Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Gut Microbiota Alterations in Children with Autism Spectrum Disorder: A Systematic Review of a Decade of Evidence (2015–2025)

Andi Alfia Muthmainnah Tanra[1,2], Martira Maddeppungeng[1,3], Ema Alasiry[1,3], Hadia Angriani[1,3], Setia Budi Salekede[1,3], Urfianty[1,3], Merlyn Meta Astari[1]

[1]Department of Pediatrics, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia;

[2]Department of Pharmacology, Faculty of Medicine, Universitas Tadulako, Palu, Indonesia;

[3]Department of Pediatrics, Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia

Andi A. M. Tanra:- Email ID: alfiamuthmainnah@yahoo.com

Martira Maddeppungeng:- Email ID: martira711@yahoo.com ORCID ID: 0000-0002-1723-2268

Ema Alasiry:- Email ID: alasiryema@yahoo.com ORCID ID: 0000-0001-6166-9902

Hadia Angriani:- Email ID: hadia_bika@yahoo.co.id

Setia Budi Salekede:- Email ID: budisalekede@yahoo.com

Urfianty:- Email ID: urfiantysaid@yahoo.com

Merlyn Meta Astari:- Email ID: merlyn290480@gmail.com

[*]**Corresponding author:**

Andi Alfia Muthmainnah Tanra

Department of Pediatrics, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia, Jalan Perintis Kemerdekaan KM 11, Makassar, South Sulawesi, 90245, Indonesia

Email ID: alfiamuthmainnah@yahoo.com

Cite this paper as: Andi Alfia Muthmainnah Tanra, Martira Maddeppungeng, Ema Alasiry, Hadia Angriani, Setia Budi Salekede, Urfianty, Merlyn Meta Astari, (2025) Gut Microbiota Alterations in Children with Autism Spectrum Disorder: A Systematic Review of a Decade of Evidence (2015–2025). *Journal of Neonatal Surgery*, 14 (32s), 5017-5025.

ABSTRACT

Background: Children with Autism Spectrum Disorder (ASD) often experience gastrointestinal symptoms, prompting interest in the gut–brain axis and the potential role of gut microbiota in ASD pathology. To systematically review human studies from 2015–2025 comparing gut microbiota in children with ASD to neurotypical controls.

Methods: We reviewed global observational and clinical studies involving children aged 2–18 that assessed gut microbiota using DNA-based methods, primarily 16S rRNA sequencing. Outcomes included microbial diversity (alpha and beta) and taxonomic composition.

Results: Most studies reported altered gut microbiota in ASD. Alpha-diversity was frequently lower in ASD, indicating reduced microbial richness, though not universally. In contrast, beta-diversity consistently showed distinct microbial community structures between ASD and control groups. Common compositional findings in ASD included reduced Bifidobacterium and Prevotella, and increased Clostridium, Desulfovibrio, Sutterella, and other Proteobacteria. These shifts may contribute to immune activation, GI inflammation, and neuroactive metabolite production (e.g., short-chain fatty acids, lipopolysaccharide), potentially influencing ASD symptoms.

Conclusions: Evidence supports the presence of gut dysbiosis in children with ASD, marked by decreased beneficial microbes and increased potentially pro-inflammatory taxa. While findings vary by region and methodology, the microbiota appears to play a role in ASD via metabolic and immune-mediated mechanisms. The gut microbiome may serve as both a biomarker and therapeutic target in ASD, but further large-scale, standardized studies are needed.

Keywords: *Autism Spectrum Disorder, gut microbiota, dysbiosis, children, 16S rRNA, microbial diversity.*

1. INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction, communication, and restricted or repetitive behaviors. Beyond these core symptoms, many children with ASD experience gastrointestinal (GI) disturbances – including constipation, diarrhea, abdominal pain, and other functional GI disorders – with prevalence estimates up to 70%. This overlap has fueled interest in the gut–brain axis, the bidirectional communication network between the gut microbiome, the GI tract, and the central nervous system.[1] The gut microbiota can influence immune function, neurotransmitter production, and metabolic signaling, all of which may impact brain development and behavior. Over the past decade, a growing body of research has focused on whether children with ASD harbor a distinct gut microbiota profile (often termed "dysbiosis") compared to neurotypical children, and how such differences might contribute to or result from ASD pathology.[2]

Should alterations in the gut microbiota transpire, this may therefore modify the synthesis of microbial metabolites [3]. Gut microbial metabolites comprise short-chain fatty acids and micronutrients vital for human health. Additional microbial products encompass molecules that function as neurotransmitters, potentially affecting synaptic activity and communication with the brain.[4] Gastrointestinal diseases and alterations in gut microbiota are recognized to influence serotonin transmission in both the gut and the brain.[5] Serotonin and other microbial metabolites, including gamma-aminobutyric acid (GABA), function as neurotransmitters, with the vagus nerve serving as a direct conduit for communication between the brain and gut.[5,6] Researchers have induced stress in mice to evaluate alterations in the gut microbiota, illustrating the brain's influence on the stomach, which may result in gut barrier disruption and inflammation due to immunological responses in the gut.[7] Anxiety-related behaviors, together with abnormalities in gut physiology and immunological development, are prevalent in germ-free mice maintained in completely sterile settings without exposure to bacterial sources.[8] Specific pathogen-free mice possess gut microbes yet are devoid of a designated array of specific pathogens. In contrast to germ-free mice devoid of gut bacteria, these mice display significant variations in behavior and neurochemical communication, indicating the critical role of gut microbial interactions.[9]

