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EXPERIENCE OF THE FIRST BRAZILIAN FECAL MICROBIOTA TRANSPLANTATION CENTER IN TREATING RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Abstract – ***Introduction:*** *Clostridioides difficile* infection (CDI) is a major cause of nosocomial diarrhea related to the use of antimicrobials worldwide. Treatment of recurrent CDI is challenging in countries where fecal microbiota transplantation (FMT) is not widely available. Furthermore, data on the effectiveness and safety of FMT in emerging countries are scarce. Thus, this study aimed to describe the initial experience of the first fecal microbiota transplantation center in Brazil for the treatment of recurrent CDI using frozen samples.
Materials and Methods: FMT was performed via colonoscopy using frozen samples from a stool bank. Donors were screened according to international guidelines and national regulatory resolutions. CDI diagnosis was confirmed in all patients. FMT success was defined as cessation of diarrhea within eight weeks. *C. difficile* isolates were subjected to ribotyping and antimicrobial susceptibility testing.
Results: Over two years, ten patients with recurrent CDI underwent FMT. The median age was 68 years (range: 23-87 years), 70% were women, 60% had severe infection. Furthermore, a median of 3 previous CDI episodes (range: 1-4) was observed. The primary resolution with a single FMT was 80%, while the overall resolution after the second FMT was 90%. Failure of treatment was not related to CDI severity ($p = 0.273$), bowel preparation ($p = 0.345$), comorbidities ($p = 0.809$), or number of previous episodes ($p = 0.457$). No serious adverse events were described during the follow-up of 26.6 months (range: 26.6-38.2 months). Mild adverse events occurred in 54.5% of the cases, which was mainly abdominal discomfort on the first day after the procedure. In addition, toxigenic *C. difficile* isolates belonged to ribotypes 106, 014/020, 131, 076, and 037. All the isolates were susceptible to metronidazole and vancomycin.
Conclusions: FMT is a safe and effective treatment for recurrent CDI in this cohort of Brazilian patients. The implementation of a stool bank allowed for the proper application of all the requirements needed to perform FMT in the country.

Keywords: Fecal microbiota transplantation, *Clostridioides difficile* infection, Stool bank, Ribotypes.

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INTRODUCTION

The human gut microbiota is a complex community of microorganisms that inhabit the gastrointestinal tract and exert marked influences on host health. Throughout life, the gut microbiota is influenced by several environmental factors, such as lifestyle, geographic location, diet, and medication use (mainly antimicrobials). When a breakdown of the local balance occurs, the composition of the resulting microbiota is altered, and diversity is reduced[1]. Dysbiosis has been associated with the pathogenesis of intestinal and extra-intestinal disorders, such as irritable bowel syndrome, inflammatory bowel disease (IBD), peripheral insulin resistance, obesity, and neurological disorders[2]. However, the greatest causal relationship between dysbiosis and illness is observed in recurrent *Clostridioides difficile* infection (CDI)[3].

CDI is the most common cause of nosocomial infectious diarrhea worldwide and is associated with significant morbidity and mortality[4]. Unfortunately, the CDI incidence in Latin America is likely to be underestimated due to limited vigilance and awareness, as well as limited availability of diagnostic tools[5]. Recently, *C. difficile* ribotype 027 (RT027) strain was isolated for the first time in Brazil[6]. This ribotype has been responsible for the increasing prevalence, severity, and recurrence of CDI cases since 2000, with outbreaks in North America, Europe, and Asia[5]. The identification of RT027 and other binary toxin-positive strains in the country highlights the need to improve awareness, disseminate diagnostic tests, and facilitate access to therapeutic measures[7,8].

