

Revealing the gut microbiome mystery: A meta-analysis revealing differences between individuals with autism spectrum disorder and neurotypical children

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SUMMARY The brain-gut axis intricately links gut microbiota (GM) dysbiosis to the development or worsening of autism spectrum disorder (ASD). However, the precise GM composition in ASD and the effectiveness of probiotics are unclear. To address this, we performed a thorough meta-analysis of 28 studies spanning PubMed, PsycINFO, Web of Science, Scopus, and MEDLINE, involving 1,256 children with ASD and 1042 neurotypical children, up to February 2024. Using Revman 5.3, we analyzed the relative abundance of 8 phyla and 64 genera. While individuals with ASD did not exhibit significant differences in included phyla, they exhibited elevated levels of *Parabacteroides*, *Anaerostipes*, *Faecalibacterium*, *Clostridium*, *Dorea*, *Phascolarctobacterium*, *Lachnoclostridium*, *Catenibacterium*, and *Collinsella* along with reduced percentages of *Barnesiella*, *Odoribacter*, *Paraprevotella*, *Blautia*, *Turicibacter*, *Lachnospira*, *Pseudomonas*, *Parasutterella*, *Haemophilus*, and *Bifidobacterium*. Notably, discrepancies in *Faecalibacterium*, *Clostridium*, *Dorea*, *Phascolarctobacterium*, *Catenibacterium*, *Odoribacter*, and *Bifidobacterium* persisted even upon systematic exclusion of individual studies. Consequently, the GM of individuals with ASD demonstrates an imbalance, with potential increases or decreases in both beneficial and harmful bacteria. Therefore, personalized probiotic interventions tailored to ASD specifics are imperative, rather than a one-size-fits-all approach.

Keywords autism spectrum disorder (ASD), gut microbiota, meta-analysis, neurotypical children

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder emerging in early childhood, marked by social interaction and communication impairments, repetitive behaviors, and potential comorbidities including sleep, immune, gastrointestinal disorders, and endocrine imbalances. Its prevalence is rising, with about 1 in 100 children affected globally as of 2022, according to the World Health Organization (WHO) (1). Nonetheless, ASD presents with heterogeneous clinical manifestations, and its etiology and pathogenesis are multifaceted and intricate. Although research suggests that ASD has a complex etiology involving both genetic and environmental factors (2), specific causes are still not well understood.

Extensive research has revealed that the development and progression of ASD may be closely linked to gut microbiota dysbiosis. Clinical investigations have

frequently observed that children with ASD often experience gastrointestinal symptoms (GIS) like diarrhea, constipation, and abdominal pain linked to disrupted GM. These GIS have been found in 9 to 91% of individuals with ASD and are correlated with the severity of clinical symptoms (3). The exact causal relationship between ASD and GIS is still unclear, but numerous studies have indicated a certain association between them. Fortunately, the "microbiota-gut-brain axis" mechanism provides novel insights into understanding this connection (4). The gut-brain axis is a crucial bidirectional communication pathway between the brain and the gastrointestinal tract, with GM acting as key regulators. They can influence brain function through the enteric nervous system (ENS), endocrine system, metabolic system, and immune system. Disruptions in the dynamic equilibrium of GM within the human body lead to peripheral neurotransmitter imbalances, abnormal secretion of metabolites, or activation of immune

responses, ultimately resulting in elevated levels of peripheral inflammatory mediators capable of affecting neurodevelopment through circulation or penetration of the blood-brain barrier (5). In other words, changes in GM composition may contribute to gastrointestinal disturbances and exacerbate ASD symptoms (6-8).

Interestingly, numerous studies have documented notable variation in the composition and quantity of GM between ASD and neurotypical children (9), but there is no consensus on the dysregulation of GM in ASD. Moreover, research on the effectiveness of prebiotics, probiotics, and fecal microbiota transplantation in managing ASD has yielded mixed results (10-12). Consequently, analyzing current clinical data and increasing sample sizes are essential to better understanding the changes in GM in individuals with ASD and to provide insights for developing treatments involving probiotics, prebiotics, or fecal transplantation.

Thus far, four published meta-analysis have examined the association between GM and ASD, yielding inconsistent conclusions. These studies reported varied findings, including a decreased presence of *Akkermansia*, *Bifidobacterium*, *Bacteroides*, *Enterococcus* and *Escherichia coli* compared to typically developing children, and an increased prevalence of *Faecalibacterium* and *Lactobacillus*, with a slight elevation in *Ruminococcus* and *Clostridium* (13). Moreover, another meta-analysis performed in 2020 noted a higher abundance of Bacteroidetes, Firmicutes, and Actinobacteria along with specific genera like *Bacteroides*, *Clostridium*, *Faecalibacterium*, *Parabacteroides*, and *Phascolarctobacterium*, but a decreased proportion of *Bifidobacterium* and *Coprococcus* (14). Conversely, a 2022 meta-analysis found no significant correlation for the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria but did report significantly lower levels of *Bifidobacterium* and *Streptococcus* in ASD (15). Moreover, the latest meta-analysis published in 2024 found decreased levels of *Bifidobacterium* and *Parabacteroides* in comparison to controls while observing elevated levels of *Bacteroides*, *Clostridium*, and *Faecalibacterium* (16). To date, published meta-analysis have covered only a limited range of GM, precluding a comprehensive understanding of GM in ASD.

To address conflicting findings regarding the composition of GM in ASD versus neurotypical controls and to provide data on the association between ASD and GM, our meta-analysis integrated data from recent studies encompassing the full spectrum of tested GM, comprising approximately 8 phyla and 64 genera, to statistically derive significant conclusions about variations in gut microbial composition. These findings are anticipated to make a valuable contribution in the advancement of a potential set of biomarkers for the diagnosis of ASD or the identification of targets for therapy.

2. Methods

To ensure the transparency and reliability of our findings, we diligently followed the guidelines outlined by PRISMA (17), which provide a comprehensive framework for performing meta-analyses in a systematic and standardized manner.

2.1. Literature search

Our meta-analysis involved an exploration of diverse databases such as MEDLINE, PubMed, PsycINFO, Scopus, and Web of Science. The search terms were combined using Boolean logic operators: (autism OR autism spectrum disorder OR ASD OR autistic disorder) AND (microbiota OR microflora OR stool OR fecal OR microbiome). The search options used in the Scopus database included "title, abstract, and keywords," whereas the PubMed database relied on searching through "title/abstract," and the "abstract" was searched for in other databases. Moreover, the searches encompassed English publications without any restrictions on the year of publication. To guarantee a thorough examination of relevant literature, we diligently examined the references of systematic reviews and meta-analysis that explored differences in GM among ASD versus neurotypical children.

2.2. Selection criteria

The studies were selected based on the inclusion criteria outlined below: (1) Participants consisted of individuals diagnosed with ASD, with neurotypical individuals constituting the control group; (2) Studies comparing the composition of GM in individuals; (3) Studies examining the relative abundance (RA) of GM, including at least the phylum and/or genus level of microbiota; and (4) Studies using stools samples for analysis.

Exclusion of studies was based on the following criteria: (1) Animal model studies; (2) Studies focusing solely on GM in blood, urine, or saliva; (3) Reviews, meta-analyses, books, conferences, or editorial materials; and (4) non-English publications.

A point worth emphasizing us that a considerable number of studies fulfilling the inclusive criteria were not included due to incomplete data or only presenting figures without specific data (*e.g.*, missing values for RA, Mean, or SD) despite attempts to obtain this information through direct communication with either the corresponding author or first author.

2.3. Data extraction and study quality

The data presented in Table 1 were extracted from included studies by two researchers working independently: First authors' surnames (publication years), the subjects' country, details on children with

ASD and NT children (sample size, gender, mean age \pm SD), samples of extracted DNA, and details on GM (microbiological assessments, units). Importantly, our meta-analysis comprehensively incorporated all relevant data on GM from the included studies to thoroughly investigate the composition of GM in ASD.