2. METHODS

Literature Search and Inclusion: We conducted a comprehensive review of human studies from 2014 to 2024 that compared the gut microbiota of children (generally ages 2–18) with ASD to that of children without ASD (neurotypical controls) (Table 1). Both observational (cross-sectional or case–control) studies and baseline data from clinical trials were included, provided they reported gut microbiota composition or diversity in pediatric ASD vs. non-ASD groups. Animal studies and adult cohorts were excluded to focus on human pediatric data, and we concentrated on papers from diverse geographic regions to capture global trends. Key sources included published systematic reviews, meta-analyses, and original research articles from this period[1,2,10].

Microbiota Analysis Methods: Nearly all studies relied on culture-independent, DNA-based profiling of the fecal microbiota. The most common approach was 16S rRNA gene sequencing of stool samples, targeting various hypervariable regions (e.g., V3–V4 or V4–V5).[10,11] This method provides taxonomic relative abundance data for bacteria present. A few studies supplemented this with quantitative PCR for specific taxa or used shotgun metagenomic sequencing to characterize the microbiome's functional potential. Differences in laboratory methodologies – such as DNA extraction protocols, 16S primer regions, and bioinformatics pipelines – were noted as potential sources of heterogeneity across studies. Most studies analyzed fecal samples, though a few examined mucosal biopsies from the gut.[11]

Data Extraction: From each study, we extracted sample size and demographics, region/country, sequencing or detection method, and the main outcomes regarding microbiota composition and diversity metrics. Specifically, we gathered information on (a) microbial diversity (alpha-diversity within samples and beta-diversity between groups), (b) microbiota composition differences at various taxonomic levels (phyla, genera, and species where available), and (c) any noted correlations with clinical features (e.g., GI symptom severity or ASD behavioral severity). Our goal was to synthesize consistent patterns and noteworthy discrepancies in gut microbiota findings for ASD vs. neurotypical children over the last decade.

3. RESULTS

Diversity of Gut Microbiota in ASD vs. Neurotypical Children

Alpha-Diversity: Alpha-diversity refers to the richness and evenness of species within a single sample. Many studies reported that children with ASD have a lower gut bacterial alpha-diversity on average than non-ASD children, suggesting a less diverse gut ecosystem, although findings are not universal. For example, an analysis of Chinese children (45 ASD vs. 45 controls) found significantly reduced species richness and diversity in ASD, as measured by phylogenetic diversity indices.[12] Similarly, a study of autistic children with chronic constipation reported lower observed species counts and Chao1 indices compared to typical children.[13] Several other cohorts have echoed this trend of decreased alpha-diversity in ASD, linking it to GI dysfunction severity in some cases.[1,14] However, a notable minority of studies found no significant

alpha-diversity difference between ASD and controls.[15] For instance, an Italian study (40 ASD vs. 40 controls) observed comparable alpha-diversity between groups despite clear compositional differences.[16] In one early investigation using sibling controls, alpha-diversity also did not differ substantially between autistic children and their neurotypical siblings, hinting that shared household factors (diet, environment) might normalize microbial richness. Interestingly, an older study in Italy even reported higher alpha-diversity in ASD children compared to siblings, though this finding stands in contrast to most other reports. Overall, while reduced gut microbial diversity in ASD is a common observation, it is not an invariant feature across all populations. Discrepancies may stem from small sample sizes and confounding factors like diet (many ASD children have restricted diets, which can independently lower gut microbial diversity).[10,16]

Beta-Diversity: Beta-diversity assesses differences in community composition between groups. Despite mixed alpha-diversity results, there is strong consensus that the overall gut microbiota composition (beta-diversity) of children with ASD differs significantly from that of neurotypical children. In roughly two-thirds of studies that measured it, fecal microbial communities from ASD children clustered apart from controls on principal coordinates analyses, indicating distinct community structures. This separation has been demonstrated using metrics like unweighted/unweighted UniFrac and Bray–Curtis distances in numerous cohorts across different countries. In practical terms, an ASD child's microbiome is often compositionally more similar to other ASD microbiomes than to those of neurotypical peers, even if species counts overlap. Notably, microbiota profile differences have been observed even when comparing ASD children to their own siblings, although sibling-pair studies sometimes show more subtle differences than case–control studies with unrelated controls.[10] The consistent finding of altered beta-diversity underscores that microbial community composition is altered in ASD, even if researchers do not always agree on which specific microbes are responsible.[2,10]

4. GUT MICROBIOTA COMPOSITION DIFFERENCES IN ASD

Numerous specific taxonomic differences in gut bacteria have been reported between ASD and neurotypical children. However, results at the genus or species level have sometimes been inconsistent across studies, likely due to geographic, dietary, and methodological differences. Below we summarize the most commonly reported microbiota alterations, noting consistent patterns and conflicting findings.[10]