Recurrent CDI usually occurs in patients with antibiotic-induced dysbiosis and commonly affects hospitalized elderly people who have poor immune responses and microbiota imbalance due to recent use of antimicrobials[9]. The first CDI was treated with metronidazole and/or vancomycin, observing a success rate of approximately 80%[9]. Recurrence can be treated with fidaxomicin, which is not available in Brazil; vancomycin for 10 days, or prolonged tapered and pulse vancomycin regimen[9]. However, the success rate of antibiotics progressively decreases as new recurrences occur. In patients with multiple relapses, 60% have a new recurrence if the antibiotic therapy strategy is maintained[10]. This finding can be explained by the persistence of dysbiosis perpetuated by antibiotics associated with the non-recovery of the microbiota. Among possible treatments, fecal microbiota transplantation (FMT) appears to be an important option.

FMT involves the transfer of healthy microbiota through the gastrointestinal tract to repopulate the digestive tract and improve dysbiosis. FMT can reshape the intestinal microbiota, restore its protective function against *C. difficile*, and achieve therapeutic effects[11]. Unlike traditional antimicrobial treatment, FMT is highly effective in treating recurrent CDI, with an overall resolution of 90%[12,13]. Although it has few side effects, which are mainly mild and transient, FMT is well accepted by patients and can improve the quality of life[3]. Although FMT remains an experimental treatment, it is now recognized as a treatment option for CDI and is therefore recommended by medical societies for multiple recurrent CDI in patients who have failed to standard therapies[9,13].

Despite the advent of recurrent CDI cases, FMT is not yet a reality in the national clinical practice. Only few reports of fecal transplantation are available in Brazil. To date, only one study describing the experience of a small cohort of patients with recurrent CDI undergoing transplantation was published in 2015[14]. However, description of the donor selection criteria, standardization process, ribotype testing, and the number of previous CDI are not yet reported. Furthermore, no national study has used frozen fecal samples from stool banks. Therefore, this study aimed to describe our initial experience with FMT in the treatment of recurrent CDI using frozen stool samples to determine the CDI resolution rate, ribotypes involved, short- and long-term occurrence of adverse events, and factors involved in therapeutic success.

MATERIAL AND METHODS

Study Design

This prospective, open, and uncontrolled pilot study was conducted at the Instituto Alfa de Gastroenterologia, Hospital das Clínicas, Federal University of Minas Gerais (IAG-HC/UFMG), to evaluate the effectiveness of FMT in patients with recurrent CDI between September 2017 and March 2020. Demographic data, clinical and laboratory variables, previous exposure to medications, symptom duration, and number of bowel movements per day were assessed. Stool shape was classified ac-

cording to the Bristol scale, and the Charlson Comorbidity Index was used to characterize sample complexity[15,16]. This study was approved by the Federal University of Minas Gerais Ethics Committee.

Patient Population

Patients aged ≥ 18 years with recurrent CDI who agreed to participate after signing the informed consent form were considered eligible for inclusion. At enrolment, CDI was characterized by the presence of diarrhea, described as having more than three daily excrements with unformed stools (Bristol 6 or 7) for a minimum period of 48 h, and microbiological confirmation for *C. difficile*. Recurrent CDI was defined as the development of a new CDI within 8 weeks of a previous episode being treated properly, in which an initial resolution of symptoms was observed. Microbiological diagnosis was performed using the positive glutamate dehydrogenase (GDH) test (GDH ECO Teste - TR.0032; Eco Diagnóstica, Nova Lima, Minas Gerais, Brazil), followed by positive toxigenic culture and/or A/B toxin detection, as recommended by the Infectious Diseases Society of America, the Society for Healthcare Epidemiology of America, and the European Society of Clinical Microbiology and Infectious Diseases[9,17].

Stool samples were subjected to toxigenic culture and enzyme-linked immunosorbent assay (ELISA) for detecting A/B toxins (*C. difficile* Tox A/B II; Techlab Inc., Radford, Virginia, USA)[18]. Toxigenic *C. difficile* strains were also subjected to PCR ribotyping, as described by Janezic and Rupnik[19]. The minimal inhibitory concentrations (MIC) of metronidazole, vancomycin, clindamycin, moxifloxacin, ciprofloxacin, erythromycin, rifampicin, and tetracycline were determined using a gradient test with the M.I.C. Evaluator™ strips (M.I.C.E.™; Oxoid Limited, Basingstoke, Hampshire, UK) in Brucella agar (Oxoid Limited) with 5% lysed blood, supplemented with hemin (Difco Laboratories Inc., Detroit, Michigan, USA) and vitamin K (Sigma-Aldrich Co., Saint Louis, Missouri, USA). The MIC values were interpreted according to clinical breakpoints from the CLSI and EUCAST guidelines[20,21].

