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Gut Microbiota in Autism Spectrum Disorder: A Systematic Review

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Abstract: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition with stereotyped behavior and deficits in communication and social interaction. There is increasing evidence of the implication of gut microbiota in ASD. We conducted a systematic review to summarize previously published data to compare the profile of gut microbiota between autistic and neurotypical subjects. The outcomes of interventions such as prebiotics, probiotics, and microbiota transplantation therapy to overcome the symptomatology of ASD were also discussed. The current review allows us to associate gut microbiota dysbiosis and ASD. To date, there is still little consensus on which bacterial species are consistently altered in individuals with autism. Further studies are required to obtain stronger evidence of the relationship between gut microbiota and the severity of ASD conjointly with the effectiveness of dietary/probiotic interventions in reducing autistic behaviors compared to their healthy siblings.

Keywords: Autism spectrum disorder; gut microbiota; dysbiosis; gut-brain-axis

1. Introduction

Over the previous fifty years, Autism Spectrum Disorder (ASD), a chronic neurodevelopmental disorder has evolved from a poorly understood, uncommon early-onset illness to a well-supported, extensively studied lifetime condition, recognized as fairly common and very heterogeneous [1]. According to the World Health Organization, the

prevalence of ASD has tremendously increased since the 1970s and is now estimated at one in 100 people worldwide. ASD is marked by social communication deficits, repetitive sensory-motor habits, and gastrointestinal (GI) difficulties [2]. Notwithstanding numerous extensive studies, the mechanisms and etiology of ASD have not been clearly explained, and there are no standardized or universally accepted therapies available [3,4].

Recently, gut-brain interactions in ASD have gained considerable attention. Increasing evidence suggests that gut microbiota has a pivotal role in the function and regulation of the central nervous system (CNS), therefore any impairment of the gut-brain axis equilibrium may elicit or aggravate the gastrointestinal and neuropsychiatric symptoms in ASD [5,6].

In an attempt to understand the link between gut microbiota and ASD via the gut-brain axis, several researches were carried out to deeply confront the gut microbiota of autistic and neurotypical individuals. Some of the studies confirmed the gut-brain axis theory by observing dysbiosis in people with ASD compared to healthy controls [7–9]. However, another study did not state any significant differences [10]. An in-depth analysis of the gut microbiota of autistics with respect to strict inclusion criteria of controls is needed to identify specific bacteria in ASD and to suggest therapeutic interventions, which could serve as a complementary treatment. Otherwise, some studies have demonstrated the role of the administration of prebiotics and/or probiotics, as well as Microbiota Transfer Therapy (MTT) in correcting gut microbiota dysbiosis and alleviating symptomatology in ASD [11–16].

Here, we aimed to update current findings on the bacterial profile of autistics to identify specific bacteria implicated in ASD, besides explaining the beneficial effect of pre/probiotics in the improvements of ASD symptoms. This review underscores the necessity for more intensive studies aimed at developing therapeutic methods and improving the quality of life for individuals with ASD.

2. Materials and Methods

2.1. Search protocol

We conducted a systematic search using the PubMed database to select papers studying the association between gut microbiota and ASD. The following Medical Subject Heading [MeSH] terms were used: Autism Spectrum Disorder AND Microbiota, Autism Spectrum Disorder AND Microbiome. Only studies published between 2010 and 2023 were considered. The collection of papers was reviewed, and duplicates were eliminated. In total, thirty articles were selected based on titles, abstracts, and full-text reading for eligibility.

2.2. Inclusion criteria

Randomized controlled trials, clinical trials, cohort studies, retrospective and prospective studies were considered. Studies comparing the gut microbiota of autistics with healthy controls and providing information on the analysis method and bacterial taxa were maintained. Studies using prebiotics, probiotics, and dietary supplementation on gut microbiota were also included.

2.3. Exclusion criteria

Exclusion criteria included studies on non-human subjects and reviews as well as participants with genetic disorders that were correlated with a high risk of ASD e.g., Rett Syndrome.

2.4. Data extraction and quality assessment

Data, including study sample size for both case subjects and controls, participant's gender and age range, criteria used for the definition of ASD, diet assessment, and antibiotic treatment were manually extracted and tabulated. Data regarding handling samples and analysis approach were also extracted. Specific data on race, educational level, and annual family income were only recorded in five out of 30 studies. The quality assessment of the included studies was conducted using the Newcastle-Ottawa scale tool for case-control and cohort studies (25 studies) and The Cochrane Risk of Bias Tool for randomized controlled trials (RCTs) (5 studies) (Supplementary Tables S1 and S2).

No ethical approval was needed for this review as the data was retrieved and analyzed from earlier published investigations in which informed consent was attained by primary investigators.

