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Review

Gut Microbiota in Autism Spectrum Disorder: A Scoping Review of Clinically Relevant Evidence and Future Research Directions

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Abstract

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition frequently accompanied by gastrointestinal manifestations. Growing interest has focused on the gut microbiota and its possible relationship with ASD, although the clinical significance of reported microbiota alterations remains uncertain. This scoping review aimed to map and synthesise human evidence on the relationship between gut microbiota and ASD, with particular attention to gastrointestinal manifestations, microbial function, microbiota-derived metabolites and microbiota-targeted interventions. The review followed Joanna Briggs Institute methodology and was reported according to the PRISMA Extension for Scoping Reviews (PRISMA-ScR). We searched PubMed/MEDLINE, Web of Science Core Collection and CINAHL Complete from inception to 28 July 2026 using database-specific strategies. Forty-five publications met the eligibility criteria. Differences in gut microbiota composition were frequently reported in individuals with ASD, but findings varied considerably across studies, and no reproducible ASD-specific microbial signature emerged. Studies of microbial function and metabolites also produced heterogeneous findings. Microbiota-targeted interventions showed variable gastrointestinal, behavioural, and microbiological outcomes, but controlled studies provided insufficient evidence to establish consistent clinical benefits. Critical appraisal using design-appropriate JBI tools identified recurring methodological limitations, including inadequate control of confounding, small sample sizes, incomplete reporting, and, in some intervention studies, limitations in blinding or follow-up. Overall, microbiome findings should be interpreted alongside gastrointestinal symptoms, diet, medication exposure and other relevant clinical characteristics. Further research would benefit from well-characterised cohorts, standardised methodologies, and longitudinal studies that integrate functional and multi-omics data.

Keywords: autism spectrum disorder; gut microbiota; gastrointestinal symptoms; microbiota–gut–brain axis; microbial metabolites; probiotics; faecal microbiota transplantation; scoping review

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1. Introduction

Autism spectrum disorder (ASD) comprises a heterogeneous group of neurodevelopmental conditions characterised by persistent differences in social communication and interaction together with restricted or repetitive patterns of behaviour, interests and activities. Current estimates suggest that approximately one in 127 people worldwide was autistic in 2021, although prevalence varies considerably according to diagnostic practices, healthcare access and case ascertainment [1]. Beyond its behavioural definition, ASD encompasses substantial variability in cognitive functioning, language development, adaptive skills, co-occurring medical conditions and support needs, reflecting a multifactorial aetiology in which genetic susceptibility interacts with developmental and environmental influences across early life [2].

Against this background, interest in the gut microbiota has expanded rapidly over the last decade. Rather than being regarded as a direct cause of ASD, the intestinal microbiota is increasingly recognised as a dynamic biological interface that can influence immune regulation, metabolic homeostasis and neural signalling through the microbiota–gut–brain axis [3–5]. Experimental studies show that intestinal microorganisms can modulate neurodevelopment, epithelial barrier integrity, and neuroactive metabolite production. However, translating these experimental observations into human ASD remains challenging. Biological plausibility alone does not establish causality.

One of the most consistent clinical observations supporting this line of research is the high prevalence of gastrointestinal symptoms in autistic individuals. Constipation, diarrhoea, abdominal pain and other gastrointestinal disturbances have been reported more frequently in autistic than in non-autistic populations and have been associated with behavioural difficulties, altered neuroimmune profiles and reduced quality of life [6,7]. Nevertheless, the relationship is likely to be bidirectional. Gastrointestinal dysfunction may influence dietary behaviour and microbial ecology, whereas selective eating, restricted dietary diversity and medication exposure, common features in ASD, may modify the intestinal environment. Consequently, gastrointestinal manifestations should not simply be considered co-occurring conditions but also an important source of biological and clinical heterogeneity within the autism spectrum.

Advances in sequencing technologies have generated substantial research on gut microbial composition in ASD. Although numerous studies have identified differences between autistic and non-autistic populations, no reproducible ASD-specific microbial signature has been consistently demonstrated across independent cohorts [8–10]. Reported findings often vary by participant characteristics, gastrointestinal phenotype, dietary habits, antibiotic exposure, geographical location, and analytical methodology. Consequently, the field has increasingly moved beyond searching for individual bacterial taxa toward understanding whether specific microbial alterations have biological relevance within clinically distinct ASD subgroups.

This evolution has also shifted attention from taxonomy to function. Recent investigations have explored intestinal permeability, immune activation, microbial metabolites, short-chain fatty acids, tryptophan metabolism and host–microbiome molecular interactions as potential pathways associated with intestinal physiology and neurobehavioural outcomes [11,12]. Although these findings suggest plausible biological pathways, their interpretation remains challenging because most available evidence comes from cross-sectional studies with relatively small sample sizes and considerable methodological variability. Moreover, taxonomic differences do not necessarily reflect functional changes. Consequently, functional microbial activity and host–microbiome interactions may provide complementary biological information beyond microbial composition alone.

Diet is a major source of variation in gut microbiota composition and may confound, mediate, or modify effects depending on the research question, temporal ordering, and causal assumptions. Food selectivity in autistic children may reduce dietary diversity and fibre intake, while gastrointestinal discomfort may reinforce restrictive eating patterns [13]. Age, medication exposure, perinatal factors, and other host characteristics also contribute to inter-individual variation [14,15]. Accordingly, it remains uncertain whether reported microbial differences contribute to ASD-related manifestations, arise in response to associated characteristics, or reflect bidirectional relationships. These factors should therefore be explicitly considered when interpreting microbiome findings.

Alongside observational research, microbiota-targeted interventions have attracted increasing attention as potential therapeutic strategies. Probiotics, prebiotics, synbiotics, dietary interventions, and faecal microbiota transplantation have all been evaluated for gastrointestinal, behavioural, and microbiological outcomes. Although several studies report encouraging results, systematic reviews generally conclude that the available evidence remains limited by small sample sizes, heterogeneous protocols, inconsistent outcome measures, and concerns about risk of bias [16,17]. Consequently, these interventions should currently be regarded as investigational rather than established therapies for ASD. At the same time, intervention studies provide an important opportunity to investigate potential mechanistic relationships and identify patient subgroups that might respond differently to personalised microbiota-based approaches.

Despite the remarkable expansion of microbiome research in ASD, consensus has not kept pace with the rapid accumulation of evidence. The central challenge is no longer determining whether differences in gut microbiota composition are observed between autistic and non-autistic populations, but understanding which microbial alterations are reproducible, biologically meaningful, and clinically relevant. Accordingly, this review integrates evidence on gastrointestinal manifestations, microbial function, microbiota-derived metabolites, microbiota-targeted interventions, and microbiota composition into a single, clinically oriented evidence-mapping framework. By bringing together these complementary domains, the review provides a broader overview of the current evidence base and helps contextualise microbiome findings within clinically relevant ASD phenotypes.

