Abstract

**Background:** Autism spectrum disorder (ASD) is a neurodevelopmental condition associated with an unclear etiologic mechanism. Following suggestions in the literature of a close relation between the gut microbiota and the central nervous system development, neuroimmune and neuroendocrine systems, new theories and strategies of the management of ASD in children focus on the brain-gut axis via microbiota transfer therapy. Despite the regular appearance in the news, the level of evidence supporting this intervention is unclear and to this date, no systematic review on this issue has been published.

**Methods:** We conducted a systematic literature review of the efficacy and safety of microbiota transfer therapy for the management of ASD in children. MEDLINE via PubMed, LILACS IBZCS via BVS, EMBASE via Ovid, Scopus and Cochrane Library were searched on 19th April 2018.

**Results:** One single study published in 2017 was identified. The intervention group included 18 patients and showed significant clinical improvements in the gastrointestinal and ASD-related symptoms. The clinical procedure was reported as safe and well-tolerated with some transitory adverse effects.

**Conclusions:** The causality and correlation of the intervention and the expected outcomes cannot be assumed with current evidence. In addition, recommendations about the effectiveness or safety of microbiota transfer therapy in children with ASD cannot be currently issued. Randomized controlled trials and clinical protocols for the intervention are needed.
Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by impaired communication, impaired reciprocal social interaction, and restricted, repetitive patterns of behavior or interests (Faras et al., 2010). In the past couple of decades, the prevalence of ASD has increased to around 1–2%, partially because of changes in the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) diagnostic criteria and patients being diagnosed at a younger age (Park et al., 2016). Standardized research assessment tools have been designed following the DSM-IV criteria. Such tools include the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) (Lyall et al., 2017).

Etiologic theories of ASD have changed over the years. It is currently accepted that ASD is not a single-cause disorder, but rather a multi-factorial disorder associated with genetic and non-genetic risk factors involved. Causes related to genetics are estimated to be present in 10% to 20% of patients with ASD (Park et al., 2016). The specific biological mechanism remains unclear, although literature suggest a close relation between the gut microbiota and the central nervous system development, neuroimmune and neuroendocrine systems (Cani & Knauf, 2011), leading to the development of new theories and strategies for the management of ASD (Estrella & Rossen, 2015). Children with ASD have been considered as a relevant interventional group for using microbiota transfer therapy. (Yang et al., 2018)(Cryan & Dinan, 2012).

The gut-brain axis describes a bidirectional communication between the gut microbiota and the central nervous system (Falsaperla et al., 2017). These bidirectional pathways control the permeability and barrier function of the gut, therefore, the composition of the microbiota. Probiotic agents, defined as living beings that positively influence health when ingested, and the gut microbiota can alter the concentration of cytokines and short-fatty acids (SCFA), which have a direct action in the brain function (Cryan & Dinan, 2012).

In the past decade, microbiota transfer therapy has gathered interest from research leading to the development of novel intervention for the management and clinical improvement of different pathologies, including ASD (Colman & Rubin, 2014). Microbiota transfer therapy aims to restore the gut microbiota of the receiver through the infusion of donor faeces to the gastrointestinal tract via oral capsules, endoscopic stomach, duodenum, jejunum, ileum, cecum or sigmoid infusions or rectal enema infusions (Rossen, 2015)(Choi & Cho, 2016). Recent evidence suggests that microbiota transfer therapy affects the pathophysiology of several brain disorders, such as ASD, Parkinson disease, chronic pain, and disorders in mood and affect (Martin & Mayer, 2017)(Yang et al., 2018). Microbiota transfer therapy has shown to cause the durable engraftment of donor microorganisms and increases the number of species present in the receiver’s gut microbiota (Kelly et al., 2015). There is already evidence showing its efficacy for different gastrointestinal diseases, such as inflammatory bowel disease, metabolic syndrome, irritable bowel syndrome, and Clostridium difficile infection (Rossen et al., 2015). As a novel therapy, there is still no protocol for using microbiota transfer therapy for the management of the different pathologies. However in 2010, a workgroup with several societies proposed a protocol for the treatment of Clostridium difficile infection with microbiota transfer therapy, including the indications, donor selection criteria, recipient exclusion criteria, means of administering stool, and the evaluation of the success (Bakken et al., 2011). There are still questions about the safety of the microbiota transfer therapy, as the possibility of transmitting infectious agents exists (Choi & Cho, 2016).