3. Results

3.1. Study Selection

The initial literature exploration revealed 336 records from the PubMed database, which were reduced automatically to 187 articles after applying filters "Free full text, Journal Article, Randomized Controlled Trial". Then, 81 studies were considered after the evaluation of titles and abstracts. Full-text review led to the exclusion of 51 articles due to incomplete eligibility criteria. Ultimately, 30 original articles were included. Figure 1 displays a flow diagram of the study process following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

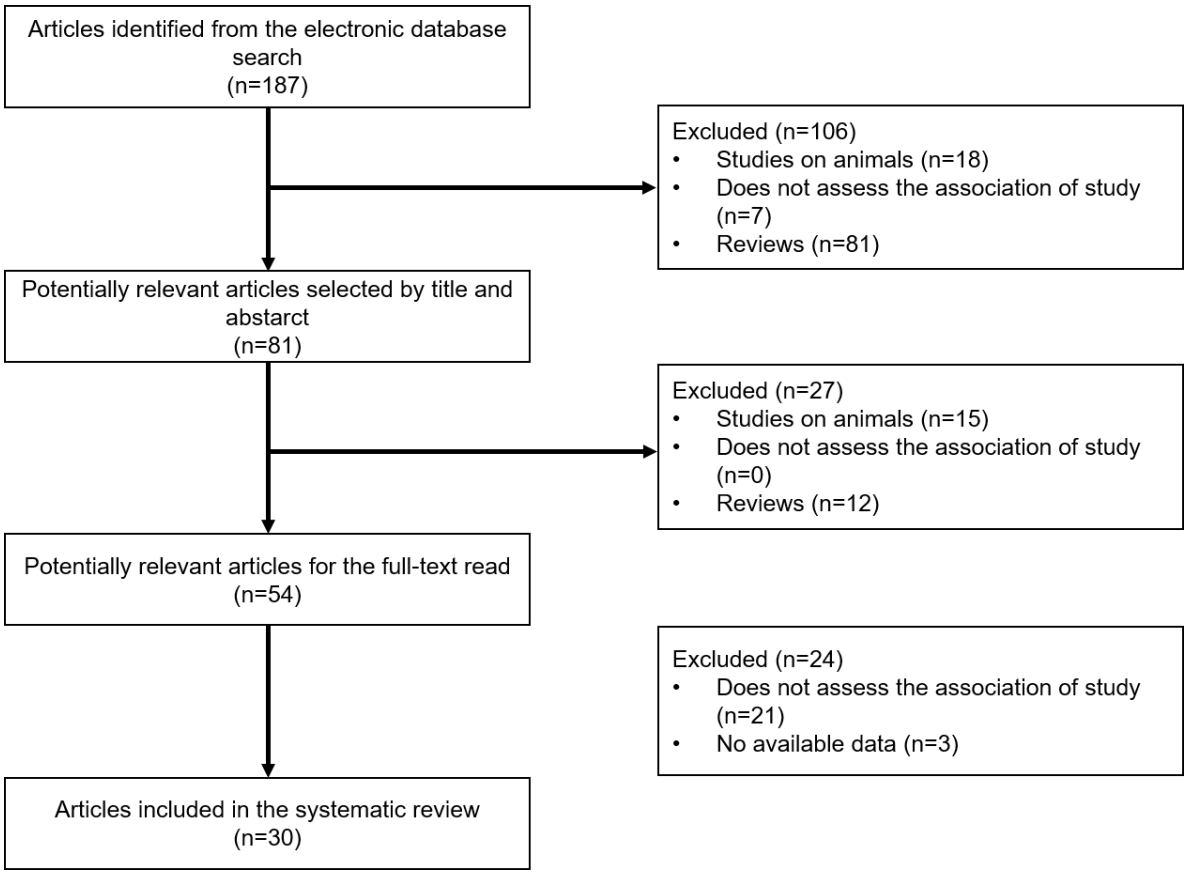

Figure 1. The PRISMA flow diagram summarizes the studies identified during the systematic literature search and review process.

3.2. Characteristics of studies

The 30 studies included a total of 1104 ASD cases (838 males and 170 females) and 698 controls (76 siblings and 622 non-related individuals). Three studies containing the remaining 96 patients did not report a gender ratio for ASD cases [12,17,18] and five studies did not have control subjects [11,12,19–21]. In a single study, the number of enrolled participants varied from 1 to 114 individuals, with ages ranging between 3 and 30 years. As regards to race, 36% of ASD cases were White, 12% were Black, 42% were Asian and 6% were Multiracial [11,22]. The parental educational level was also considered, the percentage of parents who had middle school or below education ranged from 3% to 36%, 14% to 27% had high school education and 1% to 72% were college graduates [11,22–24]. The financial income of autistic families ranged from 200$ to 90.000$ annually [11,23,25].

Regarding the diagnosis, nineteen studies used a combination of criteria, nine studies used a single one, and two studies did not mention any criteria. Demographics of the explored studies are shown in Table 1.

Table 1. Summary of data used for the analyses in the 30 articles.

