Characteristics of the gastrointestinal microbiome in children with autism spectrum disorder: a systematic review

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Background: A high prevalence of gastrointestinal (GI) symptoms has been reported in children with Autism Spectrum Disorders (ASD). However, results from studies about the GI microbiome of such children have been inconsistent.

Aim: Integrate the results of studies that examine the distribution of different GI microorganisms in children with ASD.

Methods: Studies related to the GI microbiome in children with ASD were identified through PubMed, Embase, PsycINFO, ISI web of knowledge, Ovid/Medline, the Cochrane Library, the Chinese National Knowledge Infrastructure (CNKI) database, the Chongqing VIP database for Chinese Technical Periodicals, WANFANG DATA, and the China BioMedical Literature Service System (SinoMed). Studies were screened for inclusion following pre-defined inclusion and exclusion criteria. Software Review Manager 5.2.6 was used for statistical analysis.

Results: A total of 15 cross-sectional studies, all of which had relatively small samples, were included in the final analysis. Only one of the included studies was from China. Among the 15 studies, 11 studies (with a combined sample of 562 individuals) reported significant differences between ASD children and controls in the prevalence of GI bacteria, particularly bacteria in the Firmicutes, Bacteroidetes and Proteobacteria phyla. However, due to the substantial heterogeneity in methodology and the often contradictory results of different studies, it was not possible to pool the results into a meta-analysis.

Conclusions: To date, studies on the GI microbiome in children with ASD are limited in quantity and quality. There does, however, appear to be a ‘signal’ suggesting significant differences in the GI microbiome between ASD children and children without ASD, so there would be value in continuing this line of research. To improve validity and decrease the heterogeneity of findings, future studies should enlarge sample sizes, standardize methods and assess relevant confounding variables, such as the severity of GI symptoms and the use of medications, special diets and supplements.

1. Introduction

Autism spectrum disorders (ASD) refers to a range of neurodevelopmental disorders, primarily characterized by difficulties in social interactions, verbal and non-verbal communication, and stereotypic or repetitive behaviors. Although the classification of ASD varies in different diagnostic systems, it essentially encompasses autism, childhood disintegrative disorder, Asperger’s syndrome and pervasive developmental disorder not otherwise specified (PDD-NOS). The etiological pathways and pathogenesis of ASD remain unknown.

A high prevalence (23-70%) of Gastrointestinal (GI) distress, including abdominal pain, bloating, diarrhea and constipation, has been reported among children with ASD. The high frequencies of these GI symptoms could reflect abnormal GI microflora in ASD children. There are more than 100 trillion symbiotic microorganisms in the human GI tract that are important for nutrition and metabolic processes. Some evidence suggests that these same microorganisms also play a role in brain development, behavior, and gene expression via neural, endocrine, and immune
pathways.[7] Emerging research on the gut-microbiome-brain connection in both mice and humans has shed new light on the pathogenesis of various mental diseases including ASD.[8,9] Nonetheless, whether GI microbiota contribute to the pathogenesis or the developmental course of ASD is still unclear. Previous clinical and pathological studies on microbes associated with the development and treatment of ASD have yielded inconsistent findings. To our knowledge, there has been no systematic review on this topic. The aim of the current study is to conduct a systematic review to evaluate and summarize findings from studies on the characteristics of the GI microbiome in children with ASD.

2. Methods

2.1 Data retrieval strategies

The process of identifying articles for inclusion in this review is shown in Figure 1. The following key words (in both English and Chinese) were used to search PubMed (1966-2013), Embase (1947-2013), PsycINFO (1966-2013), ISI web of knowledge (1994-2013), Ovid/MEDLINE (1970-2013), the Cochrane Library (1967-2013), the Chinese National Knowledge Infrastructure (1979-2013) database, the Chongqing VIP database for Chinese Technical Periodicals (1989-2013), WANFANG DATA (1990-2013), and the China BioMedical Literature Services System (SinoMed) (1978-2013): autism

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* A double-blind, placebo-controlled, crossover-designed probiotic feeding study was excluded because no full-text was found and sample size information was not provided. We tried to contact the corresponding author, but did not receive a reply.

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(including ‘Child Development Disorders, Pervasive’, ‘autis*’, ‘pervasive development* disorders*’, ‘child schizophrenia’, ‘kanner*’, ‘Rett*’, ‘Asperger*’, ‘zi bi zheng’ [an older term for autism in Chinese]), ‘bacteria’, ‘bacter*’, ‘microbiology’, ‘microbio*’, ‘microbiome’, ‘microbiome*’, ‘microbial’, ‘microorganism*’, ‘metagenomics’, ‘metagenome’, ‘metagenom*’). All articles published before October 5, 2013 were included in the search.