Phylum-Level Shifts: The two dominant bacterial phyla in the human gut (Firmicutes and Bacteroidetes) have been a focus of many studies. A frequent observation is an imbalance in the Firmicutes-to-Bacteroidetes ratio in ASD. Many (though not all) studies found ASD children carry a higher relative abundance of Firmicutes and/or lower Bacteroidetes, resulting in an elevated Firmicutes:Bacteroidetes ratio.[10] For example, Strati et al. reported a significantly higher F:B ratio in Italian children with ASD due to a marked depletion of Bacteroidetes (roughly 9% of sequences in ASD vs. 19% in controls).[16] Likewise, a meta-analysis noted increased Firmicutes and decreased Bacteroidetes in ASD guts in over half of studies examined.[1] Reduced Bacteroidetes in ASD is often attributable to the loss of certain fiber-degrading genera (like Prevotella, discussed below). On the other hand, a few studies have found the opposite or no phylum difference. De Angelis et al. observed more Bacteroidetes (and relatively fewer Firmicutes) in ASD children than in sibling controls – an opposite trend that highlights population differences. Additionally, some reports from Asia did not find significant phylum-level shifts after adjusting for diet and other confounders.[10,12] Beyond Firmicutes/Bacteroidetes, another phylum often implicated is Proteobacteria (a group containing many Gram-negative bacteria). Multiple studies have shown Proteobacteria to be increased in ASD relative to controls.[10] For instance, elevated levels of Proteobacteria (including families like Enterobacteriaceae) in ASD were consistently noted in early studies, and a systematic review identified Proteobacteria overabundance as one of the most reproducible findings in ASD microbiome research.[2,10] An increase in Actinobacteria phylum in ASD has also been reported in some datasets, although this is somewhat counterintuitive given that the beneficial genus Bifidobacterium (part of Actinobacteria) is often decreased in ASD (suggesting other Actinobacteria like Collinsella might be driving that increase).[2]

Genus- and Species-Level Differences: At finer taxonomic resolution, certain genera repeatedly emerge as differentially abundant in ASD children versus peers:

Bifidobacterium – Perhaps the most consistently reported change is a depletion of Bifidobacterium (a beneficial, fiber-fermenting genus) in ASD. Four independent studies in a 2019 review found significantly lower fecal Bifidobacterium in ASD children.[10] Low Bifidobacteria may contribute to GI dysfunction and has been linked to reduced production of short-chain fatty acids like acetate. Notably, some interventions that increase Bifidobacterium (e.g. certain probiotics or prebiotics) have normalized aspects of the ASD microbiome.[15]

Prevotella and Other Carbohydrate-Fermenting Genera – Numerous studies have noted that Prevotella, a genus common in high-fiber diets, is significantly reduced or nearly absent in many ASD children.[10,16] For example, Prevotella was barely detectable in one ASD cohort (0.05% of sequences, vs ~1.5% in controls).[16] Along with Prevotella, other genera that encompass fiber-fermenting, short-chain-fatty-acid-producing bacteria – such as Dialister, Blautia, Veillonella, and Turicibacter – have frequently been found at lower relative abundance in ASD. These genera are thought to support gut health (many produce butyrate or propionate), so their reduction might impact gut metabolic output in ASD. However, the exact implications of lower Prevotella and others remain under investigation. Some data suggest diet plays a role: children

with ASD often consume less diverse diets (e.g. less dietary fiber), which could directly explain the reduced prevalence of these fiber-utilizing microbes.[10]

Lactobacillus – Lactobacillus (a lactic acid bacterium) has shown contradictory trends. Several studies, including one from Slovakia, found increased Lactobacillus in ASD gut communities. Tomova et al. reported elevated levels of Lactobacillus spp. in autistic children compared to both siblings and unrelated controls.[15] This might reflect some ASD children receiving probiotic supplements or having diets (e.g. high in simple sugars) favoring lactobacilli. On the other hand, at least one recent study (in constipated ASD children) noted decreased Lactobacillus relative to controls. Thus, the Lactobacillus finding is inconsistent – it may increase in some ASD subgroups and decrease in others, possibly depending on diet, GI transit time, or probiotic use.[13]

Clostridium and Clostridiales – Several members of the Clostridiales order have been associated with ASD. Increased Clostridium (in a broad sense or specific species like Clostridium perfringens) is a recurrent finding.[10,15] Five studies summarized in one review all reported a significant rise in Clostridium (variously classified) in ASD children.[10] Certain Clostridia can produce toxins and fermentative byproducts that affect the host. Notably, higher abundance of C. perfringens was correlated with more severe ASD symptoms in at least one study.[1] Clostridiales clusters (including Clostridium, Ruminococcus, etc.) have drawn attention for their potential to produce propionate and p-cresol (see Discussion). It's worth mentioning that not all Clostridia are elevated – some butyrate-producing Clostridia (like Faecalibacterium or Roseburia) have been reported as decreased in some ASD cohorts (though increased in others), reflecting the diverse roles within this bacterial group.[10]