Study by year of publication/Country	Sample Size/Gender			Age (years)	ASD diagnosis	Assessment of diet	Assessment of antibiotics and pre-probiotics
	ASD Patients	NT Control					
		Unaffected siblings	Unrelated individuals				
1) Kang et al., 2013, USA	17♂+3♀	-	18♂+2♀	3–16	ADI-R, ADOS ATEC, PDD-BI	gluten-free/ casein-free (GF/CF) diet, probiotics use, seafood consumption, and usage of nutrient supplements (vitamins/calcium).	No antibiotics or antifungals for 1 month before sample collection
2) De Angelis et al., 2013, Italy	20	10	-	4–10	ADI-R, ADOS, CARS, DSM-IV-TR, PDD-NOS	No assessment	No antibiotics probiotics, or prebiotics for 1 month before sample collection
3) Son et al., 2015, USA	52♂+7♀	21♂+23♀	-	10	CBCL	Daily Questionnaire for 7 days prior to sample collection	No antibiotics for 1 month before sample collection
4) Lee et al., 2017, Korea	18♂+2♀	-	24♂+4♀	ASD: 22.4±4.9 NT: 21.1±9.5	DSM-V, CARS	No information	No antibiotics, probiotics, or prebiotics in the 3 months before the sample collection
5) Strati et al., 2017, Italy	31♂+9♀	-	28♂+12♀	3–17	ABC, ADOS, CARS, DSM-V	No assessment	No antibiotics probiotics, or prebiotics for 3 months before the sample collection
6) Liu et al., 2017, China	55♂+9♀	-	-	1–8	ABC, CARS, DSM-V, SRS	Questionnaires	No nutritional supplement or medication in the previous 3 months

7)	Luna et al., 2017, USA	14♂	-	18♂+3♀	3–18	ADOS	No comment	No antibiotics, steroids, or GI infection during the previous 3 months
8)	Berding et Donovan, 2018, USA	19♂+7♀	-	19♂+13♀	2–7	PDDBI-SV	Not assessed	No antibiotics probiotics, or prebiotics for 3 months before the sample collection
9)	Coretti et al., 2018, Italy	9♂+2♀	-	8♂+6♀	2–4	ADI-R, ADOS 2, CARS, GMDS, DSM-V, VABS	3-Day Diary	No antibiotics, pre-/pro- or synbiotics for 4 weeks before sample collection
10)	Rose et al., 2018, USA	42♂+8♀	-	38♂+3♀	3–12	ADI-R, ADOS, DSM-IV	Questionnaire	No antibiotics for 1 month before sample collection
11)	Zhang et al., 2018, China	29♂+6♀	-	5♂+1♀	3–8	DSM-V	No information	No antibiotics, antipsychotics, pre-/probiotics for 1 month
12)	Grimaldi et al., 2018, UK	31♂+10♀	-	-	7.7	No comment	4-Day Diary	No antibiotics, pre-/probiotics for 4 weeks before sample collection
13)	Arnold et al., 2019, USA	6♂+4♀	-	-	3–12	ABC, ADI-R, ADOS, CSHQ, DSM-V, PSI, SRS	No comment	No antibiotics for 2 months before sample collection
14)	Berding et Donovan, 2019, USA	19♂+7♀	-	19♂+13♀	2–7	PDDBI-SV	3-Food Diary Food Frequency Questionnaire	Assessed without details
15)	Inoue et al., 2019, Japan	12♂+1♀	-	-	4–9	DSM-V, M-CHAT, PARS,	No comment	No antibiotics or medication 1 month before sample collection
16)	Kong et al., 2019, USA	15♂+5♀	8♂+11♀	-	ASD 15(13–18) NT 29(11–50)	DSM-V	Questionnaire	No antibiotics for 1-month before sample collection

17)	Kang et al., 2019, USA	18	-	-	7–17	ABC, CARS, DSR, GSRS, PGI- III, SRS VABS- III	-	Vancomycin, MoviPrep, Prilosec, and Standardized Human Gut Microbiota for 10 weeks
18)	Li et al., 2019, China	50♂+9♀	-	20♂+10♀	2–10	ABC, ADOS, DSM-V	Not assessed but all on hospital cafeteria Chinese-based diet	No antibiotics for 3 months
19)	Niu et al., 2019, China	95♂+19♀	-	20♂+20♀	3–8	DSM-V	-	No antibiotics, probiotics or GI treatments for 1 month prior
20)	Sun et al., 2019, China	8♂+1♀	-	4♂+2♀	3–12	No comment	No comment	No comment
21)	Plaza-Días et al., 2019, Spain	57	-	57	2–6	ADI-R, DSM-V, PDD-BI	24 hours diary	No comment
22)	Ma et al., 2019, China	39♂+6♀	-	39♂+6♀	6–9	CARS, DSM-V	No information	No antibiotics or systemic steroids for 3 months before sample collection
23)	Liu et al., 2019, China	25♂+5♀	-	16♂+4♀	2.5–18	DSM-V, ICD-10	No information	No antibiotics, antifungals, pre-/probiotics for 3 months
24)	Wang et al., 2019, China	36♂+7♀	-	17♂+14♀	ASD: 4.51 ± 2.23 NT: 3.14±1.73	DSM-V	Questionnaire	N/A
25)	Tomova et al., 2020, Slovakia	46♂	-	16♂	ASD: 4.0–8.5 NT: 2.8–9.15	ADI-R, ADOS-2, DSM-V	Food Frequency Questionnaire (FFQ)	No comment
26)	Hazan et al., 2020, USA	1♀	1♂+1♀	-	ASD: 7 NT: 7	ATEC Physical examination and medical history	Mix of western and south-central Asian foods + lithium, olive oil and fish oil supplements	No antibiotics for 3 months before sample collection
27)	Ding et al., 2020, China	59♂+18♀	-	39♂+11♀	2-7	CARS, DSM-V	Questionnaire	No antibiotics probiotics, or prebiotics for at least 1 month