Identified articles were imported to Endnote X6 to remove duplicated records. Two authors (XC and CL) independently screened titles and abstracts of the remaining articles for potential inclusion. The full text of the articles were downloaded for further assessment when reviewers thought that the article met inclusion criteria or when reviewers were unsure about whether or not the article met the inclusion criteria. The references of all full-text articles were hand-searched to identify other potential articles. These two authors then independently read the full-text articles and evaluated them according to the pre-defined inclusion or exclusion criteria listed below. These authors had disagreements about whether or not to include four articles; these disagreements were resolved through discussion.

2.2 Inclusion and exclusion criteria
All included studies: (a) were about the GI bacteria in children diagnosed with ASD; (b) had a group of children diagnosed with ASD, regardless of which diagnostic criteria were used (including but not limited to the Diagnostic and Statistical Manual of Mental Disorders [DSM], the International Statistical Classification of Diseases and Related Health Problems [ICD], and Chinese Classification and Diagnostic Criteria of Mental Disorders [CCMD]); (c) had information about sample size and the prevalence of the specific bacteria assessed; (d) were written in English or Chinese. Studies on non-human subjects, reviews, case reports, and duplicate publications were excluded.

2.3 Data extraction and evaluation
Two authors (CX and LC) independently extracted data from identified studies including the name of the first author, year of publication, location of the study, sample size, diagnosis of ASD and criteria used to make the diagnosis, sample characteristics (GI symptoms, diet and medication use), source of bacterial samples, testing methods to identify specific bacteria, and the prevalence of different bacteria in children with and without ASD.

The evaluation of the quality of the articles was conducted by the same two authors following the guidelines listed in the article entitled ‘Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)’. For each included study, a total of 22 items were rated based on the content of different parts of the article reporting the study, including the title, abstract, introduction, methods, results, discussion, and other information. Each item counts for one point so the total quality score ranges from 0 to 22. The inter-rater reliability of the total quality score for the included studies of the two authors was high (ICC=0.90).

2.4 Statistical Analysis
The data were entered into a database and analyzed using Review Manager 5.2.6 software. Considering the discrepancies in methodology across studies, standardized mean difference (SMD) were calculated using different estimation formulas for reports with different sample sizes according to the suggestion of Hozo and colleagues. Studies were considered homogeneous when I² (i.e., the effect size variation attributable to heterogeneity) was less than 50% and the associated p>0.10; in this case a fixed effect model was used to generate pooled estimates. If I² was ≥50% or p<0.10 the studies were considered heterogeneous so a random effect model was used. No analysis was performed and no pooled estimates were generated when there were less than three studies with relevant data for a particular issue or when the I² was greater than 75%.

3. Results
3.1 General characteristics of included studies
As shown in Figure 1, a total of 3643 unduplicated reports were retrieved. After screening according to the pre-defined inclusion and exclusion criteria, 17 reports were included in the final analysis. Among the 17 studies, the single study from China was an abstract of a master’s thesis; this author was unable to provide either a full text article or any data because the relevant papers had not yet been published; the cases reported were, nevertheless, included in our main analysis. Two reports by Williams and colleagues published in 2011 and 2012 were based on the same sample, and two reports by Wang and colleagues published in 2010 and 2011 were also based on the same sample. Therefore, data from 15 separate studies were analyzed. All 15 were cross-sectional studies; among them, one (n=4) was designed as an open clinical trial, but the GI microbiome assessment was only reported at baseline.

The characteristics and main findings of the studies are shown in Tables 1 and 2. The 15 identified studies were published between 1999 and 2013 in five countries: eight in the United States, three in Australia, two in Poland, one in Britain and one in China. One of the studies had no control group, two studies used previously reported data of adults as historical controls, one study had siblings of the ASD children (SIB) as a
control group, eight studies\(^{14,16,23-25,27,28,30}\) used unrelated children without ASD as the control group (CON), and three studies\(^{17,18,25,26}\) had both sibling and unrelated children without ASD as control groups (i.e., SIB and CON). In total, the 15 studies had a cumulative sample of 805 individuals, including 437 children with ASD, 94 siblings of children with ASD, 145 concurrent child controls without ASD, and 129 historical adult controls. Only four studies reported the diagnostic criteria used to determine ASD: three used the DSM criteria\(^{15,18,19}\) and one used the ICD criteria.\(^{22}\)

Quantitative analysis on GI bacteria was carried out in 13 studies\(^{14,19,21,22,24-30}\); the other two studies\(^{20,23}\) only described the number and types of cultured bacteria and their colony morphology. Due to the large variation across studies in evaluation methods, sources of specimens, and statistical analyses, it was not appropriate to pool the main results from the studies.