Bacteroides – Bacteroides (a genus in Bacteroidetes) has also shown mixed results. In several studies, Bacteroides was increased in ASD relative to controls, contributing to the higher overall Pseudomonadota/Proteobacteria and possibly compensating for lower Prevotella.[10] A meta-analysis in 2020 found Bacteroides levels significantly higher in ASD children than controls. However, at least one meta-analysis (2019) conversely reported Bacteroides as lower in ASD.[17] These discrepancies likely arise from differences in cohorts (diet, geography) – for instance, Western vs. East Asian diets might differentially affect Bacteroides populations.[18]

Desulfovibrio and Sutterella (Proteobacteria genera) – Several studies have flagged Proteobacterial genera that are over-represented in ASD. Desulfovibrio, a sulfate-reducing bacterium, was found at higher abundance in ASD in multiple reports.[10] Tomova et al. observed a trend toward increased Desulfovibrio in autistic children, with levels strongly correlating to autism severity (particularly repetitive behavior scores).[15] Sutterella, a genus in the family Sutterellaceae (order Burkholderiales), has also been noted as elevated in ASD guts.[2,10] Sutterella was initially implicated by studies of intestinal biopsies in ASD children with GI issues and has since been reported in stool-based studies – a 2024 review identified higher Sutterella as one of the more consistent taxa differences in ASD.[2] That said, not all studies concur (one study found Sutterella decreased in ASD), but the weight of evidence leans towards an increase. Both Desulfovibrio and Sutterella are suspected to contribute to GI inflammation or barrier dysfunction, which might link them to ASD GI symptoms (discussed later).[10]

Other notable genera: Akkermansia, a mucus-degrading genus, has been inconsistently reported – some sequencing-based studies found it elevated in ASD, whereas one qPCR-based study found it lower in ASD. Faecalibacterium (a major butyrate producer) was reported as increased in a few studies of ASD children but decreased in another; likewise, Ruminococcus showed both increases and decreases across different studies. Blautia, Veillonella, and Dialister generally seem to be reduced in ASD cohorts (often alongside Prevotella, as many are linked to carbohydrate fermentation). Opportunistic or potentially pathogenic bacteria have occasionally been noted: for instance, Enterobacter and Shigella (both in Enterobacteriaceae) were found at higher levels in ASD vs. controls in one study, which could relate to gut inflammation. Table 1 provides a summary of representative studies from the past decade, illustrating these microbial differences across various populations.[10]

Regional and Cohort Variability

It is important to emphasize that geographical and cohort differences influence gut microbiota findings. Studies from North America, Europe, and Asia have all identified dysbiosis in ASD, but the exact changes often vary. Dietary patterns are a major factor – for example, children in Italy or the U.S. might have different baseline gut microbes than children in China, and ASD-related shifts can manifest differently on those distinct backgrounds. Some regional examples: a Chinese study found clear microbiota differences (lower diversity and reduced levels of certain beneficial Lachnospiraceae genera in ASD) but no significant phylum-level changes, whereas European studies often reported phylum shifts like altered F:B ratios.[10,12,16] Within-country variations also exist; for instance, one U.S. study focusing on children with ASD who had GI pain reported distinct microbiome "signatures" tied to GI symptoms, while another U.S. cohort without stratification by GI issues still showed differences but of a different nature.[10] The type of control group matters too: some studies used healthy unrelated children as controls, while others used unaffected siblings of ASD children. Sibling-controlled studies (e.g. Son et al. 2015 in the US) generally found fewer differences in microbiota, likely because siblings share diet and environment.[16] In contrast, using community controls may highlight more differences but could be confounded by diet disparities. Variation in GI comorbidities also contributes to findings – for example, ASD children with chronic constipation

may show different microbial patterns (such as the propionate-associated profile noted in one study) compared to ASD children without GI symptoms.[13] Given these sources of heterogeneity, it is not surprising that specific results differ across studies. Nevertheless, as summarized above, there are recurring themes (e.g., lower Bifidobacterium, lower Prevotella, higher certain Clostridia and Proteobacteria) that emerge in multiple regions.[10,16] Table 1 below highlights a selection of key studies from various countries, illustrating both common patterns and divergent results in gut microbiota profiles of ASD vs. neurotypical children.

Table 1. Summary of Selected Studies (2014–2024) Comparing Gut Microbiota in Children with ASD vs. Neurotypical Controls