28)	Chen et al., 2020, China	61♂+15♀	-	41♂+6♀	ASD : 3.96 ±0.12 NT: 4.25± 0.12	ADI-R, ADOS, CARS	24-h dietary record Based on China Food Composition 2009	No antibiotics or any other medications for more than 3 days in the previous month
29)	Zurita et al., 2020, Ecuador	24♂+1♀	-	32♂+3♀	5-12	ADI-R	24 h-recall form of food consumed by the children on 2 weekdays and 1 weekend day	No antibiotics or systemic steroids for 2 weeks before sample collection
30)	Kong et al., 2021, USA	26♂+9♀	-	-	3-20	DSM-IV-TR, ADI-R, ADOS-2	Not assessed	No medication, probiotics or oxytocin for at least 4 weeks before study enrolment

[1]**ADI-R**: Autism Diagnostic Interview-Revised, **ADOS**: Autism Diagnostic Observation Schedule, **ATEC**: Autism Treatment Evaluation Checklist, **PDD-BI**: Pervasive Developmental Disorder Behavioral Inventory, **DSM-IV-TR**: Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision, **CARS**: Childhood Autism Rating Scale, **PDD-NOS**: Pervasive Developmental Disorder not otherwise specified, **CBCL**: Child Behavior Checklist, **DSM-V**: Diagnostic and Statistical Manual of Mental Disorders, 5th edition, **ABC**: Aberrant Behavior Checklist, **SRS**: Social Responsiveness Scale, **PDDBI-SV**: Pervasive Developmental Disorder Behavioral Inventory-Screening Version, **ADOS 2**: Autism Diagnostic Observation Schedule, 2nd edition, **VABS**: Vineland Adaptive Behavior Scales, **GMDS**: Griffiths Mental Development Scales, **DSM-IV**: Diagnostic and Statistical Manual of Mental Disorders, 4th edition, **CSHQ**: Children's Sleep Habits Questionnaire, **PSI**: Personalized Supports Initiative, **PARS**: Pediatric Anxiety Rating Scale, **M-CHAT**: Modified Checklist for Autism in Toddlers, **GSRS**: Gastrointestinal Symptom Rating Scale, **DSR**: Delayed Self-Recognition paradigm, **PGI-III**: Parent Global Impressions-III, **VABS-III**: Vineland Adaptive Behavior Scales, 3rd edition, **ICD-10**: International Classification of Diseases, 10th revision.

Experimental design i.e., sampling strategies as well as microbiota and/or microbiome analysis included twenty-two studies assessing stool, five studies used both stool and blood, one study used both blood and intestinal biopsy, one study used both stool and saliva and one study used urine. Samples were stored at either -20 °C or -80 °C except for four studies that did not provide any storage information (Supplementary Table S3). Different total DNA extraction kits were used: QIAamp Fast DNA Stool Mini Kit (6), QIAamp DNA Stool Mini Kit (5), PowerSoil DNA isolation kit (3), FastDNA SPIN Kit for Feces (2), QIAamp PowerFecal Pro DNA Kit (1), FastDNA Pro Soil-Direct Kit (1), FastDNA SPIN Kit for Soil (1), ZR Fecal DNA MiniPre (1), NEXTfex Rapid DNA-Seq kit for Illumina (1), HR-Easy Fecal DNA Kit (1), OMEGA E.Z.N.A Stool DNA Kit (1), StoolGen fecal DNA extraction kit (1), and Cetyltrimethylammonium bromide (CTAB)-based method (1). Two studies used in addition to QIAamp Fast DNA Stool Mini Kit and QIAamp DNA Stool Mini Kit, Bead beating and 95 °C pre-treatment in lysis buffer respectively [18,26]. Five studies did not provide information on the extraction kit used. All studies used metagenomic sequencing of the 16S rRNA gene, targeting a number of hypervariable regions except for one study that used shotgun metagenomic sequencing [27].

3.3. Gut microbiota is altered in autistic subjects

Preserving enough bacterial richness and variety is crucial to ensure functional redundancy, flexibility, and thus systemic resistance to external stresses to gut microbiota. When comparing the gut microbiota of autistic individuals to neurotypical individuals, dysbiosis can be seen in ASD subjects. This dysbiosis concerned 7 phyla, 24 families, and 44 genera within 1104 ASD cases and 698 controls. We removed every phylum, family, and genus that was only found in one study or was at high levels in one study and at low levels in another. The major findings of the review are tabulated in Table 2.