The exclusion criteria were as follows: patients without laboratory confirmation, pregnancy, patients under 18 years old, clinically ill with a life expectancy of less than three months, septic shock with hemodynamic instability, need for vasoactive drugs, and unable to sign the informed consent form. Severe CDI was defined by the presence of one of the following criteria: leukocytosis with a white blood cell count ≥ 15000 cells/mL or a serum creatinine level > 1.5 mg/dL in the context of acute renal failure. Fulminant CDI is an infection that evolves with toxic megacolon, hemodynamic instability, ileus, or the need for surgical treatment. Mild-to-moderate CDI was defined as the presence of diarrhea and absence of criteria that characterize a severe or fulminant condition.

Donor Stool Preparation and Fmt

The source of the donor stool was frozen samples obtained from our stool bank at the FMT Center of IAG-HC/UFMG. Donor selection, fecal sample preparation, storage, defrosting, and pre- and post-procedure care were the same as those described in our previous report and were carried out in accordance with international guidelines on fecal microbiota transplantation and Brazilian epidemiological specificity[22].

All patients underwent FMT via colonoscopy, after bowel lavage with polyethylene glycol (PEG) solution and 10–14 days of oral vancomycin regimen, as previously described[22]. The quality of the intestinal preparation was assessed using the Boston Bowel Preparation Scale[23]. A score between 0 and 3 was considered as inadequate, between 4 and 5 considered as regular, and between 6 and 9 considered as excellent/good. A single researcher performed all colonoscopies.

Outcomes and Follow-Up

Following FMT, all patients were monitored daily via phone call with the approach to symptoms, occurrence of adverse events, and assessment of diarrhea resolution. If serious side effects or persistent complaints were detected, patients were personally assessed by the re-

searcher. After the first week, follow-up was done within eight weeks, three months, six months, and annually to assess the presence of diarrhea, use of antibiotics, hospitalization, development of new disease or complaint, and recurrence of CDI. The stool GDH test was performed whenever diarrhea occurred. If positive, subsequent toxigenic culture was performed. Participants were instructed to contact the researcher on suspicion of recurrence of CDI or in the presence of any complaints or adverse events.

Adverse events were defined as any undesired occurrence after FMT without the need for an exact causal relationship. Symptoms, disease onset, and laboratory findings were also recorded. Adverse events were classified according to severity: mild events (mild symptoms such as abdominal discomfort, diarrhea, constipation, flatulence, abdominal bloating, nausea, vomiting, and fever with spontaneous resolution) or major events (perforation, bleeding, bronchoaspiration, transmission of pathogens, exacerbation of inflammatory bowel disease, occurrence of infection, need for hospitalization, temporary or permanent functional disability, or death). Furthermore, adverse events were classified regarding the time of occurrence, as follows: short term (within one month after FMT), medium term (between one month and one year), and long term (after one year). Causality was classified as definitely related (presence of a reasonable temporal sequence, with an expected response pattern and not explained by another hypothesis), probably related (presence of a reasonable time sequence, with an expected response pattern and unlikely to be explained by the patients' characteristics or other interventions), possibly related (despite the temporal relationship, it may be caused by factors other than transplantation), and unrelated (event that is certainly unrelated to treatment).

CDI resolution rate was defined as the disappearance of diarrhea related to CDI or persistent diarrhea explicable by other causes with negative GDH and culture toxigenic at the end of eight weeks of treatment. The CDI resolution rate can be primary if achieved with a single infusion, or overall if new procedures are needed. FMT failure was defined as the recurrence of CDI within eight weeks after fecal infusion, characterized by more than three daily bowel movements with unformed stools (Bristol 6 or 7) for more than 48 h, and was associated with laboratory confirmation by positive GDH and toxigenic culture. These patients were offered a new FMT with feces from another donor.