The Newcastle-Ottawa Scale (NOS) was utilized to evaluate the methodological quality of the included studies in our meta-analysis, which primarily focused on assessing observational research such as cohort and case-control studies (18). The NOS uses a "star system" to evaluate three dimensions: Selection, Comparability, and Exposure (for case-control studies)/Outcome (for cohort studies). It consists of 8 items with a maximum rating of 9 stars. The quality was classified as high (7 – 9 stars), moderate (4 – 6 stars), or low (below 4 stars).

To ensure the reliability of extracted study data and evaluate the quality of the NOS, two researchers collaborated to extract data from a single study, resolving any discrepancies through consensus. After achieving an impressive rate of consistency of 99%, they subsequently performed the task independently. In addition, for accuracy, our meta-analysis compared the extracted microbial data with published meta-analyses and data were double-checked for any inconsistencies.

2.4. Statistical analysis

The included studies reported the relative abundance (RA), mean, standard deviation (SD), standard error (SE), or confidence interval of GM in children with ASD and NT children. RA and SE were used to standardize the data in order to calculate the overall percentage of GM from various phyla and genera in both the ASD and NT groups. In cases where SE was not directly available, we derived it using the formula $SE = SD/\sqrt{n}$.

Review Manager 5.3 was used to assess effect sizes, heterogeneity, and sensitivity analysis. (1) Heterogeneity was assessed using the Chi-Square test and I^2 . $P < 0.10$ in the Chi-square test suggests significant heterogeneity among the included studies. I^2 values of 25%, 50%, and 75% indicate slight, moderate, and high levels of heterogeneity, respectively. In situations where there are inconsistencies between the results of the Chi-square test and I^2 , priority is given to assessing studies based on I^2 . (2) Calculation of Effect Sizes. A random-effects model is utilized when $I^2 \geq 50\%$ ($P < 0.10$); otherwise, a fixed-effects model is selected. The standardized mean difference (SMD) was used as the measure of effect size in our meta-analysis. An $SMD > 0$ indicates a higher relative abundance of GM in the ASD compared to the NT group, while an $SMD < 0$ suggests a lower average abundance of GM in the ASD group. SMDs of approximately 0.20, 0.50, and 0.80 represent small, medium, and large effect sizes, respectively. (3) Sensitivity analysis in our meta-analysis was performed through a systematic exclusion of individual studies.

If the consistency of the subgroup difference remained relatively stable even after excluding a particular study, this suggested limited susceptibility and enhanced the reliability of our results. In particular, the design of our included studies, specifically cohort and case-control studies, precluded the possibility of performing a publication bias analysis in our meta-analysis. Typically, publication bias analysis is used to verify the accuracy and representativeness of study results by comparing effect sizes across different groups (19). However, our meta-analysis primarily focused on subgroup analyses comparing the abundance of GM in individuals with ASD to NT children, without the inclusion of any comparison groups.

3. Results

The step-by-step process for article screening is depicted in Figure 1. The articles underwent a rigorous review process, including the examination of titles, abstracts, and full texts. A comprehensive screening was then conducted using predefined criteria for inclusion and exclusion. As a result, 28 articles were deemed eligible for inclusion (20-47). A point of note is that although an additional 24 articles initially met the inclusion criteria of our meta-analysis, they were ultimately excluded due to the presentation of only images without providing accurate data or a lack of response despite attempts to contact the authors.

3.1. Characteristics of the included studies

The details regarding the included studies in our meta-analysis can be found in Table 1. Most of the studies were conducted in China (ten), followed by seven in the US, three in Italy, two in Australia, and one each in Japan, Spain, India, Tunisian, Uruguay, and Russia. The sample sizes ranged from 6 to 143, with 1,256 individuals with ASD and 1042 neurotypical children between the ages of 2 to 37 years. Most studies utilized 16S rRNA gene sequencing to analyze GM differences, with two studies using culture-based methods, four using a polymerase chain reaction (PCR), and two using shotgun metagenomic sequencing. Stool samples were gathered for analysis in each of the included studies. Microbiota analysis primarily focused on the phylum and genus levels, and a wide range of microbes was reported in terms of Relative Abundance (RA) or percentage.

3.2. Study quality

We performed an in-depth analysis of the sample selection and study design of the included studies, subsequently establishing criteria to evaluate their quality using the NOS. Our primary considerations regarding selection included (1) whether the studies provided comprehensive

Table 1. The characteristics of included studies in the meta-analysis

Study	ASD			NT			Sample			Details on Microbiota	
	Country	n	Gender (M/F)	Age (years)	n	Gender (M/F)	Age (years)	Sample	Microbiology Assessment	Outcomes	Unit
Finegold (2010)	USA	19	-	2-13	8	5/3	2-13	stool	Pyrosequencing	Phylum: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria, Fusobacteria, Verrucomicrobia, Tenericutes Genus: <i>Akkermansia</i> , <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Clostridium</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i>	RA
Wang (2011)	Australia	23	21/2	10.25 ± 0.75	9	4/5	9.5 ± 1.25	stool	qPCR	Genus: <i>Akkermansia</i> , <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Faecalibacterium</i> , <i>Clostridium</i>	RA
Adam (2011)	USA	58	50/8	6.91 ± 3.4	39	18/21	7.7 ± 4.4	Stool	Culture	Genus: <i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Enterococcus</i>	RA/CFU
Gondalia (2012)	Australia	51	42/9	2-12	53	19/34	2-12	Stool	Culture	Phylum: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria, Fusobacteria, Verrucomicrobia, Tenericutes Genus: <i>Anaerostipes</i> , <i>Anaerotruncus</i> , <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Blautia</i> , <i>Clostridium</i> , <i>Roseburia</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i> , <i>Sutterella</i> , <i>Veillonella</i> , <i>Coproccoccus</i> , <i>Dialister</i> , <i>Dorea</i> , <i>Phascolarctobacterium</i>	RA
Williams (2012)	USA	23	-	-	9	-	-	Stool	16S rRNA genes sequencing/PCR	Genus: <i>Sutterella</i>	RA
Angelis (2013)	Italy	10	-	4-10	10	-	4-10	Stool	Pyrosequencing	Genus: <i>Akkermansia</i> , <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Clostridium</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i>	CFU/RA
Kang (2013)	USA	20	17/3	6.7 ± 2.7	20	18/2	8.3 ± 4.4	stool	qPCR	Phylum: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria, Fusobacteria, Verrucomicrobia, Tenericutes Genus: <i>Akkermansia</i> , <i>Anaerostipes</i> , <i>Anaerotruncus</i> , <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Blautia</i> , <i>Clostridium</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i> , <i>Sutterella</i> , <i>Veillonella</i> , <i>Coproccoccus</i> , <i>Dialister</i> , <i>Dorea</i> , <i>Phascolarctobacterium</i> , <i>Roseburia</i>	RA
Son (2015)	USA	59	52/7	10.3 ± 1.8	44	21/23	10.0 ± 1.8	stool	qPCR	Genus: <i>Sutterella</i> , <i>Bacteroidetes</i> , <i>Prevotella</i>	RA

Note: ASD: Autism spectrum disorder; NT: Neurotypical children; M/F: male/female; RA: Relative Abundance; CFU: Colony Forming Unit

Table 1. The characteristics of included studies in the meta-analysis (continued)