Our analysis of the microbiota composition revealed dysbiosis at different taxonomic levels, i.e., phylum, family, genus, and species. However, we did not identify distinct patterns despite our thorough examination.

At the phylum level, the findings were somewhat contradictory. Three studies reported elevated levels of Firmicutes [23,28,29], while two other studies reported reduced levels [17,30]. Similarly, five studies reported increased levels of Bacteroidetes [17,28,29,31,32], with one study reporting the opposite trend [33]. As for Proteobacteria, five studies indicated higher levels [18,28,29,31,32], while three studies reported lower levels [21,23,34]. The situation with Actinobacteria was also inconclusive, with two studies reporting higher levels [18,31] and two studies reported lower levels [21,28].

At the family level, four studies reported a high abundance of *Enterobacteriaceae* [17,18,29,35]. Two studies reported high levels of *Lachnospiraceae* [21,29] while two other studies reported low abundancies [14,18]. Two studies reported high levels of *Ruminococcaceae* [36,37] and one study reported the opposite [35]. Two studies reported low levels of *Streptococcaceae* [30,31], while another reported high levels [23].

At the genus level, our analysis revealed a wide range of observations concerning the abundance of specific microbial genera, contributing to the complexity of dysbiosis characterization. *Akkermansia* was found to be highly abundant in four studies [17,23,25,34], potentially indicating its significance in the context of dysbiosis. On the other hand, *Veillonella* demonstrated conflicting results, with two studies reporting reduced levels [33,38], while one study suggested the opposite trend [13]. Both *Paraprevotella* and *Lachnoclostridium* were reported to be at lower levels in two studies each [24,34,39]. Similarly, *Bacteroides* showed inconsistent findings, with three studies reporting higher abundances [21,28,29], and one study indicated the opposite [39]. *Clostridium* was found to be highly abundant in three studies [5,33,39], while one study reported lower levels [21]. *Prevotella* exhibited conflicting trends, with three studies reporting elevated levels [18,21,29], and one study suggested a reduced abundance [34]. *Bifidobacterium* demonstrated variability, with two studies reporting higher levels [18,29], and three other studies reported a lower abundance [17,21,28]. *Streptococcus* was found to be highly abundant in two studies [23,32], while one study reported reduced levels [38]. Regarding *Dorea*, one study reported higher levels, while two others reported lower levels [39,40]. Similarly, *Escherichia* was reported with conflicting results, with one study indicating higher levels [33], and two other studies reporting lower abundances [13].

At the species level, one study reported high abundancies of *Clostridium bolteae* and *Clostridium difficile* [18] while another study reported high levels of *Clostridium clostridioforme* [24]. In one study, a high abundance of *Acinetobacter rhizosphaerae* and *Acinetobacter johnsonii*, and a low abundance of Prevotella melaninogenica were observed. [32]. Low levels of *Bacteroides vulgatus*, *Campylobacter jejuni* subsp. *jejuni*, *Campylobacter jejuni* subsp. *jejuni*, *Candidatus*, *Chloracidobacterium thermophilum B*, *Coraliomargarita akajimensis*, *Proteus mirabilis*, and *Spirochaeta thermophila* were reported by Wang et al. [27]. Recently, Hazan et al. reported high levels of *Bacteroides uniformis*, *Bacteroides plebius* and *Escherichia coli* as well as low levels of *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Roseburia faecis* and *Bacteroides vulgatus* [28].

Table 2. The most important observations on bacterial composition between ASD cases and controls (▲ = bacteria are more abundant; ▼ = bacteria are less abundant).

Number of patients	**20**	**20**	**20**	**40**	**64**	**14**	**11**	**50**	**35**	**20**	**59**	**9**	**57**	**45**	**30**	**92**	**1**	**77**	**76**	**25**	**35**	Total	
Studies References	**1**	**2**	**4**	**5**	**6**	**7**	**9**	**10**	**11**	**16**	**18**	**20**	**21**	**22**	**23**	**24**	**26**	**27**	**28**	**29**	**30**	▲	▼
Phylum																							
Firmicutes		▼	▲							▲					▼		▲					2	2
Bacteroidetes		▲		▼			▲			▲					▲		▲					5	1
Proteobacteria	▼		▼		▼		▲			▲	▲		▲				▲					5	3
Actinobacteria					▼		▲						▲				▼					2	2
Family																							
Enterobacteriaceae		▲								▲			▲		▲							4	0
Lachnospiraceae								▲		▲			▼	▼							▲	3	2
Ruminococcaceae								▲				▲			▼						▲	3	1
Streptococcaceae				▲			▼								▼							1	2
Genus																							
Veillonella				▼					▼												▲	1	2
Paraprevotella	▼	▼																				0	2
Lachnoclostridium																		▼	▼			0	2
Akkermansia	▲	▲	▲																	▲		4	0
Bacteroides					▲					▲							▲	▼				3	1
Bifidobacterium		▼			▼					▲			▲				▼					3	2
Clostridium		▲		▲	▼											▲						3	1
Dorea				▲		▼												▼				1	2
Escherichia				▲	▼				▼													1	2
Prevotella	▼				▲					▲			▲									3	1
Streptococcus			▲						▼		▲										▲	3	1

