



Review of fecal microbiota transplantation in autistic children and feasible techniques for fecal microbiota transplant delivery

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ABSTRACT

The review considers the implementation of fecal microbiota transplantation (FMT) to treat several kinds of diseases such as gastrointestinal disorders, diabetes mellitus, metabolic syndrome, and others. The use of FMT in the treatment of neurological disorders such as autism spectrum disorders in children is considered in more detail. We compared the techniques used to deliver FMTs, such as capsules, enemas, introduction through upper gastrointestinal tract, and colonoscopy. This article demonstrates various approaches to the preparation of capsules with lyophilisate of FMTs, such as suspending, microencapsulation, and use of capsule-in-a-capsule technology. These techniques are designed to protect the fecal microbiota microorganisms from the acidic pH of the gastric juice.

INTRODUCTION

Fecal microbiota transplantation (FMT) is a transplantation process of intestinal bacteria and bacteriophages contained in the feces of healthy individuals to the recipients suffering from gut microbiota imbalance.

The technique can be applied to treat gastrointestinal (GI) diseases, primarily recurrent *Clostridium difficile* infection (CDI) (Baunwall *et al.*, 2020; Cammarota *et al.*, 2015; Hota *et al.*, 2017; Hvas *et al.*, 2017, 2019; Jiang *et al.*, 2017, 2018; Kao, 2014; Kao *et al.*, 2017; Lynch *et al.*, 2019; Nood *et al.*, 2013; Staley *et al.*, 2018, 2019; Youngster *et al.*, 2014a, 2014b), as well as GI diseases such as colitis (nonspecific ulcerative colitis) (Adler *et al.*, 2019; Angelberger *et al.*, 2013; Cold *et al.*, 2019; Landy *et al.*,

2013; Moayyedi *et al.*, 2015; Paramsothy *et al.*, 2017; Shi *et al.*, 2016; Sood *et al.*, 2019), Crohn's disease (Bak *et al.*, 2017; Cui *et al.*, 2015, 2016; Kao *et al.*, 2014), chronic constipations, chronic diarrheas, chronic intestinal infections (chronic shigellosis, chronic salmonellosis, yersiniosis, campylobacteriosis, etc.), irritable bowel syndrome, and others (El-Salhy *et al.*, 2020). Recently, FMT is being widely used in endocrine and metabolic disorders (obesity) (Zhang *et al.*, 2019), diabetes mellitus (Wang *et al.*, 2020), and metabolic syndrome (Groot *et al.*, 2017; Zhang *et al.*, 2019), hepatic diseases (hepatic encephalopathy) (Sidhu *et al.*, 2017), neurological disorders (Parkinson's disease, Alzheimer's disease, multiple sclerosis, epilepsy, and stroke), and psychiatric disorders [autism spectrum disorders (ASD), bipolar disorder, depressive syndrome, schizophrenia, etc.] (Vendrik *et al.*, 2020; Xu *et al.*, 2021).

Table 1 shows the most common delivery techniques for fecal microbiota, such as lower GI endoscopy, introduction through upper gastrointestinal tract, and use of enemas or capsules. Lower GI endoscopy includes colonoscopy, enteroscopy, and sigmoidoscopy. Introduction through upper GIT is carried out using the nasojejunal or gastric tube and other medical devices.

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Table 1. Implementation of FMT in the management of GI diseases and nervous disorders.

Nosology	Delivery techniques	Reference
GI diseases	Capsules	Kao, 2014; Youngster <i>et al.</i> , 2017; Kao <i>et al.</i> , 2017; Staley <i>et al.</i> , 2018; Jiang <i>et al.</i> , 2018 Staley <i>et al.</i> , 2019;
	Recurrent <i>Clostridium difficile</i> infection	Lower GI endoscopy
		Enema
		Introduction through upper gastrointestinal tract (nasojunal or gastric tube, etc.)
GI diseases	Capsules	Cold <i>et al.</i> , 2019; Adler <i>et al.</i> , 2019
	Nonspecific ulcerative colitis	Colonoscopy
		Enema
		Introduction through upper gastrointestinal tract (nasojunal or gastric tube, etc.)
Neurological disorders	Crohn's disease	Introduction through upper gastrointestinal tract (nasojunal or gastric tube, etc.)
		Capsules
		Colonoscopy
		Introduction through upper gastrointestinal tract (nasojunal or gastric tube, etc.)
Neurological disorders	Parkinson's disease	Introduction through upper gastrointestinal tract (nasojunal or gastric tube, etc.)
		Transendoscopic enteral tube
		Intragastric introduction (mice)
		Colonoscopy
Psychiatric disorders	Alzheimer's disease	Colonoscopy
		Fecal microbiota infusion
		Enema
		Colonoscopy
Psychiatric disorders	Bipolar disorder	Introduction through upper gastrointestinal tract (gastroscopy)
	Depressive syndrome	

Capsules and enemas have been shown to be more easy to use for patients, while FMTs introduced by lower GI endoscopy and through upper GIT require special equipment and trained staff; however, they can be more effective and have fewer potential side effects.