Study	ASD			NT		Sample	Details on Microbiota				
	Country	n	Gender (M/F)	Age (years)	Gender (M/F)		Age (years)	Microbiology Assessment	Outcomes	Unit	
Inoue (2016)	Japan	6	-	3-5	6	-	3-5	stool	16S rRNA gene sequencing	Genus: <i>Akkermansia</i> , <i>Anaerostipes</i> , <i>Anaerotruncus</i> , <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Blautia</i> , <i>Clostridium</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i> , <i>Sutterella</i> , <i>Veillonella</i> , <i>Coproccoccus</i> , <i>Dialister</i> , <i>Dorea</i> , <i>Phascolarctobacterium</i> , <i>Roseburia</i>	RA
Strati (2017)	Italy	40	31/9	11.1 ± 6.8	40	28/12	9.2 ± 7.9	stool	16S rRNA gene sequencing	Phylum: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria, Fusobacteria, Verrucomicrobia Genus: <i>Akkermansia</i> , <i>Anaerostipes</i> , <i>Anaerotruncus</i> , <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Blautia</i> , <i>Clostridium</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i> , <i>Sutterella</i> , <i>Veillonella</i> , <i>Coproccoccus</i> , <i>Dialister</i> , <i>Dorea</i> , <i>Phascolarctobacterium</i> , <i>Roseburia</i>	RA
Berding (2018)	USA	26	19/7	4.1 ± 1.6	32	19/13	4.8 ± 1.8	Stool	Real-time PCR	Phylum: Bacteroidetes, Firmicutes, Clostridiales, Streptophyta Genus: <i>Clostridiaceae</i> , <i>Clostridium</i> , <i>SMB53</i> , <i>Blautia</i> , <i>Roseburia</i> , <i>Butyrivibrio</i> , <i>Butyrivibrio</i> , <i>Faecalibacterium</i> , <i>Dialister</i> , <i>Bilophila</i> , <i>Bifidobacterium</i> , <i>C. perfringens</i>	RA
Coretti (2018)	Italy	11	9/2	2-4	14	8/6	2-4	Stool	16S rRNA gene sequencing	Phylum: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria Genus: <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Blautia</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i> , <i>Coproccoccus</i> , <i>Roseburia</i>	RA
Kang (2018)	USA	23	22/1	10.1 ± 4.1	21	15/6	8.4 ± 3.4	Stool	16S rRNA gene sequencing	Genus: <i>Faecalibacterium</i> , <i>Haemophilus</i> , <i>Prevotella</i>	RA
Pulikkan (2018)	India	30	28/2	9.5 ± 3.25	24	15/9	9.5 ± 3.13	Stool	16S rRNA gene sequencing	Genus: <i>Lactobacillus</i>	RA
Zhang (2018)	China	35	29/6	4.9 ± 1.5	6	5/1	4.6 ± 1.1	Stool	16S rRNA sequencing	Phylum: Bacteroidetes Genus: <i>Veillonella</i> , <i>Streptococcus</i> , <i>Escherichia</i>	RA
Ma (2019)	China	45	39/6	7.04 ± 1.19	45	39/6	7.27 ± 1.07	Stool	16S rRNA gene sequencing	Phylum: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria, Fusobacteria, Verrucomicrobia, Tenericutes Genus: <i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Blautia</i> , <i>Clostridium</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i> , <i>Coproccoccus</i> , <i>Phascolarctobacterium</i> , <i>Roseburia</i>	RA

Note: ASD: Autism spectrum disorder; NT: Neurotypical children; M/F: male/female; RA: Relative Abundance; CFU: Colony Forming Unit

Table 1. The characteristics of included studies in the meta-analysis (continued)

Study	Country	ASD			NT			Sample	Details on Microbiota		
		n	Gender (M/F)	Age (years)	n	Gender (M/F)	Age (years)		Microbiology Assessment	Outcomes	Unit
Plaza-Diaz (2019)	Spain	48	-	2-6	57	-	2-6	Stool	16S rRNA gene amplicon sequencing	Phylum: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Verrucomicrobia Genus: <i>Akkermansia</i> , <i>Bacteroides</i> , <i>Veillonella</i> , <i>Bifidobacterium</i> , <i>Clostridium</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i>	RA
Dan (2020)	China	143	130/13	4.94 ± 0.16	143	127/16	5.19 ± 0.17	Stool	16S rRNA gene sequencing	Genus: <i>Bacteroides</i> , <i>Prevotella</i> , <i>Paraprevotella</i> , <i>Phascolarctobacterium</i> ,	RA
Zou (2020)	China	48	38/10	2-7	48	24/24	4	Stool	16S rRNA gene sequencing	Phylum: Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia Genus: <i>Clostridium</i> XIIIa, <i>Bacteroides</i> , <i>Prevotella</i> , <i>Eisenbergiella</i> , <i>Lachnospiraceae</i> , <i>incertae_sedis</i> ...	RA
Ding (2020)	China	77	59/18	3.21 ± 0.96	50	39/11	3.58 ± 1.21	Stool	16S rRNA gene sequencing	Genus: <i>Lachnospiraceae</i> , <i>Clostridiales</i> , <i>Dorea</i> , <i>Erysipelotrichaceae</i> , <i>Collinsella</i> , <i>Lachnoclostridium</i> , <i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Parasutterella</i> , <i>Paraprevotella</i>	RA
Averina (2020)	Russia	36	30/6	3.72 ± 0.61	21	14/7	3.58 ± 0.63	Stool	shotgun metagenome sequencing	Genus: <i>Barnesiella</i> , <i>Parabacteroides</i>	RA
Chen (2021)	China	138	117/21	6.11 ± 2.00	60	27/33	6.65 ± 2.22	Stool	16S rRNA gene sequencing	Genus: <i>Prevotella</i> , <i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Sutterella</i> , <i>Megamonas</i> , <i>Coproccoccus</i> , <i>Collinsella</i> , <i>Desulfovibrio</i>	RA
Deng (2022)	China	45	39/6	5.95 ± 2.36	45	21/24	6.13 ± 0.90	Stool	16S rRNA gene sequencing	Phylum: Bacteroidetes, Genus: <i>Agathobacter</i> , <i>Massilia</i> , <i>Proteobacteria</i> , <i>Gammaproteobacteria</i> , <i>Massilia</i> , <i>Megamonas</i> , <i>Sphingomonas</i> , <i>Agathobacter</i> , <i>Blautia</i>	RA
Wong (2022)	China	92	30/62	8.2	112	32/80	8.47	Stool	16S rRNA gene sequencing	Phylum: Firmicutes; Bacteroidetes Genus: <i>Bifidobacterium</i> , <i>Dorea</i> , <i>Blautia</i> , <i>Collinsella</i> , <i>Bacteroides</i> , <i>Alistipes</i> , <i>Parabacteroides</i> , <i>Sutterella</i>	RA
Chamtouri (2023)	Tunisian	28	22/6	7.93 ± 2.05	28	22/6	7.29 ± 2.09	Stool	16S rRNA gene sequencing	Genus: <i>Bacteroides</i> , <i>Lachnoclostridium</i> , <i>Megamonas</i> , <i>Collinsella</i> , <i>Subdoligranulum</i> ...	RA
Pang (2023)	China	19	14/5	17-32	19	14/5	19-37	Stool	16S rRNA gene sequencing	Phylum: Proteobacteria Genus: <i>Agathobacter</i> , <i>Akkermansia</i> , <i>Alistipes</i> , <i>Anaerobutyricum</i> , <i>Anaerostipes</i> , <i>Bifidobacterium</i> ... (18)	RA

Note: ASD: Autism spectrum disorder; NT: Neurotypical children; M/F: male/female; RA: Relative Abundance; CFU: Colony Forming Unit

Table 1. The characteristics of included studies in the meta-analysis (continued)

Study	ASD			NT		Sample			Details on Microbiota	
	Country	n	Gender (M/F)	Age (years)	n	Gender (M/F)	Age (years)	Microbiology Assessment	Outcomes	Unit
Dubourdieu (2023)	Uruguay	30	-	3-12	28	-	3-12	16S rDNA gene sequencing	Genus: <i>Bifidobacterium</i> , <i>Clostridium glycolicum</i> , <i>Roseburia</i> , <i>Faecalibacterium</i> , <i>Eubacterium ventriosum</i> , <i>Flavonifractor plautii</i>	RA
Wang (2023)	China Russia	43 30	- -	2-7 3-5	31 20	- -	2-7 3-5	Shotgun metagenomic sequencing	Genus: <i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Eubacterium</i> , <i>Bifidobacterium</i> , <i>Alistipes</i> , <i>Prevotella...</i> (91)	RA

Note: ASD: Autism spectrum disorder; NT: Neurotypical children; M/F: male/female; RA: Relative Abundance; CFU: Colony Forming Unit

information on the diagnostic criteria for ASD and (2) whether NT children were recruited from community settings or hospitals. In term of comparability, our key focus lay in assessing whether studies controlled for factors such as age, gastrointestinal comorbidities, probiotic or prebiotic treatments, and special diets. Our primary examination of exposure/outcome centered on the methods used for fecal sample preservation and analytical techniques. In addition, the response rate was not addressed in any of the included studies, so all studies were awarded a star in this criterion.