3.4. Effects of antibiotics, prebiotics, and probiotics on the gut microbiota of ASD patients

All studies, except for one [12], excluded subjects who reported using any medication before the sample collection. Among the included studies, four of them did not provide specific information regarding antibiotic usage [18,37,41,42]. The duration of antibiotic non-use in the remaining studies ranged from one week to three months. Notably, one study included subjects who had taken a combination of antibiotics and other components for ten weeks before the sampling date [12]. Despite the variation in antibiotic usage and inclusion criteria among the studies, it is important to consider the potential impact of pre/probiotics on the gut microbiota of autistics. Lactic acid bacteria-rich fermented foods, such as cultured milk products and yogurt, are a source of ingestible microorganisms that may benefit intestinal health and even treat or prevent inflammatory bowel disease [15,43,44].

A study randomized autistic children into a probiotic crossover trial of 8 weeks on Visbiome® that contains eight probiotic species, mostly *Lactobacillus* spp. and *Bifidobacterium* spp. [11]. While probiotics outperformed placebos in terms of improvement, the differences in the Pediatric Quality of Life Inventory (PedsQL) and the Development of the Parent-Rated Anxiety Scale for Youth with Autism Spectrum Disorder (PRAS-ASD) scores were not statistically significant, which was anticipated given the sample size. However, a specific study that examined the effects of vitamin A supplementation on the gut microbiota of 64 children with autism reported a significant increase in *Bacteroidetes/Bacteroides* spp. [21]. A notable rise in the proportion of bifidobacteria within a subset of infants was also reported. Moreover, within this subgroup, a striking correlation was observed between bacterial counts and fecal amino acids, unlike in children on an ad libitum diet. One study investigated differences between ASD and neurotypical children by conducting a 4-week Applied Behavior Analysis (ABA) training program combined with probiotic treatment [14]. Furthermore, the relative abundance of *Bacteroidetes* at the strain level was significantly lower than in the neurotypical group. At the genus level, the relative abundances of the genera *Bacteroides*, *Bifidobacterium*, *Ruminococcus*, *Rosebria*, and *Blautia* were significantly lower than the neurotypical group. After a 4-week ABA training program combined with probiotic treatment, ATEC scores and gastrointestinal symptom levels were reduced more significantly than in the control group, which received only ABA training [14].

Another study highlighted the effects of oral ingestion of the probiotic *Lactobacillus plantarum* PS128 and intranasal administration of OXT on the gut flora in a group of 35 participants with autism [13]. The findings of the study revealed a significant increase in the

abundance of certain bacterial families, including *Christensenellaceae*, *Lachnospiraceae*, *Rikenellaceae*, and *Ruminococcaceae*, as well as certain genera, such as *Blautia*, *Barnesiella*, *Veillonella*, *Streptococcus*, *Coprococcus*, *Bilophila*, *Roseburia*, *Catenibacterium*, and *Holdemanella*, when compared to the placebo group. These results suggest that the combined treatment of *Lactobacillus plantarum* PS128 and OXT may have a positive impact on the gut microbiota of autistic individuals [13].

3.5. Food intake impact on the microbiota in ASD

Dietary habits are the best-known and most powerful modifiers of brain health. Therefore, dietary patterns play a major role in the modification of the ASD profile [45]. In this review, fifteen studies assessed the dietary habits of autistics, either by using questionnaires, or daily diet records [10,18,34,36,39,42,46,19,21,25–29,31]. A low frequency of versatile carbohydrate-degrading and/or fermenting bacteria in autistic samples was reported in a study, suggesting a possible influence of abnormal dietary habits. Whereas, multivariate analysis revealed that autism-related changes in both, diversity and individual genera frequencies were linked to the presence of autistic symptoms, but not to dietary habits [34]. In one study, microbial profiles were linked to two distinct dietary patterns. The first dietary pattern, characterized by higher intakes of vegetables, legumes, nuts, seeds, fruit, refined carbs, and starchy vegetables, and lower intakes of sweets, was associated with decreased abundances of *Enterobacteriaceae*, *Lactococcus*, *Roseburia*, *Leuconostoc*, and *Ruminococcus*. The second dietary pattern, rich in fried foods, protein, starchy foods, condiments, and snacks, correlated with higher levels of *Barnesiellaceae*, *Alistipes*, and various short-chain fatty acids (SCFAs) including propionate, isobutyrate, valerate, and isovalerate, but showed reduced levels of *Streptophyta*, Peptostreptococcaceae, and *Faecalibacterium* [26]. Additionally, variations in *Streptophyta* and *Clostridium* abundances were noted alongside decreases in all SCFA concentrations over time [22]. The microbiomes of children with ASD featured *Clostridium*, *Lactococcus*, *Turicibacter*, *Dorea*, and *Phascolarctobacterium*. Dietary pattern 1 impacted species diversity and the abundance of *Erysipelotricaceae*, *Clostridium*, *Oscillospira*, and SCFAs such as propionate, butyrate, isobutyrate, and isovalerate, whereas dietary pattern 2 influenced microbiota structure, *Clostridium* and *Oscillospira* abundance, and SCFA levels [22].