3.1.1 Gender composition of the sample
Among the 15 studies, four studies\(^{14,19,26,30}\) did not report the gender of the participants, in one study\(^{15,18}\) participants were all males, and one study\(^{27}\) only reported the gender of the children with ASD but not that of the control subjects. In the studies that reported the gender of all participants, the range in the male-female ratio in the three different groups of participants were as follows: in the ASD group the male-female ratio ranged from 2.5:1 to 11:1, in the SIB group it ranged from 0.4:1 to 1.4:1, and in the CON group it ranged from 0.8:1 to 5.7:1.

3.1.2 Characteristics of the combined sample
Table 2 summarizes the clinical and dietary characteristics of participants in the studies for which this information was available. Ten (66.7%) of the 15 studies\(^{15,18,19,21,22,24-29}\) reported GI symptoms, but three of them\(^{19,26,27}\) only reported GI symptoms in the ASD group. Ten (66.7%) studies\(^{18,19,21,22,24-29}\) reported the use of antibiotic or antifungal medication. Nine (60.0%) studies\(^{15,18,21,22,24-29}\) reported the dietary restrictions of the children and six (40%) studies\(^{18,22,24,25,28,29}\) reported on dietary supplements (e.g., probiotics). Only one (6.7%) study\(^{26}\) reported on participants’ allergic diseases.

3.1.3 Source of specimens
Twelve of the 15 studies (80%) only tested fecal specimens, one study\(^{15,16}\) did ileal and cecal biopsies, one study\(^{26}\) examined digestive fluids (gastric, duodenal and empty intestinal fluids), and one study\(^{29}\) collected both biopsy specimens and fluids.

3.1.4 Methods of assessing GI microorganisms
Among the included studies, seven\(^{19-21,23,24,26,27}\) evaluated the microorganisms using cultures. Nine studies\(^{14-18,21,25,26,28-30}\) used sequencing techniques (one of these studies\(^{26}\) cultured the specimens before sequencing), including fluorescence in situ hybridization (FISH), quantitative real-time polymerase chain reaction (qPCR), high throughput SOLiD sequencing, and bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP).

3.2 Evaluation of study quality and assessment of publication bias
The quality of the reports of the studies was evaluated using the STROBE criteria.\(^{10}\) The study conducted by Zhang\(^{14}\) could not be assessed because only the abstract was available. The remaining 14 studies had scores ranging from 9 to 18 (maximum score of 22), with a mean (sd) score of 12.7 (2.7). No study was considered of high quality. The most prominent problems included inadequate or absent explanation of: (a) determination of sample size; (b) sources of the sample and method of selecting the sample (only reported in three studies); (c) timing of the study (including duration of illness, persistence of GI symptoms, concurrent treatment, etc.) and; (d) potential biases (only reported in three studies). The different studies used very different indicators of patients’ GI microbiome so it was not possible to generate a funnel plot of the results.

3.3 GI bacterial characteristics of children with ASD
As shown in Table 1, there were 11 studies\(^{14-19,23,26,28,30}\) with a combined sample of 562 individuals that found significant differences in GI microorganisms between children with ASD and controls, 3 studies\(^{21,22,27}\) with a combined sample of 215 individuals that found no differences between children with ASD and controls, and one study\(^{20}\) with 28 participants that did not have a control group.

3.3.1 Three major bacterial phyla
Four studies reported GI tract bacteria at the phylum level of classification.\(^{15,22,25,28}\) However, the studies described the results using different statistics: means without standard deviations,\(^{22}\) means with standard deviations,\(^{25}\) box graphs,\(^{25}\) and medians with inter-quartile ranges.\(^{28}\) Unfortunately, the relatively large study by Gondalia and colleagues in 2012\(^{22}\) (n=104) only reported means without standard deviations and they were unable to provide us with the original data, so the results from this study could not be combined with those of the other three studies.