Statistical Analysis

Statistical analyses were performed using SPSS (IBM Corp. Released 2013; IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA). In the descriptive analysis, categorical variables are presented as frequencies and proportions. Numerical variables are presented as means and standard deviations or as medians and ranges when the distribution was not Gaussian. To compare procedures that provided clinical remission with those that did not, we used Fisher's exact tests for categorical data and Mann-Whitney tests for continuous data. Statistical significance was defined as $p < 0.05$.

RESULTS

Between September 2017 and March 2020, 91 candidates from 17 Brazilian states and the federal district sought the FMT Center of IAG-HC/UFMG to assess their eligibility for transplantation (Figure 1). Among these patients, 77 were excluded because they did not have CDI recurrence. The majority had chronic diarrhea (with no evidence of CDI), irritable bowel syndrome, or IBD. Patients with autism spectrum disorder, graft-versus-host disease (GVHD) after bone marrow transplantation (BMT), bullous pemphigus, depression, anxiety, celiac disease, ankylosing spondylitis, small intestinal bacterial overgrowth, or food intolerance were also excluded. Ten patients received a presumptive diagnosis of recurrent CDI; however, there was no laboratory confirmation. Four patients with recurrent CDI were contraindicated for FMT due to refusal, nonage, hemodynamic instability due to septic shock by pulmonary focus, and palliative support in the frail elderly. Of the 91 patients evaluated, only 10 were eligible for FMT.