Ultimately, all included studies were assessed to be of medium to good quality. Specifically, 21 studies were deemed to be of good quality, while 7 studies were categorized as medium quality. Regarding selection criteria, the majority of included studies provided comprehensive descriptions of the screening criteria for ASD, such as DSM-5, ICD-10, or CARS, as shown in Table 3. However, two articles briefly mentioned the inclusion of diagnosed ASD without providing specific details regarding diagnostic criteria (23,37). In addition, there was insufficient information in 7 articles (21,23,29,38,42,47,48) regarding the location of the children in the control group. Regarding comparability, all studies rigorously matched age across subgroups, but 16 studies that did not explicitly address gastrointestinal comorbidities in participants (20,23-25,29,33,35,38-41,43-45,47,49), and 7 studies did not explicitly control for probiotic or prebiotic treatments or special diets (21-23,37,41,42,49). Regarding Exposure/Outcome, all studies used rigorous scientific protocols for the preservation of fecal samples and they all utilized effective analytical techniques, including culture, PCR, and pyrosequencing, in both cohorts.

3.3. Mean effect size and between-study heterogeneity

The mean effect sizes according to our meta-analysis, which includes data at both the phylum and genus levels of GM, are shown in Table 2. The meta-analysis revealed no significant differences between children with ASD and NT children across the bacterial phyla Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Fusobacteria, Proteobacteria, Tenericutes, and Verrucomicrobia. Notably, the overall effect size for two subgroups, except for Cyanobacteria, was statistically significant across Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, Tenericutes, and Verrucomicrobia, ranging from 2.18 for Fusobacteria to 34.86 for Firmicutes. This indicated that both groups might have a greater abundance of Actinobacteria, Bacteroidetes, Proteobacteria, and Verrucomicrobia, along with a lower abundance of Firmicutes, Fusobacteria, and Tenericutes. Moreover, between-study heterogeneity was high, ranging between 25% and 100%, while heterogeneity within subgroups when comparing both phyla was zero.

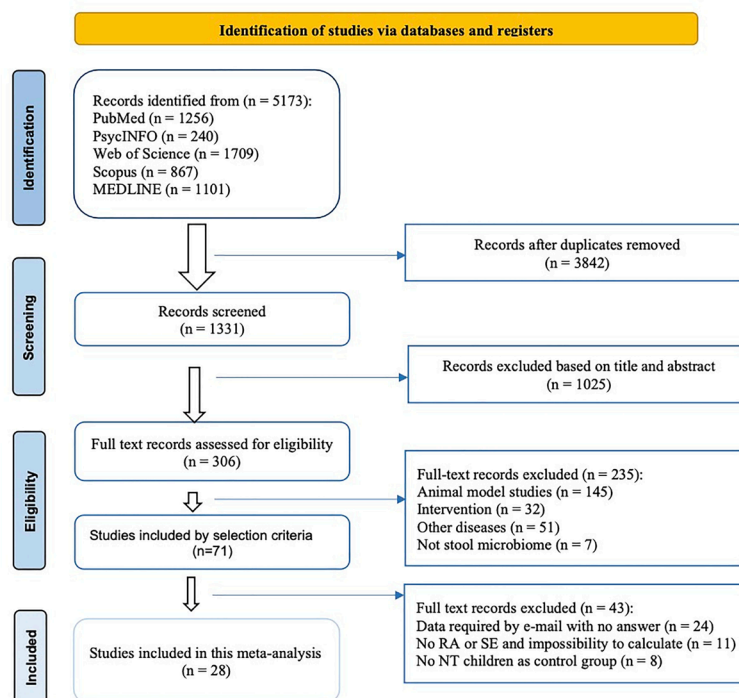


Figure 1 Flow Diagram for selection of studies (PRISMA flow diagram).

Figure 1. Flow diagram for selection of studies (PRISMA flow diagram).

3.3.1. Bacterial genera that were more abundant in individuals with ASD than controls

Parabacteroides: As shown in Table 2, 18 studies were included in the random-effects meta-analysis for *Parabacteroides*. The RA of *Parabacteroides* was 0.18% (95% CI: 0.13, 0.23) in the ASD group, compared to 0.09% (95% CI: 0.06, 0.12) in the NT group. High between-study heterogeneity was observed in both subgroups ($I^2 = 95\%$ and 96% , respectively), as well as in the comparison between the two groups ($I^2 = 89\%$). The overall effect size was large and highly significant ($Z = 7.90$, $P < 0.001$). In addition, there was a difference in the bacterial percentage of 2, suggesting higher levels of *Parabacteroides* in children with ASD compared to NT individuals.

Anaerostipes: The meta-analysis for *Anaerostipes* included 11 studies, indicating that 0.27% (95% CI: 0.19, 0.35) of the detected microbiota were attributed to *Anaerostipes* in the ASD group, while 0.08% (95% CI: 0.05, 0.11) were attributed in the NT group. Very high between-study heterogeneity was observed ($I^2 = 98\%$) in both groups. In addition, high heterogeneity persisted in the comparison between the two groups ($I^2 = 94.40\%$). The effect size was large and significant ($Z = 8.74$, $P < 0.001$). Moreover, the difference in bacterial percentage for *Anaerostipes* was 3.38, showing that children with ASD exhibited greater levels of *Anaerostipes* in comparison to NT individuals.

Faecalibacterium: The relative abundance of *Faecalibacterium* was evaluated across 22 trials. In

children diagnosed with ASD, the percentage was 2.28% (95% CI: 2.04, 2.52) in contrast to 1.04% (95% CI: 0.88, 1.19) in the NT group. High between-study heterogeneity was noted in both the ASD (99%) and NT (98%) groups, as well as between the subgroups ($I^2 = 98.60\%$). The effect size was large and significant ($Z = 22.82$, $P < 0.001$). A difference in bacterial percentage of 2.19 indicates that individuals with ASD had higher levels of *Faecalibacterium* than those without ASD.

Clostridium: Fourteen studies were included in the meta-analysis of *Clostridium*, yielding the following results: A relative abundance of 1.27% in the ASD group (95% CI: 0.97, 1.57) compared to 0.31% in the NT group (95% CI: 0.21, 0.41). Significant between-study heterogeneity was observed in both the ASD and control groups, with percentages of 97% and 98%, respectively, and heterogeneity remained very high (97.10%) when comparing the subgroups. The effect size was large and statistically significant ($Z = 9.79$, $P < 0.001$). The difference in bacterial percentage for *Clostridium* was significantly higher, by a factor of 4.10, among individuals with ASD compared to the NT group.