This review examines works that have been consequential in the field of FMT use in the treatment of ASD in children, including clinical trials and literature on various methods of FMT delivery. The main objectives of this review are as follows: (i) analysis of FMT use in ASD treatment in children; (ii) comparison of different FMT delivery methods to the intestine; and (iii) offering various methods of protecting fecal microbiota microorganisms in capsules based on literature data.

FMT IN CHILDREN WITH ASD

Recently, FMT is being extensively explored to correct ASD (Adams *et al.*, 2019), including those in children (Kang *et al.*, 2017, 2019; Yang *et al.*, 2020). Many studies reported an abnormal fecal microbiota in ASD individuals; therefore, the relationship between fecal microbiota and behavioral disorders characteristic of autism can be traced. Frequent GI problems are known to be more common in ASD children than in healthy ones; therefore, nonadaptive behavior correlates with GI problems (Chaidez *et al.*, 2014; Mazefsky *et al.*, 2014; Maenner *et al.*, 2012). Adams *et al.*

(2011) found a strong correlation of ASD and four ASD subscales (speech, sociability, sensory/cognitive awareness, and physical behavior) with GI severity.

The prevalence rate of GI diseases in ASD children ranges from 9% to 84% compared to 9%–37% in children who have no ASD (Wasilewska and Klukowski, 2015). The most frequent GI problems in ASD individuals are chronic constipation, encopresis as a complication of constipation, abdominal pain, also gastroesophageal reflux disease, bloating, disaccharidase deficiency, GI inflammation, and intestinal and nervous system anomalies (Mannion and Leader, 2014).

Children with ASD were demonstrated to suffer from clostridial infection (induced by *C. difficile*, *Clostridium bolteae*, *Clostridium perfringens*, and others *Clostridium* spp.) more frequently that aggravated ASD symptoms (Argou-Cardozo and Zeidán-Chuliá, 2018; Kandeel *et al.*, 2020). Thus, FMT, effective in CDI management, is likely to improve the condition of ASD children.

GI diseases are known to be related to problem behavior in children with ASD (Restrepo *et al.*, 2020), for example, hyperirritability (Bresnahan *et al.*, 2015), disruptive behavior (Ferguson *et al.*, 2019), increase in nonadaptive behavior and social exclusion (Chaidez *et al.*, 2014; Nikolov *et al.*, 2009), and traumatophilia (Marler *et al.*, 2017), as well as sleep problems

FMT study design

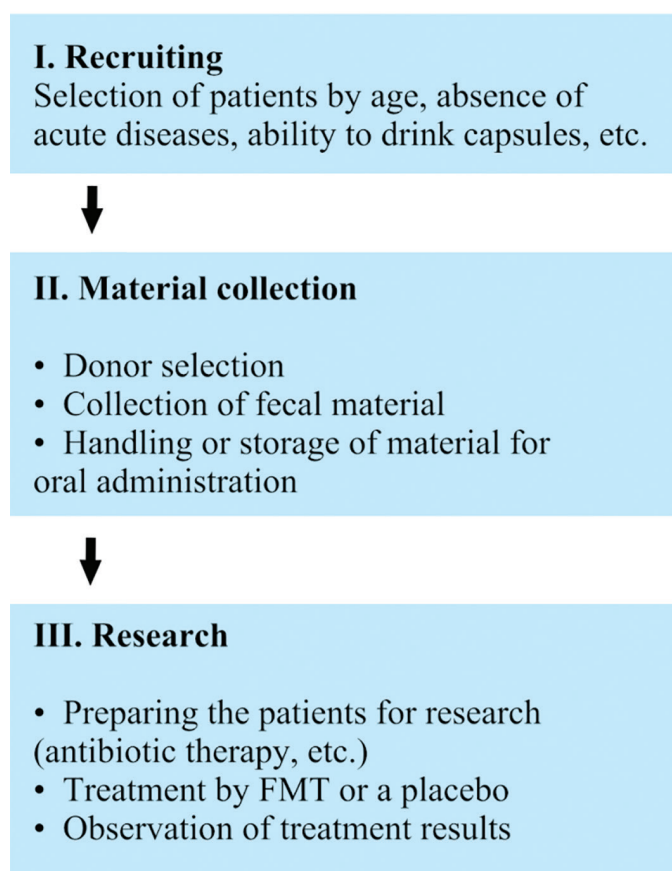


Figure 1. Study design of FMT in the management of children with ASD.