To this day, no systematic literature review of clinical trials, assessing the effects of microbiota transfer therapy in patients with ASD has been conducted. The aim of this study is to perform a systematic literature review on the efficacy and safety of the microbiota transfer therapy for the management of symptoms in children with ASD.

Methods

This study was conducted following the 27 items of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement to report a systematic review. No previous review protocol was identified for microbiota transfer therapy for the management of autism spectrum disorder in children. A protocol to identify and assess study eligibility was developed and was uploaded to the Dataverse repository as Appendix 1, Extended data (Estrella & Guillemot, 2019).

Search terms and databases

Search terms (see Appendix 1, Extended data (Estrella & Guillemot, 2019)) were developed based on other systematic literature reviews and adapted for each database (Rossen et al., 2015)(Colman & Rubin, 2014). References used in articles of peripheral relevance were reviewed. Searches were performed in five different databases: MEDLINE via PubMed, LILACS IBECS via BVS, EMBASE via Ovid, Scopus and Cochrane Library. The search was conducted on the 19th of April 2018.

Eligibility criteria and data extraction

To be included, the papers had to meet a set of eligibility criteria: Studies must be randomized controlled trials, quasi-randomized control trials or control trials. Eligible population was with children diagnosed with autism spectrum disorder (age limitation according to each study). The intervention was microbiota transfer therapy. For the data extraction, a standard data extraction form was used, which included authors, years, journal, number of patients included, eligibility criteria, study outcomes (efficacy and safety) and a final critical assessment of the trial. No language or publication date were used as exclusion criteria.

Screening

EndNote version X7.7.1 was used to extract the information of captured citations and deduplication. Two reviewers independently assessed the titles and abstracts. Discrepancies were solved with discussion until a consensus was reached.
between reviewers. If the information of the database was not enough to use the selection criteria, the full manuscript was retrieved and reviewed in full.

**Risk of bias**

To assess the risk of bias in estimates of the comparative effectiveness of the study in the included in this systematic literature review, the Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) was used (NCBI, n.d.; Sterne *et al*., 2016).

**Results**

In total, 5297 papers were identified after automatic deduplication (see PRISMA flowchart, Figure 1). From those papers, one single study fulfilled all inclusion criteria.

The study identified is an open-label trial investigating the safety, tolerability and efficacy of microbiota transfer therapy for gastrointestinal and behaviour symptoms in children with ASD and was published in 2017 (Kang *et al*., 2017). A total of 18 children participated, with ages ranging from seven to 16 years and that presented moderate to severe gastrointestinal problems, with 20 neurotypical children used as controls. After the microbiota transfer therapy intervention, children with ASD showed a greater bacteria diversity in the gut. The study showed substantial changes in gastrointestinal symptoms, such as constipation, diarrhoea, abdominal pain and indigestion. ASD symptoms showed significant improvements during the treatment and no reversion of the effects during the follow up with a decline of ASD symptoms of more than 20%. The average developmental age was found to be increased in 1.4 years with a p<0.001 with the VABS-II (Vineland Adaptive Behaviour Scale II) score. The study reported that microbiota transfer therapy was a safe and well-tolerated intervention, with some transitory adverse effects, including hyperactivity and aggression during the first phase of vancomycin treatment. The overall bias associated with the study was considered low. While the risk of bias due to confounding domains was considered serious, the risk of bias in participant selection was considered low. The bias in measurement of outcomes was considered moderate.

**Discussion**

To date, this is the only published systematic literature review on microbiota transfer therapy on children with ASD. Although the intervention has been described as effective and safe in the study found, the 18 children of this study are not enough to make recommendations for future application of the microbiota transfer therapy intervention in ASD children. The causality and correlation of the intervention and the expected outcomes cannot be assumed with the current evidence.

Most information available is not associated with randomized clinical trials. In a published observation, two children showed improvement in their ASD symptoms after faecal microbiota therapy and five children after several weeks of receiving cultured *Bacteroidetes* and *Clostridia* every day (Aroniadi & Brandt, 2013). In June 2019, the Food and Drug Administration (FDA) published a safety warning regarding the risk of adverse reactions after a microbiota transfer therapy that was not screened against an extended-spectrum β-lactamase-producing *Escherichia coli* (NEJM Journal Watch, 2019). The FDA added additional protections for investigational use of microbiota transfer therapy, that includes stool testing for multi-drug resistance organisms (MRDO) (Commissioner, 2019).