Dorea: Our meta-analysis included 12 studies on *Dorea*, revealing the following findings: the relative abundance of *Dorea* was 0.50% (95% CI: 0.33, 0.67) in children with ASD and 0.05% (95% CI: 0.03, 0.07) in the control group. Heterogeneity among studies was high at 97% and 98% in the ASD and the control group, respectively, while it decreased to 96.40% when comparing the two groups. The effect size was significant and of a large magnitude ($Z = 9.93$, $P < 0.001$). The

Table 2. Results of the meta-analysis comparing ASD and neurotypical children at the phylum and genus levels

	Included Studies			ASD		NT		Overall Effect		Subgroup Differences	
	Studies	Overall Relative Abundance	95% CI	Between-study I ²	Overall Relative Abundance	95% CI	Between-study I ²	Z	p	f	p
Bacteroidetes	5	30.35	8.21 – 52.48	100	28.55	7.04 – 50.06	99	24.85	<0.00001	0	0.91
<i>Bacteroides</i>	24	3.72	3.31 – 4.13	99	3.35	2.93 – 3.76	99	22.13	<0.00001	37.4	0.21
<i>Parabacteroides</i>	18	0.18	0.13 – 0.23	95	0.09	0.06 – 0.12	96	7.90	<0.00001	89	0.003
<i>Alistipes</i>	12	0.19	0.10 – 0.28	95	0.19	0.12 – 0.27	97	6.14	<0.00001	0	0.91
<i>Prevotella</i>	15	0.04	0.01 – 0.07	87	0.07	0.02 – 0.12	81	3.85	0.0001	19.8	0.26
<i>Fusobacterium</i>	7	0.01	-0.01 – 0.04	65	0.00	-0.00 – 0.01	78	1.91	0.06	0	0.38
<i>Barnesiella</i>	7	0.08	0.02 – 0.13	82	0.28	0.11 – 0.44	78	4.56	<0.00001	79.5	0.03
<i>Odoribacter</i>	9	0.09	0.05 – 0.13	97	0.18	0.12 – 0.24	82	7.01	<0.00001	82.8	0.02
<i>Paraprevotella</i>	8	0.00	0.00 – 0.01	11	0.03	0.01 – 0.05	52	3.08	0.002	80	0.03
<i>Pseudobutyrvibrio</i>	4	0.04	-0.01 – 0.09	98	0.01	-0.00 – 0.02	97	2.84	0.005	29.6	0.23
<i>Intestinimonas</i>	3	0.01	0.00 – 0.02	33	0.03	0.01 – 0.05	67	4.72	<0.00001	53	0.14
<i>Butyrivimonas</i>	7	0.02	0.01 – 0.03	4	0.03	0.01 – 0.06	77	3.75	0.0002	21.2	0.26
<i>Allisonella</i>	5	0.02	0.00 – 0.04	91	0.01	0.00 – 0.02	93	3.78	0.0002	16	0.28
Firmicutes	5	40.27	6.98 – 73.56	100	43.04	10.66 – 75.41	100	34.86	<0.00001	0	0.91
<i>Anaerostipes</i>	11	0.27	0.19 – 0.35	98	0.08	0.05 – 0.11	98	8.74	<0.00001	94.4	< 0.00001
<i>Anaerotruncus</i>	9	0.02	0.01 – 0.04	78	0.02	0.01 – 0.04	84	5.31	<0.00001	0	0.87
<i>Blautia</i>	14	0.09	0.06 – 0.13	99	0.20	0.15 – 0.25	99	8.35	<0.00001	91.9	0.0005
<i>Faecalibacterium</i>	22	2.28	2.04 – 2.52	99	1.04	0.88 – 1.19	98	22.82	<0.00001	98.6	< 0.00001
<i>Ruminococcus</i>	13	0.19	0.12 – 0.26	96	0.17	0.12 – 0.22	97	7.43	<0.00001	0	0.72
<i>Veillonella</i>	9	0.02	-0.00 – 0.03	79	0.05	0.01 – 0.03	87	3.47	0.0005	56.2	0.13
<i>Clostridium</i>	14	1.27	0.97 – 1.57	97	0.31	0.21 – 0.41	98	9.79	<0.00001	97.1	< 0.00001
<i>Coprococcus</i>	17	0.04	0.03 – 0.05	97	0.05	0.04 – 0.07	97	10.00	<0.00001	72.8	0.06
<i>Dialister</i>	8	0.01	-0.01 – 0.04	83	0.01	-0.01 – 0.02	88	1.64	0.10	0	0.77
<i>Dorea</i>	12	0.50	0.33 – 0.67	97	0.05	0.03 – 0.07	98	9.93	<0.00001	96.4	< 0.00001
<i>Phascolarctobacterium</i>	13	0.11	0.07 – 0.16	91	0.01	0.00 – 0.02	88	5.08	<0.00001	94.4	< 0.00001
<i>Roseburia</i>	14	0.06	0.04 – 0.09	94	0.04	0.02 – 0.06	97	6.77	<0.00001	53.8	0.14
<i>Enterococcus</i>	10	0.07	0.01 – 0.12	80	0.08	0.01 – 0.16	86	2.74	0.006	0	0.74
<i>Lactobacillus</i>	12	0.04	0.02 – 0.07	96	0.07	0.02 – 0.13	86	4.40	<0.0001	0	0.33
<i>Eubacterium</i>	11	0.23	0.16 – 0.30	97	0.25	0.15 – 0.34	98	8.88	<0.00001	0	0.84
<i>Holdemania</i>	6	0.01	0.00 – 0.02	82	0.01	0.00 – 0.01	73	4.37	<0.0001	0	0.32
<i>Lachnoclostridium</i>	8	0.47	0.36 – 0.57	99	0.24	0.17 – 0.30	99	13.90	<0.00001	92.5	0.0003
<i>Streptococcus</i>	11	0.08	0.04 – 0.13	93	0.09	0.04 – 0.14	92	4.23	<0.0001	0	0.88
<i>Turicibacter</i>	11	0.01	0.00 – 0.02	88	0.04	0.01 – 0.06	88	4.07	<0.0001	76.6	0.04
<i>Catenibacterium</i>	6	0.12	0.08 – 0.17	97	0.01	0.00 – 0.02	95	5.24	<0.00001	95.2	< 0.00001
<i>Fusicatenibacter</i>	7	0.67	0.45 – 0.89	98	0.61	0.36 – 0.87	98	10.17	<0.00001	0	0.73
<i>Holdemania</i>	5	0.03	0.01 – 0.05	99	0.01	0.00 – 0.03	89	3.30	0.0010	54.6	0.14
<i>Lachnospira</i>	8	0.07	0.02 – 0.11	89	0.26	0.08 – 0.43	95	5.86	<0.00001	76.2	0.04
<i>Lactococcus</i>	6	0.03	0.01 – 0.05	35	0.02	0.01 – 0.02	0	5.17	<0.00001	0	0.33

Note: ASD: autism spectrum disorder; NT: neurotypical children; Phyla are emphasized in bold font, while genera are italicized and organized based on their respective phyla.

Table 2. Results of the meta-analysis comparing ASD and neurotypical children at the phylum and genus levels (continued)