A scoping review is particularly appropriate in this context because it enables comprehensive mapping of the rapidly evolving and methodologically diverse literature without imposing statistical synthesis on fundamentally heterogeneous studies [18]. Rather than cataloguing taxonomic differences alone, this approach also allows the integration of clinically relevant evidence across diverse study designs. Accordingly, the review was guided by the following questions:

Q1. *What evidence is currently available regarding the relationship between gut microbiota and ASD?*

Q2. *How are gastrointestinal manifestations, microbial function, microbiota-derived metabolites and microbiota-targeted interventions represented within the available literature?*

Q3. *What methodological challenges and knowledge gaps remain in this field?*

Based on these questions, this review aims to map and synthesise current human evidence on the relationship between the gut microbiota and autism spectrum disorder, with particular emphasis on gastrointestinal manifestations, microbial function, microbiota-derived metabolites, and microbiota-targeted interventions. The review also identifies methodological challenges and knowledge gaps to inform future research and the interpretation of emerging clinical evidence.

2. Materials and Methods

2.1. Study Design

This scoping review was conducted according to the Joanna Briggs Institute (JBI) methodology for scoping reviews [18] and is reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) [19]. The completed PRISMA-ScR checklist is provided as a separate supplementary document. The review objective guided the development of the search strategy, eligibility criteria and data-charting process [20].

The review protocol was registered in the Open Science Framework (OSF) Registries (Registration DOI: 10.17605/OSF.IO/F8QD5). The protocol provided the a priori framework for review questions, eligibility criteria, and planned evidence mapping. We documented procedural clarifications, where applicable, in the manuscript and Supplementary Materials, distinguishing them from changes to the prespecified review methods.

2.2. Eligibility Criteria

We defined eligibility criteria a priori using the Population–Concept–Context (PCC) framework recommended by the Joanna Briggs Institute.

The population comprised human participants of any age with a confirmed diagnosis of autism spectrum disorder (ASD), established according to recognised diagnostic criteria (e.g., DSM, ICD or an equivalent clinical diagnosis).

The concept was the relationship between the gut microbiota and ASD, with particular emphasis on gastrointestinal manifestations, clinical phenotypes, microbial function, microbiota-derived metabolites and microbiota-targeted interventions.

The context included original observational and interventional studies conducted in clinical or community settings, with no restrictions on geographical location or healthcare setting. Studies were included if they: enrolled human participants with ASD; directly assessed the gut microbiota using culture-based or culture-independent approaches; reported original data; and investigated at least one of the following clinically relevant domains: (i) gastrointestinal manifestations or other ASD-related clinical phenotypes; (ii) microbial function; (iii) microbiota-derived metabolites; or (iv) microbiota-targeted interventions and their associated clinical and/or microbiological outcomes.

We excluded review articles, systematic reviews, meta-analyses, editorials, letters, conference abstracts, study protocols, animal or in vitro studies, and studies that did not directly assess the gut microbiota or report original human data. We also excluded duplicate records and publications that did not add information beyond an already included study population. Section 2.5 describes the procedures used to identify and manage potentially overlapping cohorts. When multiple publications were derived from the same study population, we linked them during data extraction and treated them as a single study to avoid double counting.

Because this review aimed to synthesise clinically relevant evidence, we excluded studies that reported only descriptive taxonomic comparisons without evaluating clinical phenotypes (including gastrointestinal manifestations), microbial function, microbiota-derived metabolites or microbiota-targeted interventions, as such studies did not address the predefined review objectives. This decision focused the review on evidence with potential clinical and translational relevance. Consequently, the findings reflect clinically oriented microbiome research rather than the full descriptive ASD microbiome literature.

2.3. Search Strategy

We conducted a comprehensive search of PubMed/MEDLINE, Web of Science Core Collection, and CINAHL Complete from database inception to 28 July 2026 to identify human studies investigating gut microbiota in autism spectrum disorder (ASD).

The search strategy combined controlled vocabulary (e.g., MeSH, where available) and free-text terms related to ASD, gut microbiota, gastrointestinal manifestations, microbial function, microbiota-derived metabolites, the microbiota–gut–brain axis and microbiota-targeted interventions.

We adapted search strategies to each database's indexing system and search interface while maintaining the same core concepts: ASD and gut microbiota/microbiome. The complete database-specific search strategies are provided in Supplementary Material S1.

In PubMed/MEDLINE, we limited the search to studies published in English or Spanish. We then applied eligibility criteria for study design, publication type, and population during study selection.

To maximise retrieval of relevant evidence, reference lists of included studies and relevant reviews were screened. We assessed potentially overlapping publications at the study level to minimise double counting and applied the same predefined eligibility criteria throughout study selection.

2.4. Study Selection Process

We imported all identified records into Mendeley Reference Manager (Elsevier, Amsterdam, The Netherlands), where we identified and removed duplicate records. We then exported the deduplicated records to Rayyan (Rayyan Systems Inc., Cambridge, MA, USA) to facilitate study screening. Screening occurred in two stages: title and abstract screening, followed by full-text assessment.

Two reviewers independently screened titles and abstracts in Rayyan according to the predefined eligibility criteria. The same two reviewers then conducted full-text assessments of potentially eligible records. The reviewers resolved eligibility disagreements through discussion; when they could not reach consensus, a third reviewer independently assessed the study and made the final decision. We recorded and categorised reasons for exclusion at full text according to the predefined criteria. Figure 1 summarises the study-selection process.

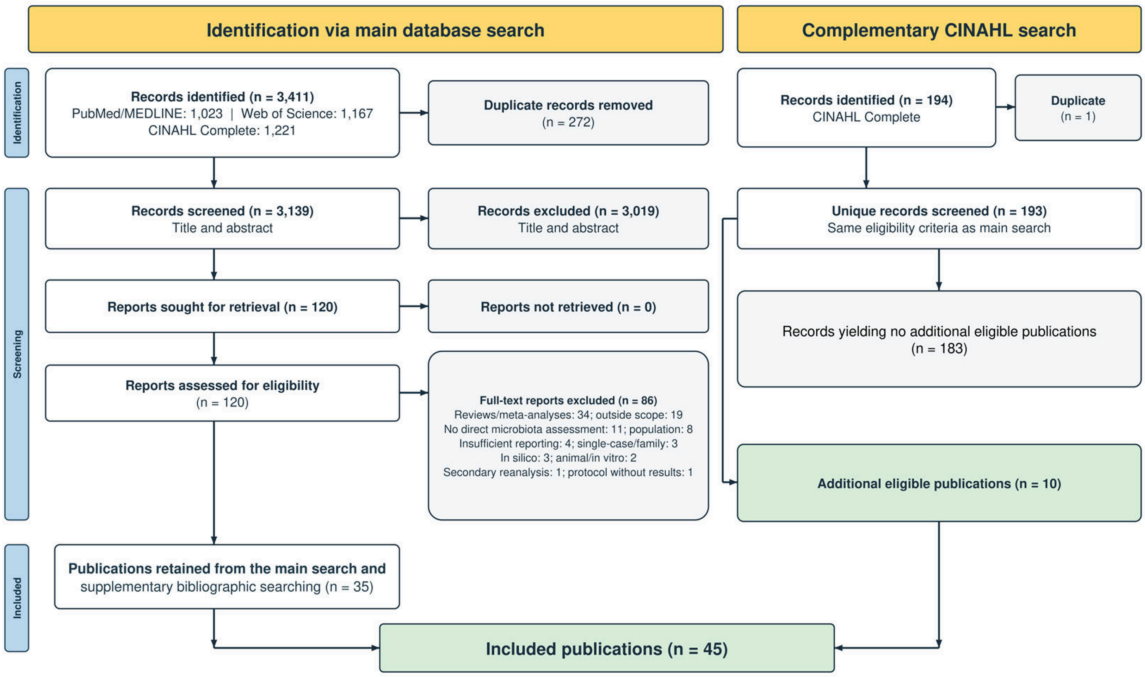

Figure 1. Flow diagram of publication selection (45 included publications).