Study (Year)	Population/Sample	Key Findings (ASD vs Controls)
Tomova et al. (2015) [15]	Case-control (Slovakia); 10 ASD vs. 10 controls (plus 9 ASD siblings)	Lower Bacteroidetes/Firmicutes ratio in ASD (due to reduced Bacteroidetes); higher Lactobacillus in ASD; trend toward increased Desulfovibrio in ASD, which correlated with greater autism severity. Noted GI symptoms severity was linked to microbiota changes.
Strati et al. (2017) [16]	Case-control (Italy); 40 ASD vs. 40 neurotypical controls	No significant alpha-diversity difference; Distinct beta-diversity (ASD microbiomes clustered separately). Higher Firmicutes:Bacteroidetes ratio in ASD due to a marked decrease in Bacteroidetes (ASD had ~9% vs 19% in controls). Notably, Prevotella almost absent in ASD group. Also observed various genus-level shifts (e.g., lower Bifidobacterium and Prevotella, higher some Clostridium spp. in ASD).
Ma et al. (2019) [12]	Case-control (China); 45 children with ASD (6–9 years of age; 39 boys and 6 girls) and 45 sex- and age-matched neurotypical children	Lower alpha-diversity in ASD (reduced richness); Altered beta-diversity (community composition differed). No significant phylum-level differences after adjusting for diet. Depletion of certain beneficial taxa in ASD, including lower families like Acidaminococcaceae and genera in Lachnospiraceae (e.g., Lachnoclostridium and others). Suggested a dysbiosis characterized by loss of some butyrate-producers in ASD.
Iglesias-Vázquez et al. (2020) [17]	Systematic review & meta-analysis (18 studies; total 493 ASD children, 404 controls)	ASD children had higher relative abundance of Bacteroidetes, Firmicutes, and Actinobacteria phyla (on average) than controls. At genus level, significantly higher Bacteroides, Parabacteroides, Clostridium, Faecalibacterium, Phascolarctobacterium in ASD, and lower Coprococcus and Bifidobacterium in ASD. Overall indicates a dysbiotic shift with both Bacteroidetes and Firmicutes members increased in ASD.
Chen et al. (2021) [19]	Case-control (China); 138 ASD (divided into ASD with developmental delay vs. ASD-only) vs. 119 control children (typical and developmental delay controls)	Lower alpha diversity in ASD groups (significantly reduced Shannon, Chao1, etc., in ASD). Gut community composition differed markedly (beta diversity separation). ASD children's stool had depleted Bifidobacterium, Prevotella, Blautia, Dialister and enriched Clostridium, Bacteroides, Faecalibacterium compared to controls. Diversity and certain genera (e.g., Bacteroides, Faecalibacterium) showed correlations with ASD symptom severity.
Ding et al. (2021)[11]	Case-control (China); 25 ASD vs. 20 neurotypical controls	No significant difference in alpha diversity between ASD and controls (Shannon and richness indices similar). However, significant differences in composition: ASD microbiota clustered separately (distinct beta diversity) and showed higher relative Firmicutes and lower Actinobacteria compared to controls. Notably, Actinobacteria (e.g., Bifidobacterium) were reduced in ASD. Authors describe an overall gut dysbiosis in ASD, and GI symptom scores were positively associated with the degree of microbiota

		imbalance.
Abuljadayel et al. (2024)[20]	Case-control (Saudi Arabia); 4 ASD children vs. 4 healthy sibling controls (3–10 years old)	Found greater microbial richness and diversity in ASD (ASD had higher observed species count). The ASD gut microbiome had dominance of Firmicutes and Proteobacteria phyla. At lower taxa: higher levels of Lactobacillaceae (family) and Bacteroides genus in ASD; lower levels of Bifidobacterium and Prevotella in ASD. Concluded that ASD children harbored more pathogenic or opportunistic genera and a different functional profile than siblings, though sample size was very small.

Note: Key findings are summarized from each study with citations to results in published papers or reviews.

Consistent Patterns vs. Discrepancies

Across these studies, several consistent patterns emerge: ASD children frequently show reduced levels of beneficial gut microbes like Bifidobacterium and Prevotella, and enrichment of certain potentially pro-inflammatory or less common taxa such as Clostridium, Desulfovibrio, Sutterella, and some Proteobacteria.[10] Many ASD cohorts also have a skewed overall community (reflected in altered F:B ratio and lower diversity) compared to controls.[1,10] These recurrent findings hint at a characteristic "dysbiosis" associated with ASD. On the other hand, there are notable discrepancies: for example, whether Bacteroidetes is decreased (majority of studies) or increased (a few studies) in ASD, or whether alpha-diversity is universally lower in ASD (often, but not always). Such differences likely result from heterogeneity in study designs and populations. Factors like dietary habits, regional microbiome baselines, use of siblings vs. unrelated controls, sample size, and even technical methods can all influence outcomes. Additionally, ASD itself is a spectrum – varying severity, comorbidities (e.g., intellectual disability or GI disorders), and prior treatments (antibiotics, probiotics) can all impact a child's gut flora.[10] Some discrepancies might reflect that subgroups of ASD have distinct microbiota profiles ("responders" vs "non-responders" to dysbiosis, or differences between ASD children with GI issues and those without). Indeed, an emerging view is that there may not be a single "ASD microbiome," but rather several microbiome phenotypes within ASD that researchers are just beginning to characterize.[19] Despite these complexities, the overarching takeaway is that the gut microbiota in children with ASD tends to differ from that of neurotypical children, supporting the idea that gut microbial communities are linked to the disorder in some capacity.[2]