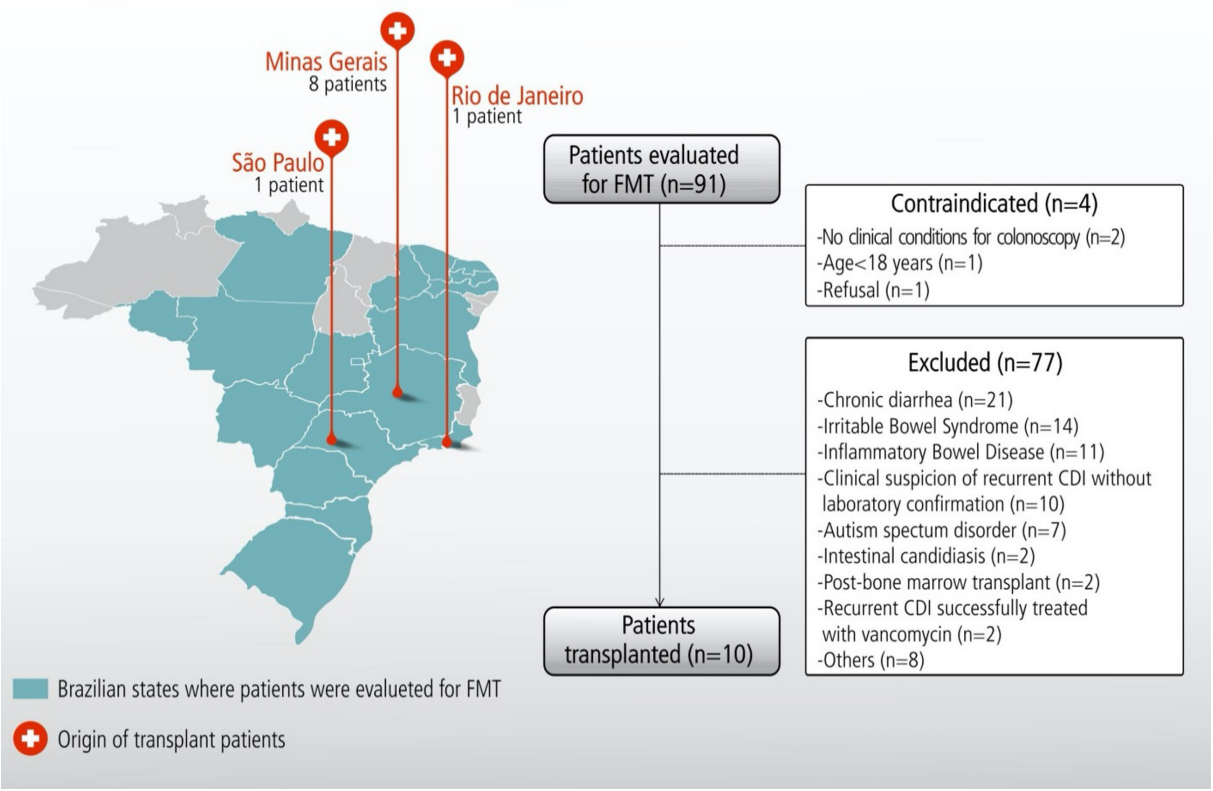

Figure 1. Evaluation of patients for fecal microbiota transplantation in Fecal Microbiota Transplant Center of IAG-HC/UFMG, Brazil, between September 2017 and March 2020.

The demographic data, comorbidities, risk factors, and clinical characteristics of the transplant patients are summarized in Table 1. Of the 10 patients included in the study, one lives in São Paulo, one in Rio de Janeiro and eight in Minas Gerais. The majority were women (70%, 7/10). The median age was 68 years (range, 23-87 years), and the median Charlson comorbidity rate was 3 (range, 0–6). All patients had a history of recent antibiotic use, of which 60% were chronic users of proton pump inhibitors, half had been hospitalized before the first CDI, and 40% had a history of malignancy. Two patients underwent immunosuppressive therapy. The first patient was a 34-year-old man who was treated with tacrolimus 3 mg/day and dasatinib 100 mg/day for graft-versus-host disease of the liver, mouth, and skin after BMT. The second patient was a 23-year-old man who was treated with azathioprine (100 mg/day), prednisone (5 mg/day), and ustequinumab (90 mg) for fistulizing Crohn's disease and overlapping syndrome with autoimmune hepatitis and primary sclerosing cholangitis.

Six of the 10 patients (60%) had severe CDI. All transplants were indicated for recurrent CDI. None of the patients had refractory CDI. The median number of recurrent CDI episodes was 3 (range: 1–4). The median stool frequency was 9 (range: 5–17) bowel movements per day, and stool consistency was classified as Bristol 6 and 7. The median time between the first CDI and FMT was 99 days (range: 51–212 days). Eighty percent were nutritionally at-risk adults, with a median involuntary loss of 10% (range 2–20%) of usual body weight within six months.

All patients had been previously treated with vancomycin. Eight patients also received metronidazole and only one received additional fidaxomicin therapy. Toxigenic *C. difficile* isolates were recovered from five patients and the following ribotypes were identified:106, 014/020, RT131, 076, and 037. Only one isolate, ribotype 131, was positive for the binary toxin encoding gene (*cdtB*). All isolates were susceptible to metronidazole, vancomycin, moxifloxacin, and rifampicin, while one strain (ribotype 131) was resistant to tetracycline and erythromycin.

TABLE 1. CHARACTERISTICS OF TEN PATIENTS UNDERGOING FMT IN THE FECAL MICROBIOTA TRANSPLANT CENTER OF IAG-HC/UFMG, BRAZIL, BETWEEN SEPTEMBER 2017 AND MARCH 2020.