2.5. Data Extraction

We charted data using a standardised data-charting form developed specifically for this review. Two reviewers charted the data independently. Reviewers resolved discrepancies through discussion and consensus; when they could not reach consensus, they consulted a third reviewer. We extracted the following information: publication characteristics (author, year and country), study design, participant characteristics, gastrointestinal manifestations, microbiome assessment methods, microbial composition and diversity, microbiota-derived metabolites, microbiota-targeted interventions and the principal findings relevant to the review objectives.

Microbiota-related data included the biological sample analysed, microbiome profiling method, sequencing or analytical approach, microbial composition, diversity measures, microbiota-derived metabolites and microbiota-targeted interventions. For intervention studies, we also collected intervention type, duration, comparator and reported clinical and microbiological outcomes.

To identify potential cohort overlap, we compared author groups, study settings, participant characteristics, recruitment periods, sample sizes, intervention characteristics, and, when available, trial-registration information.

We assessed potentially overlapping publications against the eligibility criteria and, where possible, linked reports to known parent cohorts. Unresolved overlap was documented rather than assumed absent.

Before the final synthesis, we verified bibliographic information and extracted study-level data against the original publications, and we checked consistency across the reference list, Table 1, Supplementary Table S1, and the narrative synthesis.

Table 1. Study-level characteristics and principal findings of the 45 publications included in the scoping review.

Study	Design and Participants	Microbiota Assessment	Clinical Outcomes	Principal Domain
[21] Chen et al. (2022) Taiwan	Case–control; Autistic individuals and non-autistic controls	Stool; 16S rRNA sequencing	GI symptoms; behavioural problems	Gut microbiota composition in relation to clinical phenotypes
[22] Phan et al. (2024) USA	Pilot open-label intervention; Autistic participants receiving personalised symbiotic	Shotgun metagenomics	GI symptoms	Microbiota-targeted intervention
[23] Palmer et al. (2025) Australia	Double-blind randomised controlled trial; Children with autism	Stool; Gut microbiome profiling	GI symptoms; child behaviour; parental quality of life	Microbiota-targeted intervention
[24] Wong et al. (2022) Hong Kong, China	Case–control; Prepubertal autistic boys and non-autistic controls	Stool; 16S rRNA gene sequencing	Functional GI disorders; autism-related characteristics	Gut microbiota composition in relation to clinical phenotypes
[25] Hrnciarova et al. (2024) Czech Republic	Randomised double-blind placebo-controlled pilot trial; Children with autism; neurotypical comparison group	Stool; 16S rRNA gene sequencing	Autism symptoms; GI outcomes	Microbiota-targeted intervention
[26] Zurita et al. (2020) Ecuador	Case–control; Children with autism and controls	Stool; blood; 16S rRNA gene sequencing; nutritional and immune assessment	Diet; nutritional status; immune markers; GI features	Gut microbiota composition in relation to clinical phenotypes
[27] Liu et al. (2019) China	Case–control; Children with autism and controls	Stool; 16S rRNA gene sequencing; faecal SCFA quantification	ASD clinical characteristics; GI features	Microbial function and microbiota-derived metabolites

Table 1. *Cont.*

Study	Design and Participants	Microbiota Assessment	Clinical Outcomes	Principal Domain
[28] Jung et al. (2024) South Korea	Cross-sectional observational study; Individuals with autism stratified by gut ecological structure	Stool; 16S rRNA gene sequencing	Clinical and GI characteristics	Gut microbiota composition in relation to clinical phenotypes
[29] Kang et al. (2018) USA	Case–control; Children with ASD and neurotypical controls	Stool; 16S rRNA gene sequencing; faecal metabolomics	GI symptoms; autism-related characteristics	Microbial function and microbiota-derived metabolites
[30] Chang et al. (2026) Taiwan	Sibling-matched case–control; Autistic individuals and unaffected siblings	Stool; Gut microbiota profiling	Clinical characteristics	Gut microbiota composition in relation to clinical phenotypes
[31] Rose et al. (2018) USA	Case–control with clinical subgrouping; Children with autism with and without GI symptoms; controls	Stool; blood; 16S rRNA gene sequencing; immune profiling	GI symptoms; immune markers; ASD characteristics	Gut microbiota composition in relation to clinical phenotypes
[32] Quan et al. (2026) China	Prospective microbiota-transfer intervention; Autistic participants receiving WMT	Stool; 16S rRNA gene sequencing	GI and autism-related outcomes	Microbiota-targeted intervention
[33] Williams et al. (2011) USA	Case–control mechanistic study; Children with autism and GI disturbances; controls	Intestinal mucosal biopsy; Mucosal transcriptomics; microbial profiling	GI disturbances	Gut microbiota composition in relation to clinical phenotypes
[34] Mitchell et al. (2026) Australia	Open-label randomised pilot trial; Autistic children	Stool; gut microbiome profiling	GI symptoms; behaviour; anxiety	Microbiota-targeted intervention
[35] Agrawal et al. (2026) Australia	Pilot randomised controlled trial; Children with autism	Stool; gut microbiota profiling	GI and clinical outcomes	Microbiota-targeted intervention
[36] Tomova et al. (2015) Slovakia	Hybrid pilot study: baseline analytical comparison (10 ASD, 9 siblings, 10 controls) plus uncontrolled probiotic pre–post component (9/10 ASD)	Stool; real-time PCR targeting selected bacterial groups	GI symptoms; autism severity; 4-month probiotic supplementation	Baseline microbial composition and microbial changes after probiotic supplementation
[37] Kang et al. (2020)	Secondary metabolomic report of an open-label MTT cohort; 18 ASD and 20 typically developing controls	Faecal and plasma UHPLC-MS/MS; linked parent-cohort microbiota data	GI and autism-related clinical measures from the MTT cohort; exploratory metabolite associations	Microbial function and metabolites
[38] Gondalia et al. (2012) Australia	Analytical sibling comparison; 51 ASD (23 without GI dysfunction, 28 with GI dysfunction) and 53 neurotypical siblings	Stool; bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP)	GI dysfunction	Faecal microbiota composition by GI phenotype and sibling comparison; no clinically meaningful ASD-specific differences established
[39] Arnold et al. (2019) USA	Placebo-controlled pilot trial; Children with autism and GI symptoms	Stool; gut microbiome profiling	GI symptoms; quality of life	Microbiota-targeted intervention
[40] Sanctuary et al. (2019) USA	Pilot crossover intervention study; Children with autism and GI symptoms	Stool; microbiome and metabolite profiling	GI function; behavioural outcomes	Microbiota-targeted intervention
[41] Adams et al. (2011) USA	Cross-sectional case–control; Children with autism and typical controls	Stool; culture-based microbial analysis and targeted bacterial quantification	GI status; autism severity	Gut microbiota composition in relation to clinical phenotypes

