesis that altered microbial metabolic signaling may contribute to neurodevelopmental and behavioral dysfunction.

Overall, the microbiota-gut-brain axis provides a plausible integrative framework linking gastrointestinal dysfunction, immune abnormalities, altered metabolites, and neurobehavioral manifestations in ASD [2, 3]. However, current evidence does not support a single universal mechanism operating uniformly across all individuals with ASD. Instead, gut-brain interactions may be most relevant in subgroups characterized by gastrointestinal dysfunction, altered intestinal permeability, inflammatory activation, or metabolomic abnormalities [6]. Thus, the MGB axis is best interpreted as a potential modifier of symptom severity and clinical phenotype rather than as a definitive causal explanation for ASD.

Intestinal Barrier, Immune Signaling, and Microbial Metabolites

A major hypothesis in autism spectrum disorder (ASD) research is that gut dysbiosis may influence symptom expression through disruption of intestinal barrier integrity, immune activation, and altered microbial metabolite production [1, 2]. These

processes are closely interconnected and collectively support the concept that gastrointestinal, immune, and neurobiological abnormalities in ASD may interact through shared pathophysiological pathways. Within this framework, the intestinal barrier serves as a critical interface regulating nutrient absorption, restricting translocation of luminal antigens, and maintaining mucosal immune homeostasis.

Current evidence suggests that increased intestinal permeability, commonly referred to as “leaky gut,” occurs more frequently in individuals with ASD, particularly among those with coexisting gastrointestinal (GI) symptoms [2]. Disruption of epithelial tight junctions may permit translocation of bacterial products, food-derived antigens, and inflammatory mediators into systemic circulation, thereby amplifying immune activation and inflammatory signaling. This mechanism is biologically significant because it provides a plausible route through which intestinal dysfunction may influence systemic and potentially neurobehavioral processes. However, most evidence supporting increased permeability in ASD remains associative, and it is still unclear whether barrier dysfunction represents a primary pathological mechanism or a secondary consequence of inflammation, diet, stress, or GI comorbidity.

Barrier dysfunction is closely linked to immune dysregulation, one of the most frequently reported biological abnormalities in ASD [1, 6]. Increased intestinal permeability may expose the immune system to bacterial components such as lipopolysaccharide (LPS), leading to elevated production of cytokines including interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β), and interferon gamma (IFN- γ) [2]. These inflammatory mediators may contribute to chronic low-grade systemic inflammation and potentially influence the central nervous system through altered blood-brain barrier permeability and microglial activation. Nevertheless, direct evidence linking gut-derived inflammation to ASD-related behavioral abnormalities in humans remains limited. Much of the mechanistic support for neuroimmune involvement derives from experimental and animal-based studies, highlighting the need for cautious translational interpretation [3].

Microbial metabolites represent another important mechanistic component of gut-brain interaction in ASD. Among these, short-chain fatty acids (SCFAs), particularly acetate, propionate, and butyrate, have received substantial attention because of their roles in epithelial integrity, immune regulation, mitochondrial activity, neurotransmitter synthesis, and microglial maturation [3]. However, their effects appear context-dependent rather than uniformly beneficial or harmful. Butyrate is generally considered protective because it supports epithelial barrier integrity and anti-inflammatory signaling. In contrast, excessive propionate has been associated in animal models with mitochondrial dysfunction, oxidative stress, neuroinflammation, repetitive behaviors, and impaired social interaction resembling ASD-like phenotypes [5]. Importantly, these findings derive primarily from experimental studies and should not be interpreted as definitive evidence of causality in human ASD.

In addition to SCFAs, other microbial products—including tryptophan metabolites, indoles, phenolic compounds, and neurotransmitter-related molecules—may influence serotonergic signaling, oxidative stress pathways, mitochondrial function, and neuroimmune communication [3, 4]. These findings reinforce the concept that microbial function may be more clinically relevant than microbial composition alone. Consequently, future ASD microbiome research will likely benefit from integrating metabolomics, immune profiling, and functional pathway analysis rather than relying solely on descriptive taxonomic profiling.