(Maenner *et al.*, 2012). However, currently, the effect of GI problems on the degree or functional impairments in children with ASD is not adequately investigated. Some researchers have reported that there is no significant effect of GI problems on a mentality level or learning ability (Maenner *et al.*, 2012; Nikolov *et al.*, 2009; Prosperi *et al.*, 2017), as well as the intensity of ASD symptoms (Chandler *et al.*, 2013; Mazefsky *et al.*, 2014; Nikolov *et al.*, 2009; Prosperi *et al.*, 2017), while other sources inform of the significant relationship of GIT disorders with ASD symptoms such as increased sensory sensitivity (Mazurek *et al.*, 2013), repeated behavior (Peeters *et al.*, 2013), and others (Chaidez *et al.*, 2014; Wang *et al.*, 2011).

Currently, ClinicalTrials.gov represents the data on the studies on FMT effect on the condition of children (Adams, 2015, 2019; Michail and Levitt, 2018; Youngster, 2020) and adults (Adams, 2018) with ASD; one of the researches has just been completed (Adams, 2015).

The study design (Fig. 1) includes three main stages: selection of patients; material collection and material preparation for transplants; and treatment.

FMT DELIVERY TECHNIQUES IN THE MANAGEMENT OF CHILDREN WITH ASD

The techniques chosen when designing clinical studies (Adams, 2015, 2018, 2019; Michail and Levitt, 2018; Youngster, 2020) to deliver fecal microbiota are capsules, colonoscopy, and the oral use of highly purified liquid formulation of fecal microbiota (Hamilton *et al.*, 2012). Fecal material, approximately 50 g, was homogenized in physiological saline, 250 ml; the suspension was sieved using a stainless laboratory sieve to remove indigested food and other solid particles. The obtained material was centrifuged at 6,000 g within 15 minutes and then resuspended up to half of the initial volume in physiological saline. The obtained concentrated suspension of intestinal bacteria was immediately administered to a patient, or glycerol was added (up to 10% concentration), frozen at -80°C , and stored for 1–8 weeks before usage. Fecal microbiota extract, fresh or frozen, hardly had any odor; it had low viscosity and less intense color compared to the initial material. The present purification method enabled us to administer fecal microbiota orally, the microbiota being mixed with chocolate milk; however, the technique is aesthetically questionable.

Table 2 demonstrates the most frequent FMT delivery techniques in ASD therapy, described and compared in literature.

ASD symptoms and GI symptoms in oral usage of microbiota and enema usage decreased by 47% and 80%, consequently, compared to the initial level, over the period of a 2-year follow-up (Adams, 2015; Kang *et al.*, 2017). A previous study (Kang *et al.*, 2017) used a 2-week vancomycin treatment followed by a bowel cleanse before FMT. After pretreatment, the authors used a liquid formulation of purified bacteria, with the initial dose given orally or by rectal enema and the maintenance dose given orally with a stomach acid suppressant.

Pathogenic bacteria value in gut microbiota and ASD symptoms significantly decreased in young children given enema and capsules with ultracentrifuge concentrated fecal organisms on a long-lasting basis; however, patients aged 21 showed no marked

Table 2. FMT delivery techniques in ASD therapy.