Table 1. *Cont.*

Study	Design and Participants	Microbiota Assessment	Clinical Outcomes	Principal Domain
[42] Grimaldi et al. (2018) United Kingdom	Prebiotic intervention study; Children with autism	Stool; 16S rRNA gene sequencing and metabolomics	GI and behavioural outcomes	Microbiota-targeted intervention
[43] De Angelis et al. (2013) Italy	Case–control metabolomic study; Children with autism, PDD-NOS and controls	Stool; culture-dependent and molecular microbiota profiling; metabolomics	Clinical phenotype	Microbial function and microbiota-derived metabolites
[44] Guidetti et al. (2022) Italy	Randomised double-blind crossover trial; Autistic children	Stool; gut microbiome profiling	GI and behavioural symptoms	Microbiota-targeted intervention
[45] Peñalba et al. (2026) Uruguay	Sibling-matched cross-sectional study; Autistic participants and unaffected siblings	Stool; gut microbiota profiling	GI symptoms; pharmacological treatment	Gut microbiota composition in relation to clinical phenotypes
[46] Deng et al. (2022) China	Cross-sectional case–control; children with ASD ($n = 45$) and typically developing controls ($n = 45$)	Stool; 16S rRNA gene sequencing and faecal SCFA analysis	GI symptoms; ASD characteristics	Gut microbiota composition in relation to clinical phenotypes
[47] Vernocchi et al. (2023) Italy	Cross-sectional functional profiling study; Children with ASD and controls	Stool; 16S rRNA gene sequencing and volatile organic compound functional profiling	ASD clinical phenotype	Microbial function and microbiota-derived metabolites
[48] Pan et al. (2025) China	Retrospective WMT cohort/subgroup analysis; autistic participants stratified by constipation status	Stool; gut microbiota profiling	Constipation; treatment response	Microbiota-targeted intervention
[49] Valencia-Buitrago et al. (2025) Colombia	Prospective microbiota-targeted intervention; Children with autism receiving anti-inflammatory diet and probiotics	Stool; shotgun metagenomic sequencing	Clinical and GI outcomes	Microbiota-targeted intervention
[50] Li et al. (2024) China	Open-label FMT intervention; Children with autism	Stool; 16S rRNA gene sequencing	GI and autism-related outcomes	Microbiota-targeted intervention
[51] Wan et al. (2024) China	Randomised double-blind placebo-controlled trial; 103 children with ASD (FMT $n = 52$; placebo $n = 51$)	Stool; gut microbiota diversity and donor microbiota colonisation	Autism-related behavioural and adaptive functioning outcomes	Microbiota-targeted intervention
[52] Huang et al. (2021) China	Case–control modelling study; Individuals with autism and controls	Stool; microbiome profiling with multivariable microbiome-specific modelling	Neurobehavioural outcomes; GI features	Gut microbiota composition in relation to clinical phenotypes
[53] Zhong et al. (2026) China	Cross-sectional integrated multi-omics study; Children with autism and controls	Stool and plasma; 16S rRNA gene sequencing, plasma metabolomics and integrated multi-omics	Clinical phenotype	Microbial function and microbiota-derived metabolites
[54] Yitik Tonkaz et al. (2023) Türkiye †	Cross-sectional family/control comparison; children with ASD and unaffected siblings	Stool; microbiota profiling with gastrointestinal/permeability measures	GI/permeability phenotype	Gut microbiota composition in relation to clinical phenotypes
[55] Smolko et al. (2026) Russia †	Prospective observational study; 96 children with ASD and 39 neurotypical controls; follow-up in 60 ASD participants	Stool; microbiota profiling with intestinal inflammatory/barrier markers	Dietary/eating behaviour; intestinal inflammatory/barrier markers	Gut microbiota composition in relation to clinical phenotypes

Table 1. *Cont.*

Study	Design and Participants	Microbiota Assessment	Clinical Outcomes	Principal Domain
[56] Di Benedetto et al. (2025) Italy †	Cross-sectional family comparison; children with ASD, non-ASD siblings and parents	Stool; bacterial and fungal microbiota profiling	Dietary patterns; behavioural challenges	Gut microbiota composition in relation to clinical phenotypes
[57] Kurokawa et al. (2024) Japan †	Cross-sectional ASD/ADHD/sibling/unrelated-control comparison	Stool; gut microbiota profiling	Dietary diversity; GI symptoms; neurodevelopmental phenotype	Gut microbiota composition in relation to clinical phenotypes
[58] Huang et al. (2025) China †	Case–control; 58 children with ASD and 30 healthy controls	Stool and urine; 16S rRNA gene sequencing and untargeted urinary metabolomics	Clinical phenotype; urine metabolites	Microbial function and microbiota-derived metabolites
[59] Wang et al. (2023) China †	Case–control; 42 children with ASD and chronic GI symptoms and 41 controls	Stool; 16S rRNA gene sequencing	Chronic GI symptoms	Gut microbiota composition in relation to clinical phenotypes
[60] Novau-Ferré et al. (2025) Spain †	Randomised double-blind placebo-controlled trial; 80 children, including 41 with ASD	Stool; microbiome profiling before and after probiotic intervention	Probiotic response; microbiome diversity/composition	Microbiota-targeted intervention
[61] Ha et al. (2021) South Korea †	Case–control; 54 children with ASD and 38 typically developing controls	Stool; 16S rRNA gene sequencing and faecal SCFA measurement	SCFAs; clinical phenotype	Microbial function and microbiota-derived metabolites
[62] Plaza-Díaz et al. (2019) Spain	Cross-sectional case–control; ASD with/without regression and controls	Stool; 16S rRNA gene sequencing	Regression-defined clinical phenotype	Gut microbiota composition in relation to clinical phenotypes
[63] Kushak et al. (2017) USA †	Case–control mechanistic study; 21 autistic individuals and 19 non-ASD controls	Duodenal mucosa; 16S rRNA gene sequencing and disaccharidase assays	Carbohydrate digestion; constipation	Microbial function and microbiota-derived metabolites
[64] Kong et al. (2021) USA †	Randomised double-blind placebo-controlled two-stage pilot; 35 individuals with ASD	Stool and blood; faecal microbiome and inflammatory-marker assessment	Probiotic/oxytocin intervention; socio-behavioural outcomes	Microbiota-targeted intervention
Guiducci et al. (2026) [65]	Secondary analysis of a double-blind probiotic RCT; 57 complete cases (28 probiotic; 29 placebo)	Faecal targeted qPCR and proton NMR metabolomics	ADOS-2; GI severity; exploratory subgroup analyses	Microbiota-targeted intervention

Abbreviations: ASD, autism spectrum disorder; FMT, faecal microbiota transplantation; GI, gastrointestinal; PDD-NOS, pervasive developmental disorder–not otherwise specified; qPCR, quantitative polymerase chain reaction; rRNA, ribosomal ribonucleic acid; SCFA, short-chain fatty acid; VOC, volatile organic compound; WMT, washed microbiota transplantation. Note: Studies are presented in citation order. Further details are provided in Supplementary Table S1. † Studies identified through the complementary CINAHL search.