Overall, current evidence supports a model in which dysbiosis, epithelial barrier dysfunction, immune activation, and altered microbial metabolites interact through interconnected biological pathways rather than isolated mechanisms [2, 6]. This framework strengthens the interpretation of ASD as a condition involving systemic interactions among gastrointestinal, immune, metabolic, and neurobiological processes. However, because much of the evidence remains associative and mechanistic findings are frequently derived from preclinical models, the relative contribution of each pathway to human ASD pathophysiology remains uncertain. Further longitudinal and mechanistic human studies

are therefore required, particularly in relation to GI phenotype, inflammatory status, metabolomic abnormalities, and treatment responsiveness.

Therapeutic Potential of Microbiome-Based Interventions

Given the growing evidence linking gut dysbiosis, immune activation, intestinal barrier dysfunction, and altered microbial metabolites with autism spectrum disorder (ASD), microbiome-based interventions have emerged as potential adjunctive therapeutic strategies [1, 6]. Current approaches include probiotics, prebiotics and synbiotics, dietary modulation, and fecal microbiota transplantation (FMT) or microbiota transfer therapy (MTT), all of which aim to modify microbial composition and metabolic activity to improve gastrointestinal (GI) and possibly behavioral outcomes [8].

The rationale underlying these interventions is biologically plausible. If dysbiosis contributes to ASD through altered epithelial integrity, inflammatory signaling, and abnormal metabolite production, then restoring microbial balance could theoretically influence both GI and neurobehavioral processes [2, 3]. However, mechanistic plausibility should not be equated with clinical efficacy. Current evidence does not support microbiome-targeted therapies as established treatments for core ASD symptoms, and most reported benefits remain modest, variable, and concentrated primarily in GI outcomes [8].

Among available approaches, probiotics are the most extensively studied. Several reports suggest that probiotic supplementation may increase beneficial taxa such as *Bifidobacterium* and *Lactobacillus* while reducing GI symptom burden and, in some studies, improving selected behavioral measures [10, 11]. For example, studies summarized by Kortenien et al. (2023) reported increased *Bifidobacterium longum* abundance together with reductions in Autism Treatment Evaluation Checklist (ATEC) scores and GI symptoms following probiotic supplementation. Nevertheless, interpretation of these findings remains limited by small sample sizes, heterogeneous probiotic formulations, inconsistent outcome measures, and short intervention periods [7, 8]. In addition, few studies have included follow-up beyond six months,

leaving the durability and long-term clinical relevance of these effects uncertain.

Prebiotics and synbiotics may offer a stronger conceptual approach than probiotics alone because they aim not only to introduce exogenous organisms but also to support growth of beneficial resident microbes. In the systematic review by Tan et al. (2021), probiotics alone did not demonstrate consistent behavioral improvement, whereas prebiotic and synbiotic approaches appeared somewhat more promising for selective GI and behavioral outcomes. However, these findings remain preliminary and difficult to generalize because of methodological heterogeneity, limited cohort sizes, and variation in dietary background and intervention protocols.

Dietary modification represents another major microbiome-targeted strategy because diet is a key determinant of microbial composition and metabolic activity. Several studies suggest that gluten-free, casein-free, antioxidant-rich, or microbiome-supportive diets may improve GI symptoms and selected behavioral outcomes in some individuals with ASD [4, 6]. However, interpretation is complicated by the high prevalence of food selectivity, nutritional imbalance, and heterogeneous baseline diets among children with ASD. Consequently, reported improvements may reflect broader nutritional or behavioral effects rather than direct microbiome-mediated mechanisms alone.

The most biologically transformative but still highly preliminary approach involves FMT and MTT, which seek to restructure the gut microbial ecosystem more comprehensively than strain-specific supplementation. Open-label studies have reported increased microbial diversity together with improvements in GI symptoms and selected ASD-related behaviors following microbiota transfer procedures [6]. Conceptually, this approach is important because it targets dysbiosis at the ecosystem level rather than focusing on isolated microbial taxa. However, the evidence base remains limited by small sample sizes, lack of placebo-controlled blinding, short follow-up periods, and insufficient long-term safety data.