Technique	Advantages	Disadvantages	References
Capsules	Easy-to-use; long shelf-life	Manufacturing complexity	Youngster, 2020; Ward <i>et al.</i> , 2016 Kang <i>et al.</i> , 2017
Enema	Easy-to-use	FMT fails to reach the required intestinal parts	Adams, 2015; Kang <i>et al.</i> , 2017; Kang <i>et al.</i> , 2019; Ward <i>et al.</i> , 2016
Standardized oral fecal microbiota		Non-aesthetic; FMT is partially destroyed under acidic medium of the stomach	Adams, 2015; Kang <i>et al.</i> , 2019
Gastroscopy	Homogeneous transplant distribution on large intestine walls	Requires special equipment and trained staff	Michail and Levitt, 2018; Zhao <i>et al.</i> , 2019
Colonoscopy			

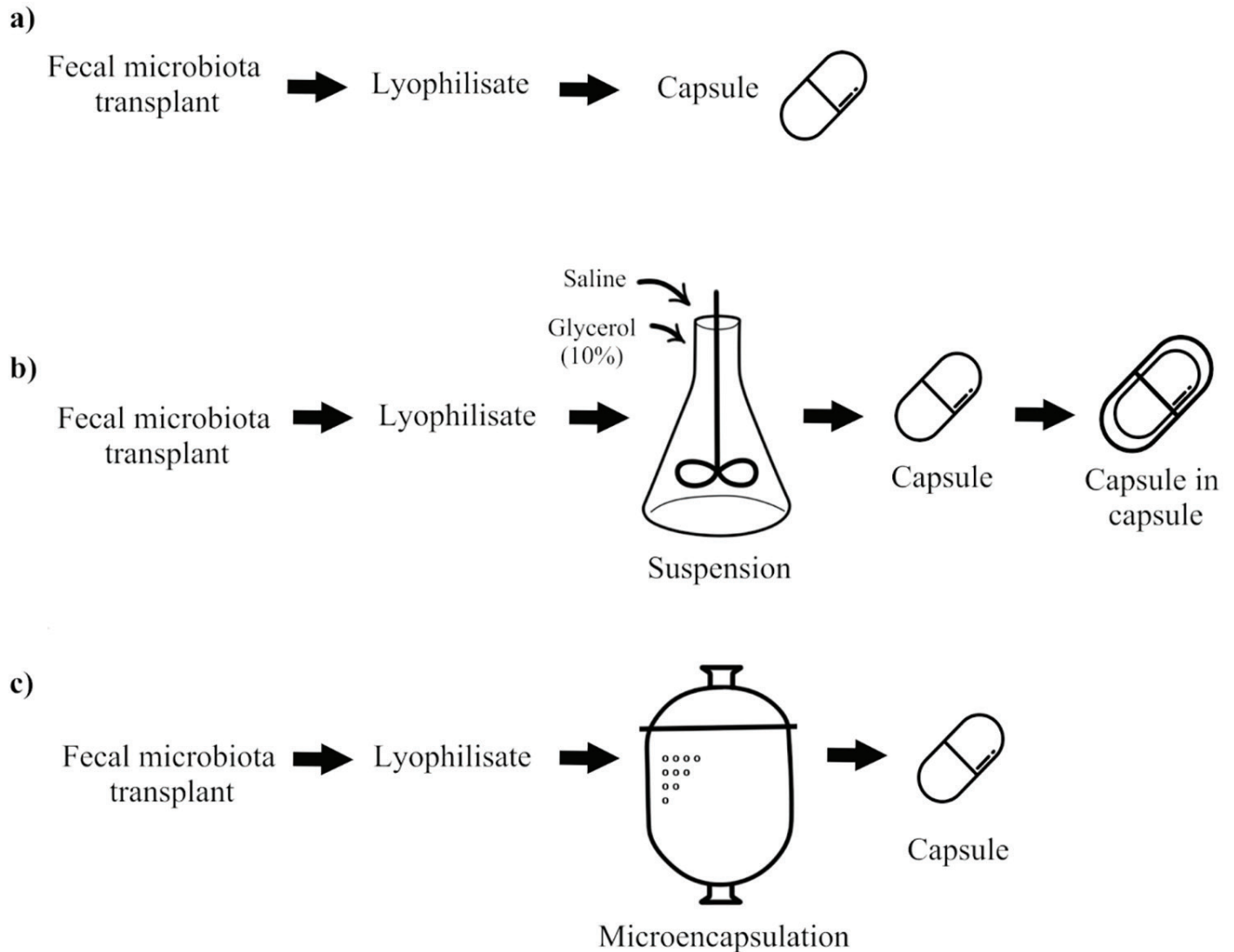


Figure 2. The schemes of possible transplant protection: (a) lyophilisate encapsulated into an enterosoluble capsule; (b) lyophilisate suspension being encapsulated into a double capsule: capsule in capsule; and (c) lyophilisate microencapsulation with the following encapsulation into a gelatin capsule.

improvement (Ward *et al.*, 2016). This study used oral vancomycin pretreatment during 7–10 days without stomach acid suppressants.