Figures 2 to 4 show the results of the ASD and CON groups for the three main phyla – Firmicutes, Bacteroides, and Proteobacteria – using the combined sample of 120 individuals in the three studies. For each study the results of the two groups are compared using standardized mean difference (SMD). However, for all three phyla the results of the three studies were quite different, the main measure of heterogeneity,
Table 1. Basic characteristics and main findings of the 15 studies included in the systematic review

| Study     | Country          | Study groups (male/female) | Evaluation methodology | Sample sources | Detected microorganisms | Findings                                                                 |
|-----------|------------------|-----------------------------|-------------------------|----------------|-------------------------|--------------------------------------------------------------------------|
| Horvath   | United States    | 36 (33/3) 0 22             | Giemsa stain; Bacterial or fungal cultures | Gastric biopsy and duodenal fluid | ----- | ASD higher than CON: none  
CON higher than ASD: none  
Other findings: No Helicobacter pylori infection was found. No fungal or bacterial overgrowth in the duodenum. |
| Sandler   | United States    | 4 0 104*                   | Routine bacterial pathogen, and ova and parasites examination; aerobic and anaerobic culture | Feces | 12 microorganisms (families or genera or species) | ASD higher than CON: none  
CON higher than ASD: none  
Other findings: Anaerobic cocci, peptostreptococcal species were absent in ASD. |
| Finegold  | United States    | 13 0 8                     | Bacterial culture; 16S rRNA gene sequencing. | Feces, gastric juice and duodenal or jejunal fluid | 2 genera (25 of Clostridium and 6 of Ruminococcus) in feces; 51 organisms in other fluid | ASD higher than CON: Clostridial counts and the number of clostridial species in feces  
CON higher than ASD: none  
Other findings: The non-spore-forming anaerobes and microaerophilic bacteria were absent in the gastric and duodenal specimens of controls but common in that of ASD children. |
| Song      | United States    | 15 0 8                     | Real-time PCR           | Feces | 1 species; 3 clusters | ASD higher than CON: Mean counts of C. bolteae and clusters I and XI  
CON higher than ASD: none  
Other findings: none |
| Parracho  | United Kingdom   | 58 (48/10) 12 10           | FISH | Feces | 5 groups or genera or species | ASD higher than CON: Clostridium histolyticum group (Clostridium clusters I and II)  
CON higher than ASD: none  
Other findings: SIB group had an intermediate (non-significant) level of the C. histolyticum group but the lowest level of Bacteroides. |
| Finegold  | United States    | 33 (24/9) 7 8             | bTEFAP | Feces | 9 phyla; 198 genera | ASD higher than CON: Phylum: Bacteroidetes and Proteobacteria; Genus: Alkaliflexus, Desulfovibrio, Acetanaerobacterium, Parabacteroides, Bacteroides; Species: Desulfovibrio spp. and Bacteroides vulgatus  
CON higher than ASD: Phylum: Actinobacteria and Firmicutes; Genus: Weisella, Turicibacter, Clostridium, Anaerofilum, Pseudoramibacter, Ruminococcus, Streptococcus, Anaeroverax, Dialister, Lactococcus, Leuconostoc, Ethanoligenens, Helcococcus, Alkaliphilus; Species: Bifidobacterium longum, Dialister invisus, Clostridium leptum  
Other findings: none |
| Gondalia  | Australia        | 28 (22/6) 0 25*           | Microbial cultivation (aerobic and anaerobic bacteria, and Candida) | Feces | 19 species of bacteria | ASD higher than CON: none  
CON higher than ASD: none  
Other findings: Some children with ASD had abnormal results: two had elevated levels of aerobic bacteria (Citrobacter spp., Enterobacter spp., Staphylococcus & Streptococcus spp.) and Bifidobacterium; one had elevated Clostridium spp; four had depressed total bacterial and total aerobes levels; and three had depressed Escherichia coli levels. |
| Study          | Country   | Study groups (male/female) | Evaluation methodology                                           | Sample sources | Detected microorganisms | Findings                                                                 |
|---------------|-----------|---------------------------|------------------------------------------------------------------|----------------|------------------------|--------------------------------------------------------------------------|
| Adams 2011[24] | United States | 58 (50/8) 0 39 (18/21)   | Bacterial/Yeast cultivation; parasitology                        | Feces          | 22 bacteria or yeast    | ASD higher than CON: Species: Lactobacillus, Bacillus                        |
|               |           |                           |                                                                  |                |                        | CON higher than ASD: Species: Bifidobacterium, Klebsiella oxytoca, Enterobacter cloacae |
|               |           |                           |                                                                  |                |                        | Other findings: Similar levels of other bacteria and yeast                |
| Wang 2010[17], 2011[18] | Australia | 23 (21/2) 22 (11/11) 9 (4/5) | Quantitative real-time PCR (qPCR)                                | Feces          | 14 species or groups | ASD higher than CON: none
CON higher than ASD: Species: Bifidobacterium spp and Akkermansian muciniphila
Other findings: ASD also had lower level of Akkermansian muciniphila than SIB. No differences between groups in levels of Faecal bacterium prausnitzii. |
| Zhang 2011[14] | China     | 6 0 2                   | SOLID sequencing                                                 | Feces          | -----                  | ASD higher than CON: Genus: Escherichia, Shigella                        |
|               |           |                           |                                                                  |                |                        | CON higher than ASD: Genus: Bacteroides                                  |
|               |           |                           |                                                                  |                |                        | Other findings: Bifidobacterium, Paenibacillus and Pseudomonas were only found in ASD. |
| Gondalia 2012[22] | Australia | 51 (42/9) 53 (19/34) 0 | bTEFAP                                                           | Feces          | 19 phyla; 666 species  | ASD higher than CON: none
CON higher than ASD: none
Other findings: Firmicutes (70%), Bacteroidetes (20%) and Proteobacteria (4%) were the most dominant phyla in samples. |
| Williams 2011[15], 2012[16] | United States | 23 (23/0) 0 9 (9/0)     | Pyrosequencing and quantitative real-time PCR; Sutterella- specific PCR, conventional PCR | Ileal and cecal biopsies | 11 phyla; 1 genus | ASD higher than CON: Phylum: Firmicutes-Bacteroidetes ratio and the cumulative level of Firmicutes and Proteobacteria; Class: Betaproteobacteria; Genus: Sutterella
CON higher than ASD: Phylum: Bacteroidetes
Other findings: Presence of Alcaligenaceae in some children with ASD children but absence in controls. High level of Sutterella genus in children with ASD. |
| Kang 2013[26] | United States | 20 (18/2) 0 20 (17/3)   | bTEFAP                                                            | Feces          | 15 phyla; 214 genera   | ASD higher than CON: none
CON higher than ASD: Genus: Prevotella, Coprococcus, and unclassified Veillonellaceae
Other findings: Autism and accompanying gastrointestinal symptoms were characterized by distinct and less diverse gut microbial compositions. |
| Martirosian 2009[20] | Poland    | 28 (20/8) 0 0          | Bacterial cultivation (anaerobic)                                | Feces          | 7 species              | ASD higher than CON: no control group
CON higher than ASD: no control group
Other findings: Clostridium spp. was isolated from 78.6% (22/28) of studied samples. The majority (28/42) of isolated strains were identified as Clostridium perfringens. |
| Martirosian 2011[21] | Poland    | 41 (32/9) 0 10 (5/5)   | Bacterial cultivation (anaerobic)                                | Feces          | 12 species             | ASD higher than CON: Species: Clostridium perfringens
CON higher than ASD: none
Other findings: Clostridium spp. was isolated with similar frequency from all samples (85.4% in ASD and 90% in CON). |