A study concluded that supplementing meals with partly hydrolyzed guar gum assisted in alleviating constipation and gut dysbiosis symptoms, which in turn reduced blood inflammatory cytokines and behavioural irritability [20]. These approaches underscore the potential of targeted dietary interventions in managing ASD symptoms by leveraging the gut-

brain axis. This aligns with findings in metabolic syndrome management, where dietary choices significantly influence gut microbiota composition and function, potentially offering therapeutic avenues beyond conventional treatments [47,48]. Children on exclusion diets reported considerably decreased levels of stomach discomfort and bowel movement. Otherwise, fewer *Bifidobacterium* spp. and Veillonellaceae family were identified, however, there were more *Faecalibacterium prausnitzii* and *Bacteroides* spp. [19].

4. Discussion

We carried out a systematic review of the existing investigations regarding the composition of gut microbiota in autistics by pooling the results of 30 studies to identify specific bacterial signatures of ASD. The sample size ranged from 1 to 114 subjects, which is a crucial asset considering the challenges associated with conducting epidemiological studies with children, particularly those with disabilities. Nonetheless, it is important to acknowledge some limitations encountered during this review. First, environmental factors and dietary habits influence the microbiota signature, and including studies from around the world may explain the variability in results. Second, measuring bacterial biodiversity at the species level is challenging; only a few studies have provided this crucial information [27,28]. Third, our analysis uniquely contributes to the field by demonstrating that each individual with ASD has a distinct gut microbiota bacterial profile. This finding is supported by a study that reported dysbiosis in a child with gastrointestinal symptoms and ASD, in contrast to her two healthy triplet siblings [28]. Furthermore, the significant heterogeneity among included studies poses challenges to conducting a meaningful subgroup analysis, especially by age groups. Future research should consider standardizing methodologies to facilitate more robust subgroup analyses.

In the present systematic review, four studies indicated higher incidences of *Clostridium* strains in autistic children's stool. Spore-forming bacteria, such as *Clostridium*, produce pro-inflammatory toxins that can enter the brain via blood flow. Similarly, several metabolites created by *Clostridiales* activity have been linked to repetitive behaviors and GI issues in ASD, underscoring the urgent need for targeted therapies [49]. A number of strategies for reducing GI symptoms in ASD children have been proposed, pointing to a significant gap in our understanding of gut-brain interactions. For instance, modulation of diet and administering oral vancomycin reduced ASD symptoms, indicating that lowering *Clostridium* spp. levels may result in significant improvements in the symptomatic treatment of ASD [50,51]. Moreover, three studies [17,21,28] reported decreased levels of the genus *Bifidobacterium* which has long been employed as a probiotic to treat a variety of disorders

by modifying the overall composition of the gut microbiota [42,52]. According to prior research, low levels of *Bifidobacterium* and metabolites of free amino acids and SCFAs in the feces seem to be involved in the development of ASD [17]. A study reported significant increases in beneficial bacteria such as *Bifidobacterium* and *Prevotella* in the gut microbiota of autistics after the treatment. Recent studies underscore the significance of diet-microbiota interactions, illustrating how specific dietary components can reshape the bacterial profile of the host, influencing immune and metabolic parameters [12]. Additionally, employing a combined dietary strategy involving prebiotics and an exclusion diet has been linked to notable improvements in antisocial behavior [46]. This body of research aligns with findings that dietary and probiotic interventions can beneficially modulate the gut microbiome, with potential implications for ASD-related neurological outcomes. For instance, *Bacillus subtilis* spores have been shown to normalize behavior in mice, highlighting the gut's influence on behavior regulation [53]. Similarly, probiotics are recognized for their ability to alleviate intestinal issues, indirectly benefiting neurological health [54]. Exploring the therapeutic capacities of *Christensenella minuta* for obesity and metabolic diseases, which frequently accompany ASD, further illustrates the gut microbiome's broad impact [55]. Moreover, the ketogenic diet's impact on the gut microbiome points to the possibility that dietary adjustments could significantly modify ASD symptoms through microbiome modulation [56].

Moreover, the investigation of the influence of dietary intake on the microbiota composition in ASD children showed an association between social deficit scores in ASD and diet [26]. Three studies reported a decrease in levels of beneficial *Bifidobacterium* and *Eubacterium* species in people consuming the Western diet that is known for high levels of animal proteins and fats with low levels of fibers, suggesting a crucial link between diet and gut health [57–59]. Sanz et al. have studied the effect of a gluten-free diet on 10 healthy subjects and reported a decrease in *Bifidobacterium* and *Lactobacillus*, while potentially unhealthy bacteria like *E.coli* and total *Enterobacteriaceae* were increased [60]. Several other studies investigating the effect of the consumption of the Mediterranean diet on gut microbiota reported increases in *Bifidobacterium*, *Lactobacillus*, and *Prevotella*, and decreases in *Clostridium*, suggesting that across the spectrum of diets, the Mediterranean diet is highly regarded as a healthy balanced diet [61–66]. Another study's results indicate a strong correlation between the abundance of the *Eubacterium hallii* group and a positive social cognition response to the combination treatment of *Lactobacillus plantarum* PS128 and oxytocin in individuals with ASD. This suggests that the combined treatment may have synergistic effects on ASD patients by promoting the growth of beneficial gut bacteria and enhancing social cognition [29]. The ability to identify and quantify the host gut bacterial profile has been of great help in understanding the influence of diet on microbial composition. Therefore, it