2.6. Critical Appraisal Methods

We conducted a critical appraisal to support interpretation of heterogeneous evidence, not to determine eligibility. We used design-appropriate JBI question sets for randomised controlled trials, quasi-experimental studies, analytical cross-sectional studies, cohort studies, and case–control studies. For hybrid studies, we used the tool corresponding to the design component contributing to the synthesised evidence. Supplementary Table S2 identifies the tools and item-level judgements.

Appraisals for reports added during reconciliation were checked against source methods, including linked parent-trial reports. Independent duplicate appraisal of these additions was not documented. Funding of individual included publications was not systematically charted. These limitations were considered when interpreting the evidence; neither appraisal concerns nor funding status was used as an exclusion criterion

We judged items as Yes, No, Unclear, or Not applicable based on information reported in the source publication. We did not calculate numerical quality scores or impose post hoc high/moderate/low thresholds. We did not use critical appraisal as an exclusion criterion; instead, we used it to inform the interpretation of findings and the degree of caution applied to causal or clinical claims. Item-level judgements for all 45 included publications are reported in Supplementary Table S2.

2.7. Data Synthesis

Because of the methodological and clinical heterogeneity of the included studies, findings were synthesised narratively and organised into four evidence domains aligned with the review objectives: (i) gastrointestinal manifestations; (ii) microbial function and microbiota-derived metabolites; (iii) microbiota-targeted interventions; and (iv) microbiota composition in relation to clinical phenotypes. We summarised the characteristics and principal findings of the included studies in tables to facilitate comparison across the evidence base. Given the substantial heterogeneity in study designs, participant characteristics, microbiome analytical methods, and reported outcomes, we did not consider quantitative synthesis or meta-analysis appropriate.

3. Results

3.1. Study Selection

The main database searches identified 3411 records (PubMed/MEDLINE, $n = 1023$; Web of Science Core Collection, $n = 1167$; CINAHL Complete, $n = 1221$). After removing 272 duplicate records, 3139 records underwent title and abstract screening, and 3019 were excluded. We sought to retrieve the remaining 120 reports; all were available and assessed for eligibility. The included-publication evidence map is documented in Tables 1 and 2 [21–65].

Table 2. Distribution of included studies by principal evidence domain.

Principal Evidence Domain	Publications, *n*	Interpretive Scope
Gut microbiota composition in relation to clinical phenotypes	19	Observational evidence relating microbial composition to gastrointestinal, dietary, behavioural or other clinical characteristics.
Microbial function and microbiota-derived metabolites	9	Studies incorporating directly measured functional, metabolomic or microbiota-derived metabolite outcomes.
Microbiota-targeted interventions	17	Intervention studies evaluating probiotics, prebiotics/synbiotics, dietary approaches, or microbiota-transfer strategies; designs and comparator structures varied.
Total	45	Each publication was assigned once according to its principal analytical focus; secondary findings could contribute to more than one narrative domain.

Note: Each study was counted once according to its principal analytical focus for descriptive totals, although secondary findings could contribute to more than one narrative domain. Critical-appraisal interpretation summarises recurrent design-level concerns; complete item-level JBI judgements are provided in Supplementary Table S2.

Of the 120 reports assessed for eligibility in the main search, 86 were excluded and 34 were retained. Exclusion reasons are summarised in Figure 1, and report-level eligibility decisions are documented in Supplementary Table S4.

The complementary CINAHL search retrieved 194 records, including 193 unique records. Ten additional eligible publications were identified beyond the main-search set [54–61,63,64]; the remaining 183 records yielded no additional eligible publications. One further eligible publication was identified through supplementary bibliographic searching [51]. Because the complementary CINAHL search overlapped with the original search, its records were not added to the original database identification total.

The final evidence map comprises 45 eligible publications, documented in Table 1 and Supplementary Table S1. Publications with potentially overlapping participants are retained separately, without summing their participant counts or treating them as independent replications. Figure 1 presents the corrected selection flow.

3.2. Characteristics of the Included Studies

Table 1 summarises the study-level characteristics most useful for comparison in the main manuscript, including design and participants, microbiota assessment, clinical outcomes and principal evidence domain. Supplementary Table S1 provides more detailed study-level data, including microbiological findings and intervention details.

For descriptive mapping, we assigned each study to one principal evidence domain while retaining secondary contributions in the narrative synthesis. Nineteen studies primarily addressed gut microbiota composition in relation to clinical phenotypes, nine primarily addressed microbial function and microbiota-derived metabolites, and 17 primarily evaluated microbiota-targeted interventions. This classification avoids double counting while acknowledging that many studies contributed evidence to more than one review domain.

Faecal samples were the most frequently analysed biological material, but analytical platforms varied substantially and included culture-based methods, qPCR, 16S rRNA gene sequencing, shotgun metagenomics, metabolomics, and integrated multi-omics approaches. This methodological diversity, together with differences in participant selection, gastrointestinal phenotyping, diet and medication assessment, limits direct comparison of taxonomic and functional findings across studies.

3.3. Critical Appraisal of Included Studies

Methodological strength varied considerably among intervention studies. Randomised placebo-controlled trials generally provided stronger comparative evidence, whereas open-label, single-arm, and non-randomised studies were more vulnerable to performance bias, confounding, and overinterpretation of causal effects. Some pilot trials also had limited sample sizes or incomplete follow-up. We incorporated these appraisal findings into the narrative synthesis by distinguishing between controlled between-group effects, within-group change, and observational associations.

Across observational studies, the most recurrent concerns involved incomplete control of confounding, particularly diet, medication exposure, gastrointestinal phenotype and other host characteristics that may influence the gut microbiome. Several studies used small or clinically selected samples, and some reports did not provide sufficient information to resolve individual appraisal items; therefore, we judged them Unclear rather than assumed them adequate.

Critical appraisal findings for all 45 included publications are presented in Supplementary Table S2. Appraisal informed the interpretation of findings and was not used to determine eligibility.