ASD, autism spectrum disorder patients group; SIB, non-autistic siblings group; CON, unrelated control group.
PCR, polymerase chain reaction; FISH, fluorescence in situ hybridization; bTEFAP, bacterial tag encoded FLX amplicon pyrosequencing.
*used previously published data on adults as historical controls.
I², was 87% for the three results for Firmicutes, 91% for the three results for Bacteroides, and 91% for the three results for Proteobacteria. Given the high level of heterogeneity (I²>75%) and the small number of eligible studies (three) it was not feasible to pool the results in a meta-analysis.

### 3.3.2 Clostridium

Although different assessment methods were employed (i.e. culture, real-time PCR or FISH), three studies [26,29,30] with a combined sample of 124 individuals, found higher numbers of some clusters or groups of Clostridium genus bacteria in the stool samples of children with ASD compared to controls. However, a subsequent study conducted by Finegold and colleagues [25] (n=48) using bTEFAP yielded the opposite result: compared to controls, a smaller proportion of Clostridium genus and a lower level of Firmicutes phylum (to which Clostridium belongs) were found in children with ASD.

### 3.3.3 Bifidobacteria

Three studies [17,18,24,25] with a combined sample of 199 individuals reported that the level of the probiotic Bifidobacterium (genus or species) was lower in children with ASD than in controls. But the study by Gondalia and colleagues [21] (n=53) found no statistically significant difference in fecal Bifidobacteria levels between children with ASD and a historical control sample of adults without ASD.