	Included Studies	ASD			NT			Overall Effect			Subgroup Differences		
		Overall Relative Abundance	95% CI	Between-study I ²	Overall Relative Abundance	95% CI	Between-study I ²	Z	p	I ²	p	p	
<i>Monoglobus</i>	3	0.01	0.00 - 0.01	3	0.00	-0.00 - 0.01	69	3.24	0.001	49	0.16		
<i>Megamonas</i>	13	0.01	-0.00 - 0.01	86	0.04	0.00 - 0.08	86	2.89	0.004	64.4	0.09		
<i>Megasphaera</i>	10	0.02	0.00 - 0.04	56	0.00	-0.00 - 0.00	24	2.37	0.02	70.1	0.07		
<i>Flavonifractor</i>	7	0.06	0.04 - 0.08	99	0.06	0.04 - 0.08	98	9.28	<0.00001	0	0.66		
<i>Acidaminococcus</i>	6	0.01	-0.00 - 0.01	44	0.00	-0.01 - 0.01	6	1.42	0.15	0	0.66		
<i>Butyrivibrio</i>	6	0.01	0.00 - 0.02	94	0.01	0.00 - 0.03	95	2.90	0.004	0	0.71		
<i>Gemmiger</i>	4	0.64	0.19 - 1.09	91	0.70	0.26 - 1.15	94	4.52	<0.00001	0	0.85		
<i>Intestinibacter</i>	5	0.03	-0.00 - 0.07	96	0.01	-0.00 - 0.03	95	2.93	0.003	9.6	0.29		
<i>Subdoligranulum</i>	7	0.02	-0.01 - 0.05	95	0.01	0.00 - 0.02	96	3.35	0.0008	0	0.40		
<i>Collinsella</i>	7	1.04	0.64 - 1.44	98	0.23	0.12 - 0.34	96	6.19	<0.00001	93.3	0.0001		
<i>Oscillospira</i>	6	0.14	0.07 - 0.22	94	0.17	0.11 - 0.24	97	6.99	<0.00001	0	0.52		
<i>Slackia</i>	5	0.19	-0.03 - 0.41	92	0.07	-0.01 - 0.16	79	3.32	0.0009	0	0.33		
<i>Dialister</i>	10	0.02	-0.00 - 0.04	90	0.06	0.02 - 0.10	89	2.57	0.01	67.7	0.08		
<i>Coprococcus</i>	6	0.01	0.00 - 0.02	64	0.04	0.01 - 0.08	88	3.40	0.0007	63.9	0.10		
Proteobacteria	5	1.89	0.32 - 3.46	97	1.28	0.41 - 2.15	98	4.93	<0.00001	0	0.50		
<i>Sutterella</i>	14	0.02	0.01 - 0.03	95	0.04	0.02 - 0.05	94	5.00	<0.00001	65.3	0.09		
<i>Escherichia/Shigella</i>	12	0.34	0.21 - 0.47	91	0.35	0.22 - 0.49	92	7.77	<0.00001	0	0.90		
<i>Pseudomonas</i>	6	0.00	-0.02 - 0.02	96	0.06	0.02 - 0.10	97	2.61	0.009	84.0	0.01		
<i>Klebsiella</i>	9	0.01	0.00 - 0.02	73	0.05	-0.01 - 0.11	75	3.19	0.001	44.1	0.18		
<i>Parasutterella</i>	8	0.03	0.01 - 0.04	87	0.11	0.05 - 0.17	80	5.98	<0.00001	85.3	0.009		
<i>Enterobacter</i>	6	0.00	-0.00 - 0.01	65	0.02	-0.01 - 0.05	72	1.83	0.07	0	0.40		
<i>Haemophilus</i>	11	0.04	0.02 - 0.06	83	0.13	0.06 - 0.20	65	5.23	<0.00001	83.8	0.01		
<i>Citrobacter</i>	5	0.01	-0.00 - 0.02	84	0.13	-0.05 - 0.30	51	2.63	0.009	44.6	0.18		
<i>Desulfovibrio</i>	9	0.01	0.00 - 0.02	39	0.01	0.00 - 0.03	61	4.67	<0.00001	0	0.90		
<i>Bifidobacteria</i>	6	0.03	0.01 - 0.05	89	0.06	0.04 - 0.09	74	5.11	<0.00001	66.2	0.09		
<i>Actinobacteria</i>	5	4.35	2.06 - 6.64	97	4.55	1.56 - 7.54	98	4.87	<0.00001	0	0.92		
<i>Bifidobacterium</i>	22	0.46	0.37 - 0.55	99	1.48	1.25 - 1.71	99	16.56	<0.00001	98.5	< 0.00001		
<i>Actinomyces</i>	6	0.03	0.01 - 0.04	96	0.04	0.01 - 0.06	96	4.51	<0.00001	0	0.46		
Cyanobacteria	3	0.00	-0.00 - 0.01	82	0.01	-0.01 - 0.04	93	1.66	0.10	0	0.43		
Fusobacteria	3	0.91	-1.23 - 3.06	80	0.50	-0.42 - 1.43	89	2.18	0.03	0	0.73		
<i>Fusobacterium</i>	3	0.03	-0.03 - 0.10	77	0.02	-0.03 - 0.08	62	1.33	0.18	0	0.80		
Verrucomicrobia	4	0.27	-0.14 - 0.68	89	0.32	-0.23 - 0.86	79	2.15	0.03	0	0.88		
<i>Akkermansia</i>	14	0.21	0.10 - 0.32	87	0.11	0.02 - 0.20	82	3.98	<0.0001	48.8	0.16		
Tenericutes	3	0.00	0.00 - 0.00	100	0.00	0.00 - 0.00	25	2.19	0.03	0	0.45		

Note: ASD: autism spectrum disorder; NT: neurotypical children; Phyla are emphasized in bold font, while genera are italicized and organized based on their respective phyla.

Table 3. Quality assessment of included studies

Study	Selection (max = ★★★★★)			Comparability (max = ★★★)		Exposure (max = ★★★)		Quality score
	Adequate definition of cases	Representativeness of the cases	Selection of controls	Definition of controls	Controls for important factor	Ascertainment of exposure	Same method of ascertainment for cases and controls	
Finegold (2010)	★	★	★	★	★	★	★	8
Wang (2011)	★	★	★	★	★★	★	★	9
Adam (2011)	★	★	-	★	★	★	★	6
Gondalia (2012)	★	★	-	-	★	★	★	7
Williams (2012)	★	-	-	-	★	★	★	4
Angelis (2013)	★	★	★	★	★★	★	★	9
Kang (2013)	★	★	★	-	★	★	★	7
Son (2015)	★	★	★	★	★★	★	★	9
Inoue (2016)	★	★	★	-	★★	★	★	8
Strati (2017)	★	★	-	-	★	★	★	6
Berding (2018)	★	★	★	★	★★	★	★	9
Coretti (2018)	★	★	★	★	★★	★	★	9
Kang (2018)	★	★	★	-	★★	★	★	8
Pulikkan (2018)	★	★	★	★	★★	★	★	8
Zhang (2018)	★	★	★	-	★★	★	★	8
Ma (2019)	★	★	-	★	★	★	★	7
Plaza-Diaz (2019)	★	★	★	★	★	★	★	8
Dan (2020)	★	-	★	★	★	★	★	7
Zou (2020)	★	★	-	-	★	★	★	6
Ding (2020)	★	★	★	★	★	★	★	8
Averina (2020)	★	★	★	★	★	★	★	8
Chen (2021)	★	★	★	★	★	★	★	8
Deng (2022)	★	★	★	★	★	★	★	8
Wong (2022)	★	★	-	-	★	★	★	6
Chamtouri (2023)	★	★	★	-	★	★	★	7
Pang (2023)	★	★	★	-	★	★	★	7
Dubourdieu (2023)	★	★	-	-	★	★	★	6
Wang (2023)	★	★	-	-	-	★	★	5

Note: each item within the Selection and Exposure categories of a study is eligible for a maximum of one star (one point). For the Comparability category, a study can earn up to two stars.

difference in bacterial percentage for *Dorea* was notably higher by a factor of 10 among individuals with ASD in comparison to the NT group.

Phascolarctobacterium: The meta-analysis of *Phascolarctobacterium*, which included 13 studies, yielded the following findings: 0.11% (95% CI: 0.07, 0.16) was observed in children with ASD, while 0.01% (95% CI: 0.00, 0.02) was observed in the control group. High between-study heterogeneity was observed in the ASD group ($I^2 = 91\%$) and the control group ($I^2 = 88\%$). Similarly, high heterogeneity was noted when comparing the two groups ($I^2 = 94.40\%$). The effect size was significant and large ($Z = 5.08, P < 0.001$).

Lachnospirillum: Eight studies were included in the meta-analysis of *Lachnospirillum*. The results were as follows: 0.47% (95% CI: 0.36, 0.57) was observed among children diagnosed with ASD, and 0.24% (95% CI: 0.17, 0.30) was observed in the NT group. Considerable heterogeneity was observed within both the ASD and control groups ($I^2 = 99\%$). Similarly, high heterogeneity was noted when comparing the two groups ($I^2 = 92.50\%$). A significant and large effect was evident in the meta-analysis of *Lachnospirillum* ($Z = 13.90, P < 0.001$).

Catenibacterium: The 6 trials included in the meta-analysis of *Catenibacterium* revealed that the level of *Catenibacterium* was 0.12% in the ASD group (95% CI: 0.08, 0.17) and 0.01% in the NT group (95% CI: 0.00, 0.02). There was very high heterogeneity observed ($I^2 = 97\%$ in the ASD group and 95% in the NT group) among the included studies and also between the subgroups ($I^2 = 95.20\%$). Nevertheless, the overall effect size was large and significant ($Z = 5.24, P < 0.001$).