Variables	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Age (year)/sex	87y Fem	76y Fem	68y Fem	68y Fem	76y Fem	43y Male	62y Fem	23y Male	75y Male	79y Fem
Comorbidities	Recurrent UTI, SAH, osteoporosis, PE and previous breast cancer	SAH, ITP, asthma, rectum cancer and previous non-Hodgkin's lymphoma	SAH, DLP, glaucoma, SIBO, colonic diverticulosis	SAH, DM, PAD with previous angioplasty, diabetic foot	Hypothyroidism, recurrent UTI, irritable bowel syndrome, functional dyspepsia, depression	Liver, mouth and eye GvHD, after bone marrow, previous BOOP, Cataract	SAH, dyslipidemia, CKD, recurrent UTI, amiodarone pneumonitis, coronary artery disease	Fistulizing Crohn's disease, autoimmune hepatitis and primary sclerosing cholangitis	DM, SAH, dialysis CKD, obstructive sleep apnea, overweight	Asthma, Alzheimer's disease, Parkinson's disease, SAH, epilepsy, GERD, previous breast cancer
CDI recurrence	3rd	2nd	1st	2nd	2nd	3rd	4th	3rd	3rd	2nd
Severity of ICD	Severe	Severe	Severe	Severe	Severe	Severe	Mild to moderate	Mild to moderate	Mild to moderate	Mild to moderate
Weight loss (%)	3%	15%	6%	10%	12%	20%	11%	6%	2%	8%
Previous treatment for CDI	Metronidazole Vancomycin Fidaxomicin	Metronidazole Vancomycin	Metronidazole Vancomycin	Metronidazole Vancomycin	Metronidazole Vancomycin	Metronidazole Vancomycin	Vancomycin	Vancomycin	Metronidazole Vancomycin	Metronidazole Vancomycin
Disease duration until FMT (days)	101 days	86 days	58 days	92 days	54 days	173 days	209 days	146 days	86 days	97 days
Toxin A/B	+	+	+	+	+	+	-	+	-	-
GDH	+	+	+	+	+	+	+	+	+	+
NAAT		+			+					+
Toxigenic culture	+		+	+			+		+	
CDI r resolution	No	1st FMT	1st FMT	1st FMT	1st FMT	2nd FMT	1st FMT	1st FMT	1st FMT	1st FMT
Follow-up after FMT (months)	25.2 m	23.1 m	22.9 m	18.7 m	18.3 m	14.5 m	3.8 m	3.7 m	2.5 m	2.3 m

BOOP - bronchiolitis obliterans organizing pneumonia; CDI r - C. difficile infection recurrence CKD - chronic kidney disease; DLP - DM - diabetes mellitus; DVT - deep vein thrombosis; FMT - fecal microbiota transplant; GDH - glutamate dehydrogenase; GvHD - Graft versus host disease; ITP - Idiopathic thrombocytopenic purpura; NAAT - nucleic acid amplification tests; PAD - peripheral artery disease; PE - pulmonary embolism; Pt - patient; SAH - systemic arterial hypertension; SIBO - small intestinal bacterial overgrowth; Toxin. - Toxigenic; UTI - urinary tract infection

Eleven transplants were performed in ten patients. Two patients underwent intestinal preparation at the hospital. The other procedures were performed at home. The sources of all stool samples were unrelated donors. The median sample storage time was 39 days (range: 1–147 days), the time between sample collection and storage was 3 h 42 min (range: 2 h 24 min–29 h), and the duration was 16 min (range: 10–25 min). Intestinal preparation was considered excellent or good in 81.8% (9/11) and regular in 18.2% (2/11) of the patients. The median fecal volume was 295 mL (range: 250 – 300 mL). All patients were discharged on the day of FMT. Despite the use of loperamide before the procedure, a portion of the infused fecal substrate was eliminated in all patients during the first 8 h of follow-up.

Nine of the 10 patients (90%) treated with FMT exhibited resolution of CDI. Primary CDI resolution with a single FMT was observed in eight patients, and overall CDI resolution after the second FMT was observed in nine patients. Two patients did not respond to the first treatment. The median CDI recurrence was 9.5 days. One patient experienced recurrence on the seventh day post-FMT, and an assistant doctor opted for a taper and pulse vancomycin regimen. The other patient experienced recurrence 12 days after transplantation and received a new course of oral vancomycin (125 mg) four times a day for 10 days and a new FMT.