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**Table 2. Supplemental information provided for 11 of the 15 studies in the systematic review**

| Study          | Diagnosis | Age range (in years) | GI symptoms                                                                 | Minimum time of no antibiotics prior to evaluation | Special diets | Use of other supplements / Allergic diseases |
|----------------|-----------|----------------------|----------------------------------------------------------------------------|---------------------------------------------------|---------------|---------------------------------------------|
| Horvath 1999 [27] | AD or PDD-NOS | 2.5-10               | **ASD**, All had one or more GI symptoms, including abdominal pain (69.4%), chronic diarrhea (58.3%), gaseousness/bloating (58.3%), nighttime awakening (41.7%), and unexplained irritability (50.0%) | 1 week (also no antifungal) | 47.2% of ASD were on CF and/or GF diets | ----- |
| Sandler 2000 [19] | AD a | 3.6-7               | **ASD**, All had diarrhea                                                | 2 month (antimicrobial) | ----- | ----- |
| Finegold 2002 [26] | Autism | --                  | **ASD**, All had GI symptoms, primarily diarrhea and/or constipation       | 1 month (antibacterial) | Many of ASD were on GF/CF diets | ----- |
| Parracho 2005 [29] | ASD | 2-16               | **ASD**, 91.4% (53/58) had GI symptoms: 75.6% had diarrhea, 55.2% had excess gas, 46.6% had abdominal pain, 44.8% had constipation and 43.0% had abnormal feces a **SIB**, 25% (3/12) had GI symptoms b **CON**, None had GI symptoms b | (89.7% of ASD, 91.6% of SIB and 50% of CON had undertaken numerous courses of antibiotic treatment from an early age) | 65.5% of ASD, 8.3% of SIB and none of CON were on GF/CF diets | 53.4% ASD, 41.7% SIB and none of CON were taking probiotics/prebiotics; 65.5% ASD, none of SIB and none of CON were taking other supplements |
| Finegold 2010 [25] | ASD | 2-13               | **ASD**, All had GI symptoms, primarily constipation, diarrhea, abdominal distention, and abdominal pain **SIB**, None had GI symptoms **CON**, None had GI symptoms | 1 month (antifungal agents allowed) | A number of ASD were on CF/GF or specific carbohydrate diets | No probiotics for at least 1 month before the study-- |
| Gondalia 2010 [21] | ASD | 2-14               | -----                                                                 | 14 days | Varied across individuals | ----- |
| Adams 2011 [24] | Autism, PDD-NOS, AS | 2.5-18             | **ASD**, 6-GSI c mean score: 3.9 (2.5) d **CON**, 6-GSI c mean score 1.3 (1.4) d | 1 month (also no antifungal) | ----- | 33% of ASD and 5% of CON took probiotics; 14% of ASD and 36% of CON consume seafood; 36% of ASD and none of CON took fish oil |
| Wang 2010 [17], 2011 [18] | AD, AS a | 3.1-18.4 | **ASD**, 39.1% (9/23) **SIB**, 27.3% (6/22) **CON**, 11.1% (1/9) | 1/23 (4.3%) ASD and 1/22 (4.5%) SIB were using antibiotics | 17.4% (4/23) of ASD were on CF/GF diets | 8.7% (2/23) of ASD and 4.5% (1/22) SIB were taking probiotics |
Table 2. Supplemental information provided for 11 of the 15 studies in the systematic review (continued)

| Study                | Age range (in years) | Diagnosis | GI symptoms | Minimum time of no antibiotics prior to evaluation | Special diets | Use of other supplements / Allergic diseases |
|----------------------|----------------------|-----------|-------------|-----------------------------------------------------|---------------|---------------------------------------------|
| Gondalia 2012 (22)   | 2-12                 | ASD, Current: 54.9% (28/51); Past: 21.6% (11/51) | 15 days (also no antifungals), but 1 SIB used antibiotics | 41.2% (21/51) of ASD and 7.5% (4/53) of SIB were on GF/CF diets, dairy lactose free diet or preservative free diet | 33.3% (17/51) of ASD and 15.1% (8/53) of SIB were taking probiotics |
| Williams 2011 (15)   | 3-5                  | ASD, Current: 54.9% (28/51); Past: 21.6% (11/51) | 15 days (also no antifungals), but 1 SIB used antibiotics | 41.2% (21/51) of ASD and 7.5% (4/53) of SIB were on GF/CF diets, dairy lactose free diet or preservative free diet | 33.3% (17/51) of ASD and 15.1% (8/53) of SIB were taking probiotics |
| Kang 2013 (28)       | 3-16                 | ASD, Mean 6-GSI score: 4.7 (2.3) | 1 month (also no antifungal) | 25.0% (5/20) of ASD and 5.0% (1/20) of CON were on GF/CF diet | 66.7% (10/15) in ASD and 71.4% (5/7) in CON had restricted diets |