The clinical applicability of FMT/MTT also remains uncertain, particularly in pediatric populations. Donor screening, infectious risk assessment, stool processing standards, and regulatory oversight represent major translational challenges. Potential risks include transmission of infectious agents, unintended metabolic or immunological effects, and unpredictable long-term alterations in microbial ecology. Moreover, regulatory frameworks governing FMT vary substantially across countries, and FMT remains investigational rather than standard therapy for ASD in most clinical settings. Consequently, although FMT/MTT may represent one of the most promising microbiome-based strategies, routine clinical implementation remains premature.

Overall, the intervention literature supports cautious optimism but not practice-changing conclusions. The most reproducible benefits involve improvement of GI symptoms, whereas evidence regarding core behavioral outcomes remains inconsistent and substantially less robust [8]. This variability likely reflects both methodological limitations and the biological heterogeneity of ASD itself. Emerging evidence suggests that microbiome-based interventions may be most relevant in ASD subgroups characterized by significant GI dysfunction, inflammatory activation, altered short-chain fatty acid (SCFA) profiles, or metabolomic abnormalities [6]. Future studies may therefore benefit from patient stratification using clinically relevant phenotypes and biomarkers such as fecal calprotectin, inflammatory cytokines, intestinal permeability markers, and metabolomic signatures.

At present, microbiome-based interventions should be regarded as investigational or supportive approaches rather than established ASD therapies. Their principal value lies in targeting biologically plausible pathways and potentially contributing to personalized supportive care. However, meaningful clinical translation will require larger randomized controlled trials, standardized intervention protocols, longer-term follow-up, rigorous safety evaluation, and integration of microbiome, immune, and metabolomic profiling [1, 8].

Knowledge Gaps and Future Directions

Despite growing evidence linking the gut microbiome to autism spectrum disorder (ASD), major scientific and clinical uncertainties remain. The most important unresolved issue is causality. Most human studies demonstrate associations between dysbiosis, gastrointestinal symptoms, immune abnormalities, altered metabolites, and behavioral severity, but they do not establish whether microbiota alterations contribute directly to ASD pathophysiology or arise secondarily from restricted diet, medication exposure, gastrointestinal dysfunction, or other ASD-related factors [1, 2]. This issue is particularly important because food selectivity and limited dietary diversity are common in ASD and may independently influence microbial composition [6].

Another major limitation is the marked heterogeneity of microbiome findings. Although altered abundance of taxa such as Proteobacteria, Actinobacteria, *Sutterella*, *Clostridium*, and *Bifidobacterium* has been reported repeatedly, no consistent microbial signature has been reproduced across studies [7, 12]. This variability likely reflects both biological diversity and methodological differences, including sequencing platforms, dietary assessment, antibiotic exposure, and participant selection. In addition, many studies continue to treat ASD as a single diagnostic category despite its substantial clinical heterogeneity.

The field is also limited by the scarcity of longitudinal and mechanistic human studies. Much of the literature remains cross-sectional, restricting interpretation of temporal relationships between dysbiosis and symptom progression [3]. Similarly, many proposed mechanisms including vagal signaling, propionate-induced behavioral abnormalities, and microglial activation are supported primarily by animal or experimental models rather than direct human evidence [2, 5]. As a result, translation of these findings into human ASD biology remains incomplete.

Another important gap is the continued emphasis on descriptive taxonomic profiling rather than functional analysis. Microbial activity, including production of short-chain fatty acids (SCFAs), tryptophan metabolites, and inflammatory compounds, may be more clinically relevant than microbial composition alone [3, 5]. Future studies should therefore integrate metabolomics, immune profiling, and other multi-omics approaches to better define mechanistic pathways.