5. DISCUSSION

The accumulated evidence over the last decade strongly suggests an association between gut microbiota alterations and ASD in children. The consistent patterns of dysbiosis – such as loss of certain beneficial microbes and gains in certain anaerobes or opportunists – may have important implications for the gut–brain axis in ASD pathology (Figure 1). Several hypotheses can be drawn to explain how these microbial differences might influence ASD symptoms or development:

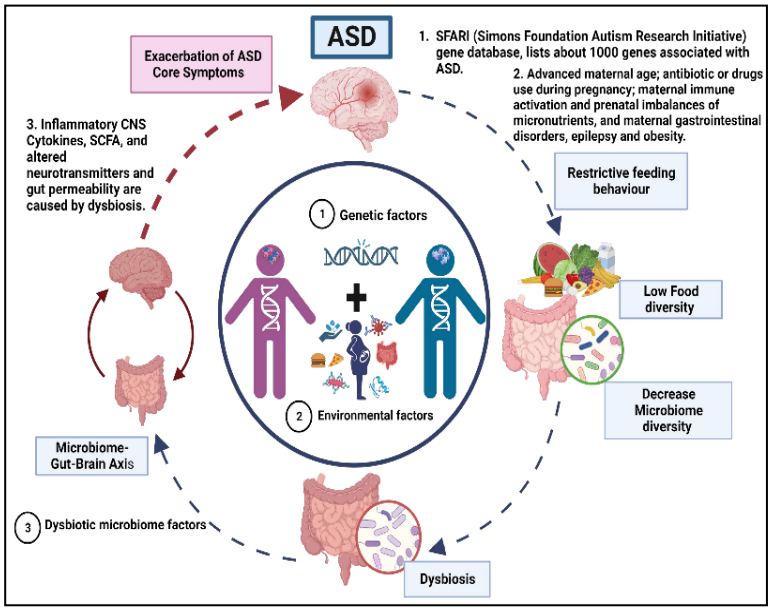

Figure 1. Gut brain axis in ASD. [21]

Microbial Metabolites and Neuroactive Compounds: Many gut bacteria produce metabolites that can affect the host's nervous and immune systems. For instance, children with ASD often harbor higher levels of Clostridia (including Clostridium spp.), which are known to produce short-chain fatty acids ([22]s) like propionate and butyrate, as well as potentially neurotoxic compounds. Elevated propionic acid in the gut has been hypothesized to contribute to ASD symptoms: animal models injecting propionate can induce autism-like behaviors, and one human study found excess fecal propionate in ASD children with GI issues, correlating with more severe ASD behaviors.[10] The depletion of butyrate-producing bacteria (like Faecalibacterium or some Lachnospiraceae) in many ASD microbiomes could mean lower levels of butyrate, a SCFA crucial for gut epithelial health and anti-inflammatory effects in the colon. Imbalances in SCFA profiles (too much propionate, not enough butyrate) might influence gut barrier integrity and neuroinflammatory signaling in ASD.[10,12]

Immune Activation and Inflammation: The frequent finding of increased Proteobacteria (e.g., Desulfovibrio, Enterobacteriaceae) in ASD is noteworthy because many Proteobacteria are Gram-negative and produce lipopolysaccharide (LPS) in their cell walls. LPS can trigger systemic inflammation. Higher Proteobacteria in ASD guts might contribute to a pro-inflammatory state – indeed, LPS from gut microbes can prompt immune responses that potentially affect neurodevelopment. One review noted that an abundance of Proteobacteria in ASD is associated with inflammatory processes, as LPS can alter brain antioxidant levels and immune function. For example, in animal studies LPS from gut microbes decreased brain glutathione and caused immune dysregulation similar to that seen in ASD. Thus, an ASD microbiome enriched in LPS-producing bacteria could conceivably influence the developing brain through immune-mediated pathways. Additionally, some ASD studies have found direct correlations between gut microbiota profiles and peripheral immune markers or GI inflammation. The presence of pro-inflammatory gut bacteria could exacerbate GI symptoms (common in ASD) which themselves correlate with worse behavioral outcomes, forming a vicious cycle linking gut dysbiosis, inflammation, and behavior.[14,15]

Gut Barrier and Metabolic Function: Several gut microbes implicated in ASD can affect the intestinal barrier and metabolic function. Desulfovibrio, for example, produces hydrogen sulfide which at high levels can damage the gut lining. Sutterella, often higher in ASD, has been linked to IgA deficiencies and intestinal inflammation in other contexts.[2] A "leaky gut" (increased intestinal permeability) has been reported in some individuals with ASD, which might allow microbial metabolites or fragments (like LPS) to more readily enter circulation and impact the nervous system. Although causal links are unproven, one can speculate that dysbiosis in ASD could contribute to a leaky gut and abnormal metabolic signaling to the brain.[10]