3.4. Gastrointestinal Manifestations and Gut Microbiota

A substantial proportion of included studies evaluated gastrointestinal manifestations, one of the most frequently investigated clinical domains related to gut microbiota in

ASD [21,24,27–29,31,36,38,41,43,46,47,52,53]. The most assessed gastrointestinal symptoms were constipation, diarrhoea, abdominal pain, bloating, flatulence, and altered bowel habits, although criteria and measures varied across studies.

Several studies compared autistic individuals with and without gastrointestinal symptoms and evaluated differences in gut microbiota composition, microbial diversity, immune markers or microbiota-derived metabolites [21,24,27–29,31,36,47,52]. Overall, studies reported differences in gut microbiota between clinical subgroups; however, the specific microorganisms and the magnitude of these differences were not consistent across studies.

Constipation was among the most frequently analysed gastrointestinal symptoms. Some studies examined its relationship with gut microbiota composition or metabolite profiles, while others assessed its influence on outcomes after microbiota-targeted interventions [27,31,34,47,48]. Although studies described associations between constipation and gut microbiota characteristics, findings differed between studies.

Several observational and intervention studies also examined the relationship between gastrointestinal symptoms and behavioural characteristics, including autism symptom severity, anxiety, irritability and repetitive behaviours [21,28,32,42,47,52]. These studies reported associations between gastrointestinal and behavioural outcomes, although measures and reported relationships varied across cohorts.

Overall, the included studies focused heavily on gastrointestinal manifestations. However, differences in study design, participant characteristics, and gastrointestinal assessment methods limited direct comparison across studies. Most evidence in this domain came from observational studies, primarily cross-sectional and case–control designs. In addition, gastrointestinal symptoms were assessed using heterogeneous definitions, questionnaires and outcome measures, making it difficult to determine whether differences between studies reflect biological variability, measurement variability or both.

3.5. Microbial Function and Microbiota-Derived Metabolites

Nine publications primarily investigated microbial function or microbiota-derived metabolites in individuals with ASD [27,29,37,43,47,53,58,61,63], while several intervention and observational studies also incorporated functional analyses as secondary outcomes. Measured domains included short-chain fatty acids (SCFAs), faecal and urinary metabolites, bacterial volatile organic compounds (VOCs), intestinal disaccharidase activity assessed alongside mucosal microbiota, and integrated multi-omics profiles. Predicted functional pathways derived solely from taxonomic data were not treated as direct functional evidence. Urinary 5-HIAA is a host serotonin-metabolism marker and is not, on its own, evidence of microbial production or direct gut microbiota profiling.

SCFAs were the most frequently assessed microbial metabolites. Several studies reported differences in SCFA concentrations between autistic participants and comparison groups; however, the metabolites evaluated and the reported findings varied across studies [27,43,61].

Metabolomic analyses identified differences in measured metabolite profiles and metabolic pathways in individuals with ASD compared with control groups [27,29,43,53]. In addition, one study evaluated bacterial VOC profiles, while another incorporated an integrated multi-omics approach combining microbiome and metabolic data [47,53].

Overall, studies assessed a wide range of functional and metabolic measures using different analytical approaches and outcome measures [27,29,37,43,47,53]. Although several studies reported differences in SCFAs, metabolic pathways, and other microbiota-associated metabolites, specific biomarkers and the direction of change varied across studies, preventing identification of reproducible functional patterns associated with ASD.

3.6. Microbiota-Targeted Interventions

Seventeen included studies were classified primarily as microbiota-targeted intervention studies [22,23,25,32,34,35,39,40,42,44,48–51,60,64,65]. The synthesis distinguishes four outcome domains: gastrointestinal symptoms, associated behavioural outcomes, core autistic characteristics, and microbiological measures. Interpretation was based on study design and comparator structure; within-group improvement alone was not considered evidence of comparative efficacy.

Among controlled studies, between-group effects were distinguished from within-group changes. Palmer et al. [23] observed no between-group difference in behavioural outcomes, although both groups improved from baseline. For selected outcomes, the authors reported between-group effects for gastrointestinal symptoms and parental quality of life, while *Bifidobacterium* abundance increased after prebiotic supplementation. These findings were therefore interpreted as limited evidence of clinical benefit.

In Wan et al. [51], the primary SRS-2 outcome showed no significant between-group difference, although a secondary Vineland-3 socialisation outcome favoured FMT at Week 17. Changes in gut microbial diversity and donor microbiota colonisation were also reported.

Uncontrolled, open-label studies reported changes over time in gastrointestinal, behavioural, and, in some cases, microbiological outcomes. Without a concurrent comparator, these findings cannot distinguish intervention effects from natural variation, regression to the mean, or other concurrent influences.

Prospective observational studies reported changes in gastrointestinal symptoms, associated behaviours and/or microbiological measures following microbiota-targeted interventions. Without random allocation or adequate control of confounding, these changes cannot establish treatment effects.

Microbiological outcomes included changes in measured microbial composition and, in some reports, metabolite profiles [23,32,35,40,42,44,48–51,65]. Researchers considered these biological endpoints separately from gastrointestinal symptoms, associated behaviours, and core autistic characteristics. A change in microbial composition, even when observed after an intervention, does not by itself demonstrate clinical efficacy or an aetiological role in ASD.

Interventions differed in formulation, duration, comparator, sample size and outcome measurement. These differences, together with the limited statistical power of several pilot studies, restrict cross-study comparisons and require caution when interpreting clinical efficacy.

3.7. Gut Microbiota Composition in Relation to Clinical Phenotypes

Nineteen included studies primarily examined gut microbiota composition in relation to clinical phenotypes (Tables 1 and 2).

Several of these studies explicitly examined gastrointestinal symptoms, dietary patterns, regression-defined phenotypes or control-group effects, reinforcing the importance of clinical contextualisation when interpreting taxonomic differences.

Most observational studies reported differences in gut microbiota composition between autistic participants and comparison groups. However, the identified microorganisms varied across studies, and no consistent microbial profile emerged across studies [21,24,26,28,30,31,36,38,41,45,46,52].

Microbial diversity also varied by study population, comparison group, and analytical methods [21,24,28,30,31,45,46,52].

Researchers also examined associations between gut microbiota composition and clinical characteristics, including gastrointestinal symptoms, behavioural characteristics, pharmaco-

logical treatment, and clinically defined ASD subgroups [21,24,28,30,31,41,45,46,52]. The variables evaluated and the reported associations differed across studies.

Some studies compared participants with unaffected siblings, while others used neurotypical control groups [30,38,45,54,57]. Comparison groups and matching strategies varied among studies.

Overall, observational studies reported differences in gut microbiota composition between study groups, although the identified taxa and the direction of those differences varied across populations and analytical approaches.

Reported compositional differences involved genera such as *Bifidobacterium*, *Prevotella*, and *Clostridium*, although their associations with ASD were not consistent across cohorts.

Taken together, these heterogeneous findings do not support identifying a reproducible ASD-specific microbial signature.