Comparing the procedures that provided clinical remission with those that did not, no difference in relation to the donor employed ($p = 0.164$), quality of intestinal preparation ($p = 0.345$), CDI severity ($p = 0.273$), presence of comorbidities ($p = 0.809$), fecal volume used ($p = 0.618$), sample preparation time ($p = 0.478$), and storage time ($p = 0.814$) were observed. Similarly, no difference was observed in the occurrence of adverse events between successful and unsuccessful transplants. The characteristics of the two groups are summarized in Table 2.

The median follow-up time after transplantation was 432 days (range: 70–782 days). No major adverse events occurred during the study. Mild adverse events were observed in 54.5% of procedures. Most events are probably related to FMT, with a short duration and spontaneous resolution without the need for hospitalization. The two immunosuppressed patients did not experience any infectious adverse events. The details of each adverse event, occurrence, and causality are presented in Table 3. Regarding patient acceptance, 9 out of 10 stated that they would undergo a new FMT if necessary.

The only patient who did not respond to treatment was an 87-year-old woman with a history of breast cancer and recurrent urinary tract infection, who underwent FMT after her fourth CDI episode. After therapeutic failure with FMT, a successful tapering and pulse vancomycin regimen was chosen. After one year and nine months of follow-up, the patient developed a urinary infection caused by *Escherichia coli* and was treated with amoxicillin/clavulanic acid. In less than a month, she developed a new episode of CDI, which was treated with vancomycin.

DISCUSSION

FMT plays a well-established role in the treatment of recurrent CDI, mainly for second or subsequent recurrence[3,9,13]. However, the development of a protocol that can provide adequate treatment is mandatory. In this scenario, a transplant center with a stool bank at our institution is a significant factor[22]. A frozen stool bank allows quick access to FMT, eliminates logistical barriers related to fresh stools, and adds to security by allowing traceability and monitoring of adverse events[13]. In addition, fecal samples can be stored at –80 °C for a long period without compromising safety or therapeutic response[24,25]. Randomized clinical trials have shown that the use of frozen fecal suspensions is as effective as fresh suspensions for the treatment of CDI[12,13,25].

In our study, all transplant patients had a confirmed diagnosis of recurrent CDI. However, a significant number of patients who sought our service did not have laboratory confirmation of CDI. Despite the suggestive clinical presentation and presumptive diagnosis, these patients started empirical antimicrobial treatment at their reference hospital without laboratory assurance. This practice shows the scarcity and unavailability of diagnostic methods in certain Brazilian regions. The implementation of an FMT center is remarkable; however, more actions are needed to improve epidemiological analyses and diagnostic measures in the country.

TABLE 2. FACTORS AFFECTING FMT THAT PROVIDED CLINICAL REMISSION AND THOSE WITH THERAPEUTIC FAILURE.

Variables		Failed FMT		Successful FMT		*p*-value
		n (%)	median	n (%)	median	
Age in years, median			60.5		68	0.722
Female sex		1 (50)		6 (66.7)		0.618
Charlson comorbidity index, median			3.0		3.0	0.809
Previous neoplasia		2 (100)		3 (33.3)		0.182
WBC count (10³/dL), median			7.9		8.6	0.478
Creatinine (mg/dL), median			0.8		0.9	0.408
Hospitalization before acquiring CDI		0 (0)		5 (55.6)		0.273
PPI usage		1 (50)		5 (55.6)		0.727
Percentage of body weight loss, median			10		10	0.906
Recurrences of CDI, median			3.6		3	0.457
Positive toxin A/B test		2 (100)		5 (55.6)		0.467
Severity of CDI	Mild/Moderate	0 (0)		5 (55.6)		0.273
	Severe	2 (100)		4 (44.4)		
CDI prior therapy	Van	0 (0)		2 (22.2)		0.200
	Met +Van	1 (50)		7 (77.8)		
	Met + Van + Fid	1 (50)		0 (0)		
Stool donor	Donor 1	1 (50)		0 (0)		0.164
	Donor 2	0 (0)		1 (11.1)		
	Donor 3	0 (0)		5 (55.6)		
	Donor 4	1 (50)		3 (33.3)		
Intestinal preparation	Excellent/good	1 (50)		8 (88.9)		0.345
	Regular	1 (50)		1 (11.1)		
Sample storage time in days, median		70		39	0.814	
Time between sample collection and storage (h), median8		16		3.7	0.47	
Colonoscopy duration in minutes, median			17		16	0.812
Infused volume in mL, median			292.5		295	0.618
Follow-up time in days, median			591.5		426	0.346
Presence of adverse events		1 (50)		5 (55.6)		0.727