FMT administered through gastroscopy and colonoscopy resulted in 10.8% reduction of ASD symptoms (Zhao *et al.*, 2019).

In terms of treatment before FMT, there has been little research on the use of FMT in ASD to make a detailed analysis; however, similar studies on the treatment of CDI have shown that there is not enough clinical data on the use of pre-FMT bowel lavage, antibiotic, and antacid treatment (Kelly and Tebas, 2018).

Thus, FMT can significantly alleviate patients' conditions suffering from ASD, including children; for this reason, the technique of ASD therapy is being extensively studied recently. Capsules are a promising delivery method in ASD therapy due to the fact that they are easy to use and easy to store.

ENCAPSULATION METHODS FOR FMT

Canadian researchers, University of Alberta, compared standard colonoscopy with most promising oral capsules. They

aimed at developing a less unpleasant technique for patients and significantly improving patients' comfort (Kao *et al.*, 2017). In their study, the authors reported that oral administration can achieve comparable results with the existing rectal techniques, and the cost, at a rough estimate, was thrice as less for oral administration (Kao *et al.*, 2017).

A standard technique of FMT capsule preparation involves obtaining lyophilisate of fecal microbiota followed by its encapsulation into an enteric-coated capsule (Fig. 2a).

However, when preparing oral products, it may be a problem that a microbiota transplant will fail to reach the intestine intact; it is broken in the stomach. To solve such problems, protective mechanisms should be developed for transplants.

Previous studies on the FMT for the RAS treatment have used coadministration of stomach acid suppressants (Kang *et al.*, 2017, 2019). They are also used in the treatment of CDI by the introduction of FMT through the upper GI (Bakken *et al.*, 2011; Kelly and Tebas, 2018). Thus, the use of stomach acid

suppressants is one way to protect the fecal microbiota from premature destruction in the stomach environment.

Youngster *et al.* (2016) suggested another interesting protective technique: a donor transplant of lyophilized microbiota was mixed and suspended as concentrated material in sterile physiological saline with 10% glycerol, being treated under normal conditions and under environmental air. The suspension was twice encapsulated into hypromellose capsules. These capsules were stored at a temperature of -80°C for 6 months pending further usage (Fig. 2b). Previous studies by Youngster *et al.* (2014a, 2014b), as well as some other authors (Jiang *et al.*, 2017; Youngster *et al.*, 2016), reported the efficacy and safety of oral dosage forms that contained frozen encapsulated fecal microbiota from nonrelative donors in CDI treatment, the total positive effect frequency being 91% cases, determined as the absence of relapsing diarrhea 8 weeks after 30 capsules administered once or twice (Youngster *et al.*, 2016).

Microencapsulation could be one of the protection mechanisms (Fig. 2c). Microencapsulation is used rather commonly for probiotics and is widely recognized as a kind of protection (Petukhova and Krynicka, 2014). Microencapsulation using gastroresistant material can be applied to accelerate and enhance the positive effect. The microencapsulation concept enables us to separate the main functional ingredient from the environment using a protective covering. The major task of encapsulation technology in pharmacy is a controlled and continuous delivery of cells to the intestine preserving their viability when coming through an acidic gastric medium (Krolevets *et al.*, 2013; Petukhova and Krynicka, 2014).

It is our opinion that additive agents and microencapsulation technologies used for probiotic production can be of help for microbiota transplant microencapsulation.

Biomaterials used for probiotic encapsulation contain natural and synthetic polymers. Due to the low pH in the gastric medium, these polymers should be acid-resistant to preserve microcapsule structure when coming to the large intestine. In addition, the materials should be biocompatible and easily disintegrate in the bowel. For that purpose, various biomaterials were developed, including alginate (Gombotz and Wee, 2012), Eudragit (Barros *et al.*, 2014), chitosan (Yeung *et al.*, 2016), and pectin (Dafe *et al.*, 2017), designed for encapsulation and effective delivery of probiotic microorganisms existing in the intestinal medium (Dong *et al.*, 2013). The biomaterial should be biocompatible and biodegradable since it is in direct contact with living cells. Alginate is the most common biomaterial used for probiotic encapsulation.

Alginate is often mixed with carob gum and starch to form capsules or gel balls (Ivleva and Kanarskaya, 2014). When mixing the polymers, certain interactions seem to occur. Proportions of each biomaterial before mixing are of primary importance.