3.8. Synthesis of the Included Studies

The 45 included publications mapped a broad but uneven evidence base. Observational studies predominated, particularly for microbiota composition and gastrointestinal phenotype, while controlled intervention evidence was less frequent. The critical appraisal reinforced the need to interpret cross-sectional associations cautiously because confounding was not consistently addressed and to distinguish controlled treatment effects from changes observed in uncontrolled or open-label studies.

Overall, the evidence mapped in this review indicates that researchers have investigated gut microbiota in ASD using diverse observational, mechanistic and interventional approaches. Most available evidence came from observational studies, whereas controlled intervention studies remained comparatively limited. Across the included studies, considerable heterogeneity in participant characteristics, gastrointestinal assessment methods, microbiome analytical approaches, and outcome measures made it difficult to assess the consistency of findings across cohorts. The current literature identified gastrointestinal manifestations, microbial function, microbiota composition, and microbiota-targeted interventions as the principal evidence domains.

Table 2 summarises the three principal study-level evidence domains, the number of studies assigned to each domain and their interpretive scope. This structure complements the detailed study-level characteristics in Table 1 and the item-level JBI judgements in Supplementary Table S2.

4. Discussion

The most important observation from this review is that, despite the rapid expansion of ASD microbiome research, no reproducible microbial signature has yet been identified. While this finding may reflect biological complexity and methodological heterogeneity, it may also suggest that some reported microbiota differences may be associated with, or arise downstream of, ASD-related characteristics, including dietary selectivity, gastrointestinal dysfunction, medication exposure, and other host-related factors. Consequently, the lack of a consistent microbial profile does not show that microbiota alterations are a primary mechanism in ASD pathophysiology. Instead, current evidence supports associations between the gut microbiota and clinically relevant manifestations in ASD, while leaving substantial uncertainty about their causal and temporal relationships. Their potential relevance therefore appears better understood in the broader context of host–microbiome interactions than through taxonomic alterations alone [66–68].

Across the included studies, alterations in microbial diversity, community composition and bacterial abundance were frequently reported, but the specific taxa associated with ASD varied markedly between cohorts [21,24,26,28,30,31,33,36,38,41,45,46,52]. Recent systematic

reviews and meta-analyses emphasise heterogeneity and limited reproducibility of specific microbial alterations. Observed host–microbiome associations may reflect individual clinical phenotypes more than ASD diagnosis alone, supporting the investigation of biologically and clinically defined subgroups [66–68].

Host-related characteristics, particularly gastrointestinal phenotype and dietary behaviour, contribute substantially to this heterogeneity. Yap et al. [69] found negligible direct associations between ASD diagnosis and the gut microbiome and supported a model in which ASD-related dietary preferences were associated with reduced dietary diversity, lower microbial diversity, and altered stool consistency. This finding reinforces the need to model diet explicitly. Its analytical role may differ by research question, temporal ordering, and causal framework; diet may act as a confounder, mediator, or effect modifier rather than occupying a single fixed role. Systematic reviews also emphasise substantial heterogeneity across ASD microbiome studies [70]. An alternative interpretation is that some microbial differences may arise downstream of ASD-related characteristics, including dietary selectivity, gastrointestinal dysfunction, medication exposure, sleep disturbance, and other host-related factors [69]. In this review, "dysbiosis" denotes a study-defined alteration in microbial composition, diversity, or function relative to an explicit comparator or baseline, not a validated ASD-specific pathological state. Such alterations may still be clinically relevant, but their presence does not establish that they are primary to ASD.

From a clinical perspective, gastrointestinal manifestations emerged as a frequently investigated clinical feature across the included studies. Observational studies examined associations between gastrointestinal symptoms and microbiota alterations, although the direction and magnitude of these associations varied across studies [21,24,28,30,31,38,41,45,46,52].

This pattern is consistent with evidence indicating that gastrointestinal symptoms are frequently reported among autistic individuals, although prevalence estimates vary considerably across studies [71–73]. These symptoms have also been examined in relation to behavioural and other clinical outcomes. Rather than being viewed solely as secondary comorbidities, gastrointestinal manifestations may help identify clinically meaningful subgroups in which host–microbiome interactions warrant further investigation, although the directionality of these relationships remains uncertain.

The relevance of gastrointestinal manifestations extends beyond symptom burden and may provide insights into potential host–microbiome interactions without implying causality. Accumulating evidence has proposed the microbiota–gut–brain axis as a biologically plausible framework through which intestinal microbial activity could interact with immune, metabolic, endocrine and neural pathways relevant to neurodevelopment [74–76].

Alterations in intestinal barrier function and microbial metabolite production have been proposed as mechanisms linking changes in gut microbial ecology with central nervous system processes [74–76]. However, the available human evidence remains predominantly associative. Although current evidence does not establish a causal or etiological role for gut microbiota alterations in ASD, a potential contribution cannot be ruled out. It remains unresolved whether these associations reflect causal mechanisms, downstream consequences, bidirectional interactions, or shared environmental and host-related determinants. Longitudinal and mechanistic studies are needed to clarify these relationships.

Microbiota-derived metabolites provide complementary approaches to investigating the biological relevance of host–microbiome interactions, alongside taxonomic and clinical information [27,29,43,47,53].

In the studies included in this review, functional investigations examined short-chain fatty acids, volatile organic compounds, other metabolites, and integrated metabolic profiles, with heterogeneous findings [27,29,37,43,47,53,58,61], while several intervention studies

used functional or metabolite outcomes as secondary analyses [48,50]. Although no reproducible functional biomarker has emerged, these findings suggest that microbial metabolism warrants further investigation as a potential source of complementary information on host–microbiome interactions in ASD beyond microbial composition alone. Experimental and mechanistic studies suggest that microbial metabolites participate in immune regulation, intestinal barrier maintenance and neurotransmitter synthesis, thereby providing biologically plausible links between intestinal microbial activity and neurodevelopment [77–80]. However, biological plausibility should not be interpreted as evidence of causality. Whether these functional alterations precede ASD-related manifestations or arise as secondary consequences of ASD-associated characteristics remains unclear. Available evidence is also insufficient to determine whether functional biomarkers are more reproducible or clinically informative than taxonomic profiles. Overall, the evidence supports integrating taxonomic, functional and clinical data rather than prioritising any single analytical approach.

Despite their growing relevance, functional analyses should be interpreted cautiously. Most studies investigating microbial function included relatively small cohorts and differed substantially in analytical platforms, targeted metabolites and functional outcomes, limiting direct comparison across studies [27,29,37,43,47,53,58,61,63]. For this reason, while alterations in microbial metabolism appear biologically plausible, current evidence does not yet support identifying reproducible functional biomarkers applicable to routine clinical practice. This limitation aligns with recent reviews identifying methodological standardisation as a principal barrier to translating microbiome research into clinically applicable biomarkers and therapeutic strategies [66,68,81].

Future studies should characterise symptom severity, gastrointestinal phenotype, dietary habits, medication exposure and associated comorbidities. Incorporating gastrointestinal phenotype into participant selection and analysis may help identify clinically relevant microbial and metabolic subgroups and improve reproducibility [21,24,28,31,38,41,45,46,52].