AD, Autistic disorder; ASD, Autism Spectrum Disorder; AS, Asperger’s Syndrome; PDD-NOS, Pervasive Developmental Disorder—not otherwise specified; SIB, non-autistic siblings; CON, unrelated control subjects; GI, gastrointestinal; GF, gluten-free; CF, casein-free.

a used diagnostic criteria specified in the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV)

b GI symptoms were significantly more frequent in ASD than in SIB or CON (p<0.05)

c The 6-GSI scale asks about constipation diarrhea, stool consistency, stool smell, flatulence and abdominal pain

d GI symptoms in ASD more frequent than in CON (p<0.001)

e used diagnostic criteria specified in the Diagnostic and Statistical Manual-Fourth Edition, Text Revision (DSM-IV-TR)

3.3.4 Other bacteria

As shown in Table 1, single studies also identified other abnormalities in the GI microbiome of children with ASD: the lack of the anaerobic cocci peptostreptococcus species,[19] and the presence of non-spore-forming anaerobes,[26] microaerophilic bacteria,[26] Paenibacillus,[14] Pseudomonas,[14] and Alcaligenaceae.[16]

3.4 Association between the GI symptoms and the severity of autism

Two studies[24,28] examined the association between GI symptoms and the severity of autism. Both studies used the Autism Treatment Evaluation Checklist (ATEC) to measure the severity of autism and assessed the severity of GI symptoms using the Gastrointestinal Severity Index (6-GSI)[20] (which assesses constipation, diarrhea, stool consistency, stool smell, flatulence and abdominal pain). The study by Kang and colleagues[28] also assessed the severity of autism using the Autism Diagnostics Interview-Revised (ADI-Revised), Autism Diagnostics Observation Schedule (ADOS), and Pervasive Developmental Disorder Behavior Inventory (PDD-BI). The study by Adams and colleagues,[24] which included 58 children with ASD, found a strong correlation between the 6-GSI score and the ATEC score (Pearson’s correlation coefficient [r]=0.60, p<0.001). However, the study by Kang and colleagues[28], which included 20 children with ASD, did not find a significant correlation between the 6-GSI score and any of the four measures of autism severity (Spearman’s correlation coefficient [r_s] ranged from 0.09 to 0.28; all p>0.05).
4. Discussions

4.1 Main findings

The characteristics of the GI microbiome in children with ASD is an under-studied field, particularly in China where we were only able to identify the abstract of one small study. The 15 studies that met inclusion criteria for this systematic review varied in the taxonomic level of the assessed organisms (Phylum, Genus, Species) and used different methods to assess the GI microbiome, varying from traditional bacterial cultures to genome sequencing (reflecting the evolution in techniques for assessing GI microflora). Methodologically, the studies were generally of poor quality, primarily because of the use of small, unrepresentative samples and little consideration of potential confounders.

Despite the relatively small size of most of the studies (7 of the 15 studies had results for less than 50 individuals), 11 of the 14 studies that had control groups (79%) reported significant differences in the prevalence of different GI microorganisms between children with ASD and controls. However, the variety of assessment methods and differences in the reported organisms made it difficult to pool the results of the different studies. Sufficient information to allow for cross-study comparison was available for 3 studies that used genome sequencing techniques, but these 3 studies reported conflicting results about the differences in the prevalence of the three main phyla of GI bacteria – Firmicutes, Bacteroidetes and Proteobacteria – between children with ASD and controls. The results about the relative abundance of other GI microorganisms between children with ASD and controls were also inconsistent across the 15 studies. Given the different target organisms assessed, the variety of assessment methods employed, and the heterogeneity of the results for the small number of studies that used similar methods to assess the same class of organisms, it was not possible to conduct a meta-analysis of the results of the studies.