The brain-gut axis involves complex communication and metabolic mechanisms that are still not fully understood. Several pathophysiologic pathways have been proposed, hypothesizing the relation between the microbiota composition with different neurological conditions, nevertheless further evidence to clarify the function of this axis is needed. Taking in consideration that children with ASD present a pattern of dysbiosis, the microbiota transfer therapy has raised attention as a novel intervention for the management of ASD patients. However, it is still not clear if there is a direct relation between ASD and the alteration in their gut microbiota and therefore, if the change in the microbiota directly influences the different gastrointestinal and clinical symptoms of ASD. Besides, microbiota transfer therapy is a growing field in medicine for management of several diseases. It is not a new therapeutic modality however it has received public attention from the media in the past years. The first report of faecal material given to patients with gastroenterological illness was in China by Ge Hong (Aroniadi & Brandt, 2013). The use of faecal enemas was reported in 1958 as an adjunct treatment of pseudomembranous enterocolitis (Eiseman *et al*., 1958). Today, microbiota transfer therapy is extensively used for *C. difficile* infection, inflammatory bowel disease, and irritable bowel syndrome management (Sha *et al*., 2014). The evidence is growing around faecal microbiota therapy and its applications in other fields of medicine, including ASD patients. Efficacy and safety data have been recorded mainly in adults, while it has not been studied so widely in children.

The selection of microbiota transfer therapy donor’s is an important step to assure the safety of this intervention. There is not a standardized procedure for selection and screening of donors, and varies from study to study (Owens *et al*., 2013). The evaluation can include but is not limited to past medical history, questionnaires and a physical examination and serologic for bacteria, parasites and viral infections. Some studies have used exclusion criteria for donors, such as history of gastrointestinal pathologies, use of immunosuppressive drugs, prior use of antibiotics, and gastrointestinal symptoms (Paramsothy *et al*., 2015).

This systematic literature review shows that the evidence is not strong enough to make recommendations about the use of microbiota transfer therapy because of its effectiveness or safety in children with ASD. Randomized, placebo-controlled, double-blind control trials and clinical protocols for the intervention are needed. However new evidence is expected to be released as three clinical trials related to microbiota transfer therapy with ASD patients. Are currently online. One targets ASD and gastrointestinal disorders in children (The National Library of Medicine, 2019a), another focuses on adults (The National Library of Medicine, 2019b) and the third looks into children and young adults (The National Library of Medicine, 2019c).
Figure 1. PRISMA Diagram. Identification, screening, eligibility and inclusion of studies.
Data availability
Underlying data
All data underlying the results are available as part of the article and no additional source data are required.

Extended data

This project contains the following underlying data:

- Screening Database (all studies initially identified in the search)
- Appendix 1 (Protocol and Search Terms for each Database)
- PRISMA Checklist (reporting guidelines)

References


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This is an important and timely systematic review of the efficacy and safety of microbiota transfer therapy (MTT) use for autism spectrum disorder in children. MTT is a current subject of interest and controversy among researchers and clinicians. Thorough and unbiased, this systematic review's findings provide a very limited evidence base of one study; with early outcomes and a small sample size warranting cautious interpretation of the findings. Moreover, the authors note that MTT is still considered an experimental treatment with the FDA warning of unknown risk factors and specifying safety guidelines for donor recruitment and assessments of treatments.

This paper would have benefited from a more detailed discussion and critique of the one study identified. In particular, clarification of the low bias stated in the study's results required greater discussion. For example, an explanation of the 'open label' methodology, which does not include a blinded comparison group and has been demonstrated to result in a potentially high placebo effect seems to contradict the low risk of bias reported. A greater critique of the study would have strengthened and supported the authors concluding statements for the need for randomized, placebo-controlled, double-blinded control trial. Additionally, while the authors accurately state that causality and correlation of the intervention and outcomes cannot be assumed based on current evidence, a more detailed discussion of confounding factors in this study may have raised further evidence-based limitations.

This procedurally detailed, well written systematic review appropriately concludes that more specific and controlled research is needed to determine the safety and efficacy of MTT as an intervention modality for autistic children with moderate to severe gastrointestinal problems. Furthermore, it provides an opportune contribution to this subject matter.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes
Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Autism

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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