Correlation vs. Causation: It is crucial to note that most studies to date are cross-sectional, meaning they show association but not causation. Children with ASD often have restricted diets (e.g., preference for certain foods, avoiding others), which could cause some of the microbiome differences – for instance, low fiber intake could explain reduced Prevotella and Bifidobacterium. Additionally, many ASD children have taken frequent antibiotics (for infections or GI issues) or probiotics, which could directly shape their microbiota. So, an altered microbiome might be partly a consequence of ASD behaviors or treatments, rather than a cause. Researchers are actively exploring this chicken-and-egg problem. Intriguingly, some recent experiments provide evidence that the microbiota can influence neurobehavioral outcomes: one study transplanted gut microbes from ASD children into germ-free mice, and those mice developed autism-like behaviors, unlike mice colonized with microbes from neurotypical children. This provocative finding suggests the microbiome can contribute to ASD-related traits (at least in an animal model), lending weight to a causal hypothesis. Still, such results must be interpreted cautiously; they highlight potential microbiome-driven mechanisms but do not prove that microbiome alterations initiate ASD in humans.[19]

Toward Therapeutic Interventions: The gut–brain axis link in ASD has spurred investigations into microbiome-targeted therapies. If certain microbial imbalances exacerbate ASD symptoms, then correcting those imbalances might offer relief. Trials of probiotics (live beneficial bacteria) and prebiotics (fiber supplements to nourish good microbes) in ASD have shown promising though mixed results. For example, probiotic supplementation has been reported to increase Bifidobacterium and Prevotella levels and even modestly improve GI and behavioral symptoms in some studies. Fecal microbiota transplantation – termed Microbiota Transfer Therapy (MTT) – was tested in an open-label study where ASD children received gut microbiota from healthy donors; the results indicated long-term increases in microbial diversity and beneficial bacteria, alongside improvements in GI and ASD symptoms that persisted for two years post-treatment. While these early therapeutic studies need confirmation in larger controlled trials, they underscore the principle that modulating the microbiota can influence ASD-related outcomes. This is strong, albeit indirect, evidence that the gut microbiome is not just a bystander but likely an active player in the gut–brain axis of ASD.[1]

It should be acknowledged that studies of the ASD gut microbiome face several limitations. Sample sizes in many studies have been relatively small (dozens of children), making it hard to generalize findings or detect subtle effects. There is also considerable heterogeneity – ASD is a spectrum with genetic and environmental diversity, and microbiome profiles are likewise influenced by many factors. The lack of consistency in some results (e.g., one study finds a microbe up, another finds it down) reflects this complexity.[10] Additionally, many studies did not control rigorously for diet, antibiotic history, or GI symptoms, which can confound interpretations. There is a need for more longitudinal studies (following children over time) to determine if microbiota changes precede ASD symptom changes or vice versa. Despite these challenges, the

recurring observations of dysbiosis cannot be dismissed as noise – rather, they call for more standardized, multi-center research. Large-scale studies controlling for diet and environment, and integrating other data (metabolites, immune markers, genetics), will be crucial to untangle cause and effect and to identify whether a core "microbial signature" of ASD truly exists.

6. CONCLUSION

In summary, a decade's worth of research from around the globe indicates that children with ASD frequently exhibit an altered gut microbiota compared to non-ASD children. Common findings include a trend toward lower microbial diversity, reduced levels of beneficial genera like Bifidobacterium and Prevotella, and overrepresentation of certain Firmicutes and Proteobacteria (e.g., Clostridium, Desulfovibrio, Sutterella) associated with metabolite production and inflammation. These differences, though variable across studies, support the notion that the microbiome-gut-brain axis plays a role in ASD. The gut microbiota may influence ASD symptoms via production of neuroactive compounds, modulation of the immune system, and effects on GI health, thereby potentially exacerbating or even driving some of the neurological features of ASD. Importantly, this line of research has practical implications: if specific microbial imbalances consistently correlate with ASD, they could serve as biomarkers for earlier diagnosis or stratification (e.g. identifying subtypes of ASD). Moreover, the microbiome represents a promising target for intervention – early trials of probiotics and fecal transplants have shown improvements in GI and behavioral symptoms, hinting that correcting dysbiosis might alleviate some ASD difficulties. Nevertheless, much remains to be learned. The field must address inconsistencies by conducting larger, more rigorous studies and exploring mechanistic pathways in greater depth. Going forward, a multidisciplinary approach integrating microbiology, neurology, immunology, and nutrition will be essential to fully decode how the gut microbiota influences ASD. Such research will not only clarify the pathophysiological role of the microbiome in autism but may also open the door to novel microbiome-informed therapies to improve the health and quality of life of children with ASD.

Acknowledgments: The authors would like to thank Dr. Wahidin Sudirohusodo Hospital, Makassar for the support and facilities that enabled this research to be conducted.

Found or Financial Support: None

Author's Contributions

AMT, MM, EA, HA, SBS, UR, and MMA conceived and designed the analysis;

SBS, UR, and MMA collected the data;

AMT, MM, EA, and HA contributed data or analysis tools;

AMT and MM performed the analysis;

AMT, MM, EA, HA, SBS, UR, and MMA wrote the paper.

All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

[1] Lewandowska-Pietruszka Z, Figlerowicz M, Mazur-Melewska K. Microbiota in Autism Spectrum Disorder: A Systematic Review. Int J Mol Sci 2023;24:16660. doi: 10.3390/ijms242316660.