Collinsella: The meta-analysis of *Collinsella*, using a random-effects model and incorporating 7 studies, indicated a proportion 1.04% (95% CI: 0.64, 1.44) in the ASD group and 0.23% (95% CI: 0.12, 0.34) in the NT group. However, there was considerable heterogeneity among the included studies, with high levels noted in both the ASD group ($I^2 = 98\%$) and NT group ($I^2 = 96\%$), as well as between the subgroups ($I^2 = 93.30\%$). Despite these variations, the overall effect size was found to be large ($Z = 6.19, P < 0.001$).

3.3.2. Bacterial genera that were less abundant in individuals with ASD than controls

Barnesiella: The relative abundance of *Barnesiella* was evaluated in 7 trials. In children with ASD, the percentage was 0.08 % (95% CI: 0.02, 0.13), while it was 0.28% in the NT group (95% CI: 0.11, 0.44). Considerable heterogeneity was observed both between studies (82% and 78%, respectively) and within subgroups ($I^2 = 79.50\%$). The effect size indicated a moderate yet significant impact ($Z = 4.56, P < 0.001$).

Odoribacter: The meta-analysis of *Odoribacter*, which included 9 studies, yielded the following findings:

the relative abundance of *Odoribacter* in children with ASD was 0.09% (95% CI: 0.05, 0.13), while it was 0.18% (95% CI: 0.12, 0.24) in the NT group. High heterogeneity was noted both within the ASD group ($I^2 = 97\%$) and the control group ($I^2 = 82\%$). Similarly, when comparing the two groups, a significant and substantial effect size was noted ($Z = 7.01, P < 0.001$), accompanied by considerable heterogeneity ($I^2 = 82.80\%$).

Paraprevotella: Meta-analysis of *Paraprevotella* revealed no presence of *Paraprevotella* in individuals with ASD (95% CI: 0.00, 0.01), while it accounted for approximately 0.03% in the NT group (95% CI: 0.01, 0.05). The included studies exhibited a low to medium level of heterogeneity ($I^2 = 11\%$ in the ASD group and 52% in the NT group) both within and between subgroups ($I^2 = 80\%$). Despite this variability, there was a significant and substantial overall effect size ($Z = 3.08, P = 0.002$).

Blautia: A total of 14 studies were included in the meta-analysis performed on *Blautia*. The findings indicated that the percentage was 0.09% (95% CI: 0.06, 0.13) among children diagnosed with ASD, while a relative abundance of 0.20% (95% CI: 0.15, 0.25) was found in the NT group. Both the ASD and control groups exhibited significant heterogeneity ($I^2 = 99\%$). When these two groups were compared, a high level of heterogeneity was also noted ($I^2 = 91.90\%$). The meta-analysis revealed a substantial and statistically significant effect for *Blautia* ($Z = 8.35, P < 0.001$).

Turicibacter: We performed a meta-analysis of 11 studies on *Turicibacter* and found that the relative abundance in individuals with ASD was 0.01% (95% CI: 0.00, 0.02), while it was 0.04% (95% CI: 0.01, 0.06) in NT children. Both groups showed significant heterogeneity among studies, with percentages of 88%, which remained high at 76.60% when comparing subgroups. The effect size was large and statistically significant ($Z = 4.07, P < 0.001$). In addition, a lower relative abundance of Clostridium bacteria was observed in individuals with ASD compared to the NT group.

Lachnospira: The relative abundance of *Lachnospira* was assessed in 8 trials. Among ASD children, the relative abundance was 0.07% (95% CI: 0.02, 0.11), while it was 0.26% (95% CI: 0.08, 0.43) in the NT group. High heterogeneity between studies was observed both in the ASD (89%) and NT (95%) groups, as well as among subgroups ($I^2 = 76.20\%$). The effect size was large and significant ($Z = 5.86, P < 0.001$). The difference in bacterial percentage for *Lachnospira* (-3.71) indicated that individuals with ASD exhibited lower levels compared to NT children.

Pseudomonas: As shown in Table 2, the random-effects meta-analysis of *Pseudomonas* included six studies. The findings revealed that the levels of *Pseudomonas* in the ASD group were zero (95% CI: -0.02, 0.02), while they were slightly higher at 0.06% (95% CI: 0.02, 0.10) in the NT group. Both subgroups

exhibited considerable heterogeneity among studies ($I^2 = 96\%$ and 97% , respectively), as the comparison between the two subgroups also indicated ($I^2 = 84\%$). The overall effect size was large and highly significant ($Z = 2.61$, $P = 0.009$).

Parasutterella: Our meta-analysis included 8 studies on *Parasutterella*, which yielded the following findings: the relative abundance of *Parasutterella* was estimated to be 0.03% (95% CI: 0.01, 0.04) in children with ASD and 0.11% (95% CI: 0.05, 0.17) in the control group. Notably, there was a considerable level of heterogeneity among the studies, which was as high as 87% and 80% for children with ASD and the control group, respectively; however, this heterogeneity decreased to approximately 85.30% when comparing these two groups together. The effect size revealed significant results of a large magnitude ($Z = 5.98$, $P < 0.001$). Moreover, individuals with ASD had a lower factor (-3.67) difference in bacterial percentage for *Parasutterella* compared to NT children.

Haemophilus: A meta-analysis of *Haemophilus* was performed in 11 studies, yielding the following results: 0.04% in the ASD group (95% CI: 0.02, 0.06) and 0.13% in the NT group (95% CI: 0.06, 0.20). Both the ASD and control groups exhibited significant heterogeneity among studies, with percentages of 83% and 65% , respectively, which remained consistently high (83.80%) even when comparing subgroups. The effect size was found to be substantial and statistically significant ($Z = 5.23$, $P < 0.001$).

Bifidobacterium: In the meta-analysis of *Bifidobacterium*, which included 22 studies, a significantly lower level of *Bifidobacterium* was observed in children with ASD (0.46% , 95% CI: 0.37, 0.55) compared to NT children (1.48% , 95% CI: 1.25, 1.71). Despite substantial heterogeneity among both ASD group studies ($I^2 = 99\%$) and NT group studies ($I^2 = 99\%$), as well as subgroups ($I^2 = 98.50\%$), the overall effect size remained large and statistically significant ($Z = 16.56$, $P < 0.001$).

3.3.3. Bacterial genera that did not differ between individuals with ASD and controls

As shown in Table 2, the comparison between ASD and NT children did not yield statistically significant differences concerning specific bacterial genera. However, *Bacteroides* was more abundant in both children with ASD (3.72% , 95% CI: 3.31, 4.13) and the control group (3.35% , 95% CI: 2.93, 3.76), with a consistent heterogeneity of 99% within both groups. *Fusicatenibacter* and *Gemmiger* were found in lower percentages among the studied microbiota, accounting for $0.50\% - 1.00\%$ overall in both the ASD and control groups, respectively, whereas all other genera were found in even lower proportions ($< 0.50\%$) within the studied microbiota across both groups collectively. High

heterogeneity was noted within subgroups, while slight to moderate heterogeneity was observed in intergroup comparisons.

3.4. Sensitivity analysis

The consistency of the effect size was evaluated in our meta-analysis by performing a sensitivity analysis, systematically excluding each study. As detailed in Table 4, significant differences in *Faecalibacterium*, *Clostridium*, *Dorea*, *Phascolarctobacterium*, *Catenibacterium*, *Odoribacter*, and *Bifidobacterium* between children with ASD and NT children persisted even after sequentially excluding each study. In addition, near-significant differences between children with ASD and NT children persisted in *Anaerostipes*, *Collinsella* and *Paraprevotella* following the sequential exclusion of each study. A point worth noting is that the subgroup differences in *Turicibacter* and *Lachnospira* were found to be highly sensitive, as non-significant differences within these two subgroups were observed in more than five of the excluded studies. Moreover, the studies conducted by Strati (29), Dan (37), and Deng (43) were most frequently excluded due to their potential influence on the significant difference between children with ASD and NT children.