Evidence supporting microbiome-based interventions also remains preliminary. Although probiotics, dietary modification, and microbiota transfer therapies may improve gastrointestinal symptoms, findings remain inconsistent and are often derived from small, short-term, and methodologically heterogeneous studies [8]. Long-term efficacy, safety, and optimal patient selection remain unclear.

Future progress will require large longitudinal studies, improved patient stratification, integration of multi-omics approaches, and rigorous randomized controlled trials with standardized protocols and long-term follow-up. Greater focus on biologically relevant ASD subgroups such as individuals with significant GI dysfunction or inflammatory abnormalities may improve both mechanistic understanding and therapeutic targeting [6]. Overall, future research should focus less on identifying a single “autism microbiome” and more on understanding how microbial, immune, metabolic, and neurobehavioral pathways interact across distinct ASD phenotypes [3].

DISCUSSION

This review suggests that the relationship between gut microbiota and autism spectrum disorder (ASD) is biologically relevant but clinically heterogeneous. Three major themes emerged from the literature. First, individuals with ASD frequently exhibit alterations in microbial composition, although no single disease-specific microbial signature has been identified. Second, the microbiota–gut–brain (MGB) axis provides a plausible framework linking dysbiosis with gastrointestinal, immune, metabolic, and

neurobehavioral abnormalities. Third, microbiome-based interventions show potential therapeutic value, particularly for gastrointestinal (GI) symptoms, although current evidence remains insufficient to support routine clinical application [1, 2, 8]. Collectively, these findings support the interpretation that the gut microbiome is unlikely to represent a primary cause of ASD but may instead function as a modifier of symptom severity, inflammatory activity, and clinical phenotype.

The reviewed literature consistently demonstrates microbial differences between ASD and neurotypical populations [7, 12]. However, the most important conclusion is not the existence of an “autism microbiome,” but rather the presence of disrupted microbial ecology. Recurrent alterations involving Proteobacteria, Actinobacteria, *Sutterella*, *Clostridium*, and related taxa suggest that microbial imbalance may influence epithelial integrity, immune signaling, fermentation, and metabolite production [1, 4]. Nevertheless, taxonomic findings remain inconsistent across studies, likely reflecting differences in diet, GI phenotype, medication exposure, sequencing methodology, geography, and ASD heterogeneity itself [6]. Restricted dietary behavior, which is highly prevalent in ASD, further complicates interpretation because it may independently alter microbial composition. Consequently, current evidence supports dysbiosis as a context-dependent and potentially clinically relevant phenomenon rather than a definitive diagnostic biomarker.

The MGB axis provides a biologically plausible explanation for how gut dysbiosis could influence ASD-related physiology. Neural, endocrine, immune, and metabolic pathways collectively offer mechanisms linking gastrointestinal dysfunction with neurobehavioral manifestations [2]. Proposed mechanisms include vagal signaling, hypothalamic-pituitary-adrenal (HPA) axis dysregulation, inflammatory cytokine production, altered intestinal permeability, and abnormal short-chain fatty acid (SCFA) metabolism. However, much of the mechanistic evidence supporting these pathways derives from animal or experimental models rather than direct human studies [3, 5]. Therefore, although the MGB axis is conceptually compelling, its precise

contribution to human ASD pathophysiology remains incompletely established. Current evidence also suggests that gut-brain mechanisms may be particularly relevant in ASD subgroups characterized by significant GI dysfunction, inflammatory activation, increased permeability, or metabolomic abnormalities [6].

The intervention literature supports cautious optimism but not practice-changing conclusions. Probiotics, prebiotics, dietary modification, and fecal microbiota transplantation (FMT)/microbiota transfer therapy (MTT) are biologically attractive because they target pathways implicated in dysbiosis and immune dysfunction. However, the most reproducible benefits involve GI symptom improvement rather than core ASD behaviors. Reported behavioral effects remain inconsistent and may reflect indirect improvements mediated through reduced GI burden or altered inflammatory and metabolic signaling. In particular, FMT/MTT may suggest that ecosystem-level microbiome restructuring is more effective than isolated strain supplementation. Nevertheless, existing studies remain limited by small sample sizes, short follow-up periods, lack of placebo-controlled blinding, and insufficient long-term safety data [8]. Concerns regarding donor screening, infectious risk, regulatory oversight, and long-term safety further limit current clinical applicability, especially in pediatric populations.