Encapsulation technology requires a safe and nonaggressive attitude to cells. To capture and protect probiotics, the following techniques were used: spray drying, lyophilization, and fluid-bed drying (Bakhtin *et al.*, 2009; Gordienko, 2006; Nezhuta *et al.*, 2020). Using the mentioned techniques, probiotics were processed into a concentrated powder form that extends probiotic shelf life.

Spray drying has some disadvantages. For example, probiotic viability can be reduced under the action of high temperatures necessary to water evaporation. The air temperature at the entry is in the range of 100°C – 170°C , and air temperature at the output varies from 45°C to 105°C , in accordance with the literature data (Nazzaro *et al.*, 2012; Sitenkov, 2020). At such temperatures, cells are unlikely to preserve their probiotic activity. Probiotic activity is not the same as probiotic survival. Probiotic activity is the cell's resistance to the GIT medium and ability to adhere to the mucosal surfaces of the intestines. Therefore, the encapsulation technique should not reduce probiotic activity. The use of probiotics with a physical barrier against unfavorable conditions provokes great interest. For this purpose, different methods were introduced for further probiotic protection improvement. The techniques were designed for developing gel balls or capsules made of hydrocolloids by means of extrusion molding or emulsification (Nazzaro *et al.*, 2012). Hydrocolloids are water dispersions of biomaterials (natural or synthetic polymers). Li *et al.* (2016) used cellulose microgels (CMs) to encapsulate *Lactobacillus plantarum*. CMs having porous structures were shown to have better bacteria bearer capability. The resistance to acidic medium and survivability can be increased due to the conjugation of bacteria with alginate. In 2017, pectin-starch hydrogels were synthesized to encapsulate *L. plantarum* ATCC:13643 cells by Dafe *et al.* (2017).

Yeung *et al.* (2016) developed a microgel “nucleus membrane” containing an alginate nucleus and a chitosan membrane to encapsulate *Bifidobacterium longum*. It has been shown that viability and resistance of *B. longum* were significantly improved after encapsulation in aerobic storage and gastric fluid modeling. In 2015, protein–polysaccharide capsules were made for *B. adolescentis* by Varankovich *et al.* (2015). The encapsulated bacteria show significant resistance at 37°C on a gastric medium model in comparison with free bacteria. Alginate or iota-carrageenan-contained capsules were readily soluble in a modeled intestinal fluid and released 70%–79% bacterial cells within 3 hours. In addition, the use of the lyophilized capsules increased the number of the released living bacterial cells. Another research used alginate and chitosan for layer-by-layer bacteria coating for *Bifidobacterium breve* (Cook *et al.*, 2013). These multilayered alginate matrices enhanced *B. breve* viability in a low pH medium and delivered bacterial cells to the bowel, being released there subsequently. Moreover, chitosan-coated alginate microcapsules were developed for *Bifidobacterium animalis* subsp. *lactis* PBS075, *Lactocaseibacillus rhamnosus* PBS070, and *L. plantarum* PBS067 by D’Orazio *et al.* (2015). They revealed encapsulated probiotics to exhibit significantly high resistance on a gastric medium model and other unfavorable conditions.

It is essential to preserve the viability of microorganisms as a part of FMT; however, due to the fact that fecal microbiota contains a large number of different bacteria, it is difficult to determine which of them are of great importance in therapy and which have no effect or even reduce the key probiotic function. Despite the fact that FMT usage exhibited positive clinical consequences, currently, it is separated the activity of microorganisms forming a part of fecal microbiota depending on nosology and individual characteristics of patients.

CONCLUSION

Thus, the control of intestinal bacteria by means of FMT encapsulation can become an effective strategy for many diseases, including ASD in children. Future work should focus on investigating FMT action on the ASD symptoms and its dependence on the dosage form. The use of orally administered especially encapsulated FMT will improve access for patients and make the design of placebo-controlled trials more comfortable. A convenient dosage form is very important in the treatment of children with ASD. It is necessary to find optimal dosage form for FMT administration and to continue the study of capsule preparation techniques that can help to protect FMT microorganisms from the acidic medium of the stomach. In this review, it was suggested to obtain lyophilisate of fecal microbiota; after that, lyophilisate is protected using suspending or microencapsulation followed by encapsulating the prepared lyophilisate in an enterosoluble capsule. The present delivery technique of fecal microbiota is characterized by an extended storage life, and it is easy to use.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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AUTHORS' CONTRIBUTIONS

All the authors substantially contributed to the conception, compilation of data, checking, and approving the final version of the manuscript.

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