might give critical evidence for the development of therapeutic methods for microbiota-related disorders such as autism spectrum disorder.

The findings of previous studies are heterogeneous and often contradictory. Our review suggests that the relationship between gut microbiota and ASD exists, however, there are no taxa specific to ASD. This could be explained by the sample size of the current review which is larger than the previous reviews, the more we gathered information on the subject, the more we found it hard to draw a clear trend. Also, this conclusion could be explained by the fact that twenty-five of the studies analyzed used unrelated individuals as controls. To this end, we focused on four studies that have used healthy siblings as controls [10,17,28,29]. Accordingly, we suggest carrying out a study using a low to medium sample size of ASD patients and healthy siblings as controls with the same dietary habits and lifestyle. This may provide clear evidence of bacteria associated with ASD and guide the implementation of dietary and probiotic therapies aimed at improving gut flora in ASD patients.

In Figure 2, we present a concise schematic representation of our key findings, illustrating the intricate relationships between gut microbiota composition and autism spectrum disorder, including the impacts of dietary habits, specific bacterial profiles, treatment approaches, and their correlation with behavioral symptoms in ASD.

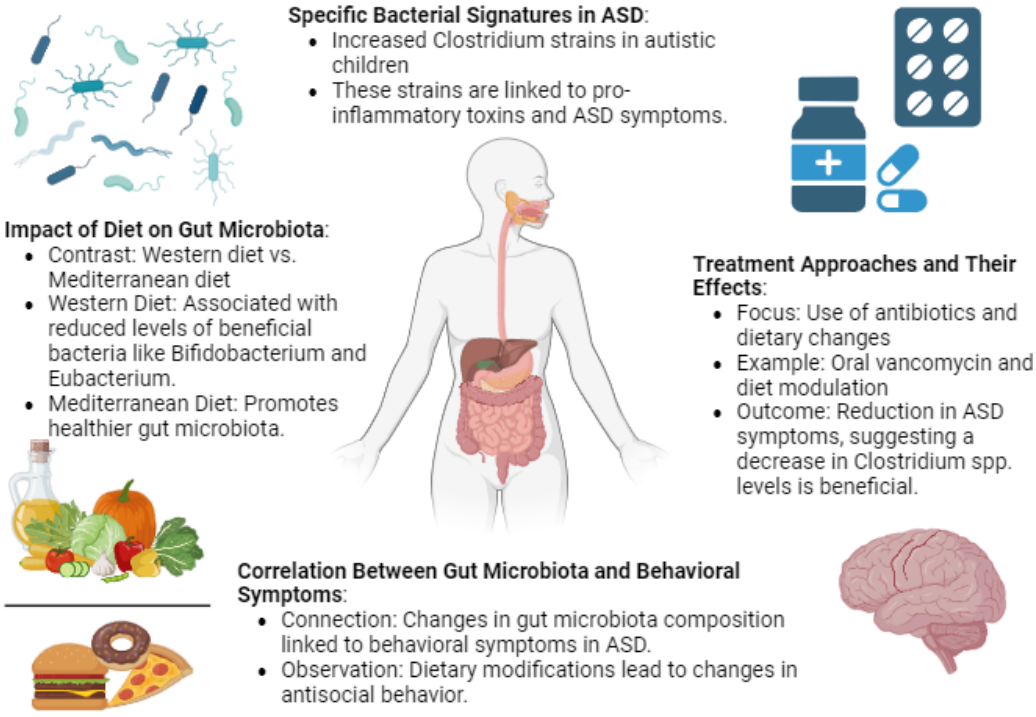

Figure 2. Overview of Gut Microbiota's Impact on ASD, highlighting specific bacteria, dietary effects, and treatment outcomes. Created with Biorender.com.

5. Conclusions

In conclusion, the present review provides evidence for the changes in the intestinal microbial diversity in ASD. Clinical evidence indicates that antibiotics, probiotics, prebiotics as well as diets influence the gut bacterial composition and affect ASD. The specific bacterial signature that may be perceived as "ASD-promoting bacteria" has yet to be determined. Despite advancements, further comprehensive studies are needed to elucidate the specific bacterial species implicated in ASD and to establish a consensus on the effectiveness of dietary and probiotic interventions in alleviating autistic behaviors. Future research should aim to correlate gut microbiota dysbiosis with the severity of ASD, providing a more nuanced understanding and paving the way for targeted therapeutic approaches.

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