An important implication of the reviewed literature is the need for patient stratification. ASD is biologically heterogeneous, and microbiome-related mechanisms are unlikely to operate uniformly across all individuals. Emerging evidence suggests that microbiome-targeted approaches may be most relevant in subgroups characterized by prominent GI dysfunction, inflammatory abnormalities, altered SCFA profiles, or metabolomic dysregulation [6]. Future studies may therefore benefit from distinguishing clinically meaningful phenotypes such as “high-GI ASD” and “low-GI ASD” while integrating biomarkers including fecal calprotectin, inflammatory cytokines, intestinal permeability markers, and metabolomic signatures.

Overall, current evidence supports the gut microbiome as a biologically relevant component of ASD pathophysiology, particularly through its interactions with gastrointestinal, immune, metabolic, and neurobehavioral systems. However, the evidence base remains heterogeneous, largely associative, and heavily dependent on cross-sectional and preclinical studies. Future progress will require larger longitudinal human studies, improved mechanistic characterization, integration of multi-omics approaches, rigorous randomized controlled trials, and clearer identification of biologically relevant ASD subgroups. Such advances will be essential for translating microbiome research into clinically meaningful and evidence-based therapeutic strategies

CONCLUSION

This review highlights that gut microbiota alterations are common in autism spectrum disorder (ASD), although the reported microbial patterns remain clinically and biologically heterogeneous across studies. Current evidence supports the microbiota–gut–brain axis as a plausible framework linking dysbiosis with neural, immune, endocrine, and metabolic pathways, suggesting that gut microbial imbalance may contribute to gastrointestinal dysfunction, inflammatory activity, metabolite dysregulation, and variability in symptom expression [2, 3]. However, the available evidence remains largely associative, and many mechanistic pathways continue to rely heavily on animal and experimental models rather than direct human evidence.

Microbiome-based interventions, including probiotics, dietary modification, and microbiota transfer approaches, show potential therapeutic value, particularly for gastrointestinal symptoms. Nevertheless, current findings remain inconsistent and insufficient to support routine clinical application [8]. The most defensible interpretation is therefore not that ASD is caused by a single “autism microbiome,” but rather that the gut microbiome may function as a biologically relevant and clinically heterogeneous modifier of ASD phenotype and symptom severity.

Importantly, the future of this field will likely depend on identifying biologically meaningful ASD subgroups and integrating microbiome, metabolomic, and immune biomarkers into more precise and personalized therapeutic frameworks. A deeper understanding of how microbial ecology interacts with gastrointestinal, immune, metabolic, and neurobehavioral pathways may ultimately contribute to more targeted supportive strategies for individuals with ASD.

RECOMMENDATION

Future research should prioritize several key areas. First, large longitudinal human studies are needed to clarify whether dysbiosis precedes symptom progression or instead reflects secondary effects of ASD-related behaviors, diet, medication exposure, or gastrointestinal dysfunction. Second, future work should emphasize patient stratification because microbiome-related mechanisms and therapeutic responses are unlikely to operate uniformly across all individuals with ASD. Greater attention should be given to clinically relevant subgroups defined by gastrointestinal phenotype, inflammatory status, metabolomic profile, and dietary characteristics.

Third, future studies should integrate microbiome sequencing with metabolomics, immune profiling, and other multi-omics approaches to improve mechanistic understanding beyond descriptive taxonomic analysis. Finally, microbiome-based interventions should be evaluated through larger randomized controlled trials using standardized protocols, longer follow-up periods, rigorous safety monitoring, and clinically meaningful outcome measures. Such approaches will be essential for determining not only whether microbiome-targeted therapies are effective, but also which patients are most likely to benefit and through which biological mechanisms.

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