Few human studies integrate microbiome analyses with immune, metabolic and neurodevelopmental outcomes in the same cohort. Longitudinal studies combining these measures are needed to test the temporal relationships proposed by experimental models of the microbiota–gut–brain axis [74–78].

A key clinical question is whether modulating the gut microbiota can influence gastrointestinal or behavioural outcomes in ASD. The intervention studies included in this review evaluated probiotics, prebiotics, dietary approaches and faecal microbiota transplantation, reporting variable effects on gastrointestinal symptoms, microbial composition and behavioural measures [22,23,25,32,34,35,39,40,42,44,48–51,60,64,65]. However, adverse-event reporting varied across studies, and long-term safety data remain limited, preventing firm conclusions about safety or clinical effectiveness. Importantly, improvements observed after microbiota-targeted interventions should not be interpreted as evidence that dysbiosis causes ASD. Instead, reported changes following these interventions may reflect gastrointestinal symptoms or other clinical outcomes, regardless of whether the underlying microbiota changes are primary pathogenic mechanisms or secondary consequences of ASD-related characteristics. Improvements in gastrointestinal symptoms, associated behavioural measures, core autistic characteristics, and microbiological outcomes should be interpreted separately. Differences in study design, intervention protocols, treatment duration, and outcome assessment further limit comparisons, particularly when findings are based on uncontrolled within-group changes rather than between-group effects.

In controlled studies, the absence of statistically significant between-group differences in selected primary behavioural outcomes [23,51] highlights the need to distinguish exploratory secondary findings from established treatment effects.

Recent systematic reviews and meta-analyses report heterogeneous findings and insufficient evidence for establishing microbiota-targeted interventions as routine ASD treatments [82–84]. Gao et al. [82] found no statistically significant overall pooled effect on gastrointestinal or behavioural symptoms. These findings reinforce the need for well-controlled studies with clearly defined clinical outcomes before conclusions about therapeutic efficacy can be drawn.

Clinically, this review's findings do not support the routine use of microbiota-based diagnostic tests or microbiota-targeted therapies for individuals with ASD. Nevertheless, they emphasise the importance of considering intestinal health as part of comprehensive clinical assessment. Gastrointestinal symptoms, dietary habits, nutritional status, and medication exposure should be evaluated systematically because they not only affect quality of life but may also shape how microbiome findings are interpreted. This integrated approach aligns with the growing movement toward more individualised clinical and microbiome research, in which biological, clinical, and environmental information is considered collectively rather than independently.

Strengths and Limitations

The main strength of this review is integrating microbial composition, gastrointestinal manifestations, microbial function, directly measured microbiota-derived metabolites, and microbiota-targeted interventions within a single clinically oriented evidence map. The review followed JBI methodology and PRISMA-ScR reporting, incorporated a complementary CINAHL search, documented known parent reports and unresolved potential overlap, and added design-appropriate JBI critical appraisal to support more transparent interpretation of the evidence.

Several limitations need to be considered. The included studies varied widely in participant characteristics, gastrointestinal phenotyping, microbiome methods, and outcome measures, limiting direct comparison. Many studies were cross-sectional and based on relatively small or selected samples. Critical appraisal identified recurrent concerns regarding confounding control and incomplete reporting in observational studies, while some intervention studies were limited by open-label or uncontrolled designs, small samples, blinding constraints or incomplete follow-up. Although appraisal informed interpretation, we did not use it to exclude studies because this scoping review aimed to map the extent and nature of the evidence.

The 45 included publications must not be interpreted as 45 demonstrably independent cohorts. Pan–Quan and Chen–Chang share centres, investigators or recruitment periods, but participant overlap could not be confirmed. Known secondary reports were linked to their parent trials. We did not sum participants across potentially overlapping reports or use their findings as independent replication.

Independent duplicate appraisal was not documented for the two reports added during reconciliation, and funding of individual included publications was not systematically charted. These aspects limit the appraisal process and reporting completeness, respectively, without changing the eligibility criteria or the distinction between associations and intervention effects.

The eligibility criteria also shape the review's scope. We excluded studies reporting only descriptive taxonomic comparisons without clinical, functional, metabolic, or intervention-related information. Therefore, the conclusions about ASD-associated microbial patterns should be understood in relation to the clinically oriented evidence examined here, not as a synthesis of the entire descriptive microbiome literature. Diet, gastrointestinal symptoms, medication use and other host-related factors may also contribute to differences between

studies. Furthermore, the search was restricted to three bibliographic databases, and relevant studies indexed elsewhere may not have been retrieved.

Finally, unpublished studies, the grey literature and selective reporting may have affected the available evidence. Further research would benefit from larger longitudinal studies with better characterisation of gastrointestinal symptoms, diet and medication exposure, together with more standardised microbiome methods and, where possible, integrated multi-omics approaches.

5. Conclusions

Gut microbiota differences are frequently reported in individuals with ASD, but findings remain inconsistent and do not support a reproducible ASD-specific microbial signature. Differences in gastrointestinal symptoms, diet, medication exposure, participant characteristics, and microbiome methods may contribute to this variability. The available evidence does not establish temporal or causal direction: observed microbiota differences may relate to gastrointestinal phenotype, diet, medication exposure or other host characteristics, and bidirectional relationships remain plausible.

Current evidence does not support the routine use of microbiota-based biomarkers or microbiota-targeted treatments in ASD. Intervention studies have reported variable findings across gastrointestinal, behavioural, and microbiological outcomes, while controlled evidence remains limited. Differences in study design, intervention protocols, and outcome measures prevent firm conclusions about clinical effectiveness, particularly regarding core autistic characteristics. The design-specific critical appraisal further supports this cautious interpretation, especially when associations come from cross-sectional data or intervention effects come from small, uncontrolled, or incompletely blinded studies.

Future studies would benefit from larger, well-characterised cohorts, longitudinal designs and greater consistency in microbiome sampling, analysis and outcome assessment. Careful assessment of gastrointestinal symptoms, diet and medication exposure, together with functional and multi-omics approaches, may help clarify which microbiome findings are reproducible and clinically meaningful.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life16111895/s1, Material S1: Electronic Search Strategies and Complementary Search; Table S1: Complete Data Charting of the 45 Included Publications in the Scoping Review; Table S2: JBI Critical Appraisal of the 45 Included Publications; Table S3: Provenance of the 10 Additional Studies Identified Through Complementary CINAHL Searching; Table S4: Report Reconciliation.

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Abbreviations

The following abbreviations are used in this manuscript:

5-HIAA	5-Hydroxyindoleacetic Acid
ASD	ASD Autism Spectrum Disorder
FMT	Faecal Microbiota Transplantation
GI	Gastrointestinal
JBI	Joanna Briggs Institute
MeSH	Medical Subject Headings
OSF	Open Science Framework
PCC	Population–Concept–Context
PRISMA-ScR	Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Review
SCFAs	Short-Chain Fatty Acids
VOCs	Volatile Organic Compounds
WMT	Washed Microbiota Transplantation

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