Clearly, in the future more consistency in the methodology across studies will be needed to determine...
whether or not the GI microbiome of children with ASD is, in fact, significantly different from that of other children. At a minimum studies need to (a) use widely accepted diagnostic criteria for ASD; (b) include a representative sample of ASD children; (c) use validated methods for assessing the severity of ASD and the severity of GI symptoms; (d) select an appropriate control group of children without ASD; (e) use the same sample source (stools) collected in the same manner; (f) report on a common set of organisms (starting with the three main phyla—Firmicutes, Bacteroidetes and Proteobacteria); and (g) collect information on other potential confounding variables including, gender, age, use of antibiotics or antifungal agents, history of allergic and autoimmune conditions, dietary restrictions, and dietary supplements (e.g., probiotics and vitamins). It is certainly possible that an abnormal GI microbiome only occurs in certain subgroups of ASD children or at certain phases in the course of their condition, so there will be need to be sufficiently large samples of subjects included in these studies to identify potentially important subgroups.

4.2 Limitations

The search strategy was quite exhaustive so we expect that there were relatively few relevant studies that were not identified, unless they were published in languages other than English or Chinese. Three high-income countries (i.e., United States, United Kingdom, Australia) accounted for 80% (12/15) of the identified studies and for 89% (718/805) of the combined samples from the identified studies, so it is uncertain whether or not the results would be different if available studies were more internationally representative. The limitations of the results are primarily determined by the limitations in the included studies, which we assessed to be of poor to fair quality (none were good quality). Of the 15 studies included in the review, 7 studies\(^\text{[14,15,20,25,26,28,30]}\) had fewer than 50 subjects (including children with ASD and controls), 1 study did not have a control group,\(^\text{[20]}\) 2 studies\(^\text{[19,21]}\) used historical control groups of adults, 1 study was only available as an abstract,\(^\text{[14]}\) and 1 study only provided mean prevalence figures of the different microorganisms without standard deviations (so it could not be compared to other results). As stated previously, the small size, different methods, and heterogeneity of results made it impossible to combine the results of different studies in a meta-analysis.

4.3 Significance

The high reported prevalence of GI symptoms in children with ASD has focused attention on the potential role of GI microorganisms in the onset and development of ASD. To help elaborate a theoretical model that could suggest potential mechanisms for the proposed connection between GI microorganisms and ASD, a number of studies have compared the GI microbiome in children with ASD to that of control subjects. To the best of our knowledge, this paper reports on the first systematic review of these studies. Despite identifying 15 studies that met our inclusion criteria, the small sample sizes, lack of standardization of methods and generally poor methodological quality of the studies makes it impossible to come to definitive conclusions. The majority of studies identified significant differences between children with ASD and controls, but it remains to be proven whether or not the GI microbiome of children with ASD is significantly different from that of controls and, if it is different, what the difference is. Resolution of this theoretically important question will require larger studies of higher quality that use standardized methods for the selection of subjects and the assessment of the GI microbiome.

Conflict of interest

The authors report no conflict of interest related to this manuscript.

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儿童孤独症谱系障碍胃肠道细菌学研究的系统综述

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摘要

背景：有报道指出，孤独症谱系障碍 (ASD) 患儿的胃肠道 (GI) 症状发生率较高。然而，有关 ASD 患儿胃肠道微生物的研究结果不尽一致。

目的：系统复习相关研究结果，分析 ASD 患儿各种胃肠道微生物的分布特征。

方法：检索 PubMed、Embase、PsycINFO、ISI web of knowledge、Ovid/Medline、Cochrane Library、中国知识资源总库、中国科技期刊数据库、万方数据检索系统，以及中国生物医学文献服务系统，收集有关 ASD 患者胃肠道微生物的文献，按照预先制定的纳入及排除标准筛选相关研究。采用 Review manager 5.2.6 软件进行统计分析。

结果：最终共纳入 15 项小样本横断面研究，其中 1 项来自于中国。在 15 项研究中，11 项研究（合并样本量为 562 例）报道 ASD 患儿组与对照组的胃肠道细菌患病率有显著性差异，尤其是厚壁菌门、拟杆菌门和变形菌门。但是，由于方法学上较大的异质性以及不同研究结果之间的相互矛盾，我们无法汇集结果进行 meta 分析。

结论：目前对 ASD 患儿胃肠道微生物的研究数量和质量都非常有限。然而，似乎有迹象表明 ASD 患儿的胃肠道微生物和没有 ASD 的儿童是有显著差异的。因此，继续开展此方面的研究是非常有价值的。为了提高研究的效度、减少研究结果的异质性，将来的研究需要增大样本，应用标准化的研究方法并评估相关混杂因素，例如胃肠道症状的严重程度，以及药物、特殊饮食和营养补充品的使用情况。