[2] Korteniemi J, Karlsson L, Aatsinki A. Systematic Review: Autism Spectrum Disorder and the Gut Microbiota. Focus: Journal of Life Long Learning in Psychiatry 2024;22:242. doi: 10.1176/appi.focus.24022008.

[3] Bakhtiar B, Ahmadwirawan A, Makkadafi M, Qanitha A, Faruk M, Laidding SR. Gut microbiota analysis in patients with Hirschsprung's disease: A genomic pilot study. Acta Biomedica Atenei Parmensis 2025;96:16151. doi: 10.23750/abm.v96i1.16151.

[4] Wong GC, Montgomery JM, Taylor MW. The Gut-Microbiota-Brain Axis in Autism Spectrum Disorder. In: Grabrucker AM, ed. Autism Spectrum Disorders. Brisbane (AU): Exon Publications; 2021.

[5] Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, *et al.* Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015;161:264–76. doi: 10.1016/j.cell.2015.02.047.

[6] Breit S, Kupferberg A, Rogler G, Hasler G. Vagus Nerve as Modulator of the Brain–Gut Axis in Psychiatric and Inflammatory Disorders. Front Psychiatry 2018;9:44. doi: 10.3389/fpsyt.2018.00044.

[7] Gao X, Cao Q, Cheng Y, Zhao D, Wang Z, Yang H, *et al.* Chronic stress promotes colitis by disturbing the gut microbiota and triggering immune system response. Proc Natl Acad Sci U S A 2018;115:E2960–9. doi: 10.1073/pnas.1720696115.

[8] Grover M, Kashyap PC. Germ free mice as a model to study effect of gut microbiota on host physiology.

Neurogastroenterol Motil 2014;26:745–8. doi: 10.1111/nmo.12366.

[9] Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil 2011;23:255–64, e119. doi: 10.1111/j.1365-2982.2010.01620.x.

[10] Liu F, Li J, Wu F, Zheng H, Peng Q, Zhou H. Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. Transl Psychiatry 2019;9:1–13. doi: 10.1038/s41398-019-0389-6.

[11] Ding H, Yi X, Zhang X, Wang H, Liu H, Mou W-W. Imbalance in the Gut Microbiota of Children With Autism Spectrum Disorders. Front Cell Infect Microbiol 2021;11:572752. doi: 10.3389/fcimb.2021.572752.

[12] Ma B, Liang J, Dai M, Wang J, Luo J, Zhang Z, *et al.* Altered Gut Microbiota in Chinese Children With Autism Spectrum Disorders. Front Cell Infect Microbiol 2019;9:40. doi: 10.3389/fcimb.2019.00040.

[13] He J, Gong X, Hu B, Lin L, Lin X, Gong W, *et al.* Altered Gut Microbiota and Short-chain Fatty Acids in Chinese Children with Constipated Autism Spectrum Disorder. Sci Rep 2023;13:19103. doi: 10.1038/s41598-023-46566-2.

[14] Ho LKH, Tong VJW, Syn N, Nagarajan N, Tham EH, Tay SK, *et al.* Gut microbiota changes in children with autism spectrum disorder: a systematic review. Gut Pathog 2020;12:6. doi: 10.1186/s13099-020-0346-1.

[15] Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, *et al.* Gastrointestinal microbiota in children with autism in Slovakia. Physiol Behav 2015;138:179–87. doi: 10.1016/j.physbeh.2014.10.033.

[16] Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, *et al.* New evidences on the altered gut microbiota in autism spectrum disorders. Microbiome 2017;5:24. doi: 10.1186/s40168-017-0242-1.

[17] Iglesias-Vázquez L, Van Ginkel Riba G, Arija V, Canals J. Composition of Gut Microbiota in Children with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Nutrients 2020;12:792. doi: 10.3390/nu12030792.

[18] Xu M, Xu X, Li J, Li F. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry 2019;10:473. doi: 10.3389/fpsyt.2019.00473.

[19] Chen Z, Shi K, Liu X, Dai Y, Liu Y, Zhang L, *et al.* Gut Microbial Profile Is Associated With the Severity of Social Impairment and IQ Performance in Children With Autism Spectrum Disorder. Front Psychiatry 2021;12:789864. doi: 10.3389/fpsyt.2021.789864.

[20] Abuljadayel D, Alotibi A, Algothmi K, Basingab F, Alhazmi S, Almuhammadi A, *et al.* Gut microbiota of children with autism spectrum disorder and healthy siblings: A comparative study. Exp Ther Med 2024;28:430. doi: 10.3892/etm.2024.12719.

[21] De Sales-Millán A, Aguirre-Garrido JF, González-Cervantes RM, Velázquez-Aragón JA. Microbiome–Gut–Mucosal–Immune–Brain Axis and Autism Spectrum Disorder (ASD): A Novel Proposal of the Role of the Gut Microbiome in ASD Aetiology. Behavioral Sciences 2023;13:548. doi: 10.3390/bs13070548.

[22] De Angelis M, Francavilla R, Piccolo M, De Giacomo A, Gobbetti M. Autism spectrum disorders and intestinal microbiota. Gut Microbes 2015;6:207–13. doi: 10.1080/19490976.2015.1035855.