4. Discussion

Our meta-analysis encompassed 28 studies, with a particular emphasis on the most recent studies (44,45,47,49), and it offered a most comprehensive overview of the GM in children diagnosed with ASD, highlighting their differences. By pooling data from these medium- to high-quality studies, we analyzed the relative abundance of GM across 8 phyla and 64 genera within a sample size of 1,256 children with ASD and 1,042 NT children. Our findings revealed that individuals with ASD exhibited a significantly higher relative abundance of *Anaerostipes*, *Catenibacterium*, *Clostridium*, *Collinsella*, *Dorea*, *Faecalibacterium*, *Lachnoclostridium*, *Parabacteroides*, and *Phascolarctobacterium* and a lower relative abundance of *Barnesiella*, *Blautia*, *Bifidobacterium*, *Haemophilus*, *Odoribacter*, *Paraprevotella*, *Pseudomonas*, *Parasutterella*, *Lachnospira*, and *Turicibacter*. Importantly, the significant differences in the relative abundance of *Faecalibacterium*, *Clostridium*, *Dorea*, *Phascolarctobacterium*, *Catenibacterium*, *Odoribacter*, and *Bifidobacterium* between individuals with ASD and NT controls were systematically confirmed by individually excluding studies. Given that this study exclusively examined GM data from individuals diagnosed with ASD, data were for single groups without comparison groups, and a non-normal distribution was evident. Therefore, we decided not to evaluate publication bias in our meta-analysis.

4.1. Persisting significant differences in GM between individuals with ASD and controls

Our findings consistently confirmed significant differences between children with ASD and NT children in *Faecalibacterium*, *Clostridium*, *Dorea*, *Phascolarctobacterium*, *Catenibacterium*, *Odoribacter* and *Bifidobacterium*. In these GM, the differences in *Faecalibacterium*, *Clostridium*, *Phascolarctobacterium* and *Bifidobacterium* between the two groups were in line with those in previous meta-analyses (13-16). Notably, our meta-analysis is the first to consistently find significant differences in bacterial *Dorea*, *Catenibacterium* and *Odoribacter* between the two groups.

Regarding *Dorea*, some studies have suggested that it might have an inflammatory effect in ASD (50) since it has been positively correlated with pro-inflammatory cytokines like TNF- α and it has been negatively correlated with the anti-inflammatory cytokines TGF- β and IL-10 (51). However, other studies suggested a protective effect of *Dorea* against ASD, possibly related to its ability to alleviate tropomyosin (Tm)-induced allergic responses (52,53). Regarding *Catenibacterium*, Wu *et al.* previously proposed *Catenibacterium* as a potential biomarker in patients with ASD (54). However, scant attention has been devoted to elucidating the mechanism between *Catenibacterium* and ASD. An animal study found that phobic dogs exhibited an increased abundance of *Catenibacterium* (55). Moreover, studies revealed significant differences in the abundance of *Catenibacterium* across nativity, race/ethnicity (56), and socioeconomic status (57). Regarding *Odoribacter*, several studies suggested a potential association between a higher percentage of *Odoribacter* and ASD (58). Wang *et al.* suggested that *Odoribacter* might play a role in regulating serotonergic and glutamatergic synapse metabolism in mice with VPA-induced ASD (48). Other studies suggested that *Odoribacter* is involved in the production of short-chain fatty acids (SCFAs), which exhibit neuroactive and anti-inflammatory effects and which have been linked to worsening ASD symptoms at high levels (59,60). However, research into the association between *Dorea*, *Catenibacterium*, and *Odoribacter* and ASD is still in its preliminary stages. Caution is advised when interpreting these results.

4.2. Imbalance of gut microbiota in children with ASD

The main issues associated with dysbiosis in ASD involve an increased presence of harmful bacteria along with decreased levels of beneficial bacteria (13,14). Contrary to this perspective, our findings revealed an increasing abundance of certain beneficial bacteria, including *Faecalibacterium*, *Phascolarctobacterium*, and *Lachnoclostridium*, while some harmful bacteria like *Pseudomonas*, *Parasutterella*, and *Haemophilus*

tended to decrease. In addition, significant differences in certain bacteria with indeterminate functions, such as *Catenibacterium* and *Odoribacter*, were also noted. Our study suggested that dysbiosis in the GM of individuals with ASD may manifest as either an increase or decrease in the abundance of beneficial or harmful bacteria, thereby disrupting the overall structure of the microbial community.

This dysbiosis is believed to play a significant role in the pathophysiology of ASD. An important point to note is that while an overabundance of beneficial bacteria might intuitively seem positive, it can, in fact, disrupt the delicate balance of the gut microbial community. This imbalance can lead to a range of issues including digestive disturbances, immune reactions, and nutritional deficiencies (61). Many pathogens can exist within a normal, healthy microbiome for extended periods without causing harm. Contrary to expectations, commensal organisms can also cause disease and often carry genes associated with virulence (62). These findings challenge the traditional division between pathogens and commensals, revealing instead a dynamic spectrum of microbial behaviors. Therefore, simply boosting beneficial bacteria without addressing the specific dysbiosis present in ASD may not be effective and could potentially exacerbate existing problems.

This underscores the necessity for a more targeted approach to probiotic therapy in ASD. Rather than using conventional probiotics or prebiotics in a generalized manner, supplementation needs to be tailored to address the specific microbial imbalances observed in individuals with ASD. This targeted supplementation should aim to restore a healthy balance of gut bacteria, which may involve introducing specific beneficial strains that are deficient or underrepresented in the GM of individuals with ASD.

By addressing the dysbiosis in a more precise and tailored manner, probiotic therapy holds the potential to alleviate gastrointestinal symptoms and improve immune function and overall health and well-being in individuals with ASD. However, additional research is required to deepen our understanding of the complex interplay between the GM and ASD and to identify the most effective probiotic interventions for this population.

5. Limitations of this study

Our meta-analysis had several limitations. First, due to the unavailability of data from a substantial portion of the studies that met our inclusion criteria, our meta-analysis could not fully capitalize on the breadth of available study data. Second, considering the dynamic nature of GM composition and its susceptibility to various factors such as host region, sex, age, disease, drug treatment, dietary habits, lifestyle, and BMI, the inclusion of studies from diverse geographical locations worldwide might account for the high between-study

heterogeneity observed. Third, our meta-analysis was limited to assessing the abundance of bacteria in fecal samples from individuals with ASD at the phylum and genus levels, potentially underestimating the overall diversity of GM. A point worth emphasizing is that fecal samples exclusively collect bacteria released from the intestinal lining, potentially offering a narrower view compared to that obtained through biopsies. In addition, focusing solely on evaluating microbial abundance might lead to an underestimation of bacterial diversity.

6. Conclusion

Our meta-analysis indicated that dysbiosis of the GM in ASD may involve more intricate changes beyond a simple reduction in beneficial bacteria and an increase in harmful bacteria. In essence, children with ASD exhibited a higher abundance of *Parabacteroides*, *Anaerostipes*, *Faecalibacterium*, *Clostridium*, *Dorea*, *Phascolarctobacterium*, *Lachnoclostridium*, *Catenibacterium*, and *Collinsella* and a lower abundance of *Barnesiella*, *Odoribacter*, *Paraprevotella*, *Blautia*, *Turicibacter*, *Lachnospira*, *Pseudomonas*, *Parasutterella*, *Haemophilus*, and *Bifidobacterium* compared to NT children. Notably, significant differences in the relative abundance of *Faecalibacterium*, *Clostridium*, *Dorea*, *Phascolarctobacterium*, *Catenibacterium*, *Odoribacter*, and *Bifidobacterium* between individuals with ASD and NT controls remained consistently stable even after sequentially excluding single studies. However, given the complex pathophysiology of ASD and the susceptibility of GM to factors such as living conditions, lifestyle, and diet, validating our findings is imperative, particularly by taking into account potential factors that may influence the composition of human GM, such as geographical location, dietary patterns, medication usage, and underlying diseases, and exploring whether disruption of GM is associated with specific subpopulations of ASD.

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