FMT, fecal microbiota transplantation; WBC, white blood cell; PPI, proton pump inhibitors; CDI, C. difficile infection; Van, vancomycin; Met, metronidazole; Fid; fidaxomycin; (h), in hours.

TABLE 3. OCCURRENCE OF EARLY ADVERSE EVENTS PER FMT.

FMT	Adverse Events	Causality	Follow-up day
1	None	–	–
2	Hyporexia and bloating	Probably	day 2
3	None	–	–
4	Dehydration	Probably	day 1
5	Abdominal cramps; bloating	Probably	day 1 to 7; day 1
6	Abdominal discomfort; bloating	Probably	day 1 to 2; day 2 to 3
7	Abdominal pain	Probably	day 1
8	None	–	–
9	Fever, abdominal pain and nausea; diarrhea	Probably	day 1; day 1 to 7
10	None	–	–
11	None	–	–

FMT, fecal microbiota transplantation

Among the patients with recurrent CDI, two were immunosuppressed. One had IBD, and the other had GVHD after BMT. CDI is common in patients with IBD and is associated with an increased risk of colectomy and mortality[26]. Similarly, post-BMT individuals are predisposed to dysbiosis, with a nine times greater risk of developing CDI than other hospitalized patients[27]. In this context, FMT appears to be safe and effective even in immunosuppressed patients[28-30].

The elevated treatment acceptance found in this study (90%) was similar to that described in the literature[31]. This may be due to the favorable judgment between benefits and risks of treatment in the face of a debilitating disease with a great impact on patients' quality of life. Several factors, such as a high level of education and family support, are crucial for better acceptance[32].

No administration route has been proven to be more effective than the other routes. Meta-analysis showed a tendency towards higher efficacy rates in lower gastrointestinal administration compared to upper gastrointestinal administration, but the results were not statistically significant[12,25]. Although colonoscopy is more invasive and may be inappropriate for critically ill patients, it is associated with higher cure rates (78% with single infusion vs. 98% with multiple infusions)[25]. In addition, colonoscopy allows the infusion of a larger amount of fecal substrate and identification of some risk factors for failure, such as pseudomembranous colitis or inadequate bowel preparation.

To the best of our knowledge, this is the first complete report of a Brazilian cohort treated with FMT using colonoscopy with frozen samples. FMT achieved 90% overall CDI resolution, and these findings are consistent with those reported in the literature[3,12,13]. To date, only one Brazilian study has been reported[14]. Ganc et al[14] published a successful experience with FMT by infusing fresh samples via enteroscopy in 12 patients with recurrent CDI. Despite the growing number of CDI cases in Latin America, only few reports of FMT are available, wherein most are pilot studies with a small number of patients and without a transplant center with a stool bank[33,34].

In the present study, most patients achieved clinical remission after their first transplant. Some studies have indicated factors related to patients and procedures that may predict an inadequate response and the need for new procedures. Factors, such as severe CDI and FMT in hospitalized patients predict the need for second treatment[35]. Other predictors include surgery before FMT, female sex, low stool volume, pseudomembranous colitis, concomitant use of other antibiotics, and previous hospitalization[3,36]. Ianiro et al[36] evaluated 64 patients with recurrent CDI who underwent FMT using colonoscopy. Most were female, with an average age of 74 years; 40% had severe CDI, and 59% were hospitalized. The remission rate with only one infusion was 69%. Severe CDI and inadequate bowel preparation were considered as predictors of failure after a single infusion. However, these findings were not confirmed in the present study. It is possible that the small sample size was insufficient to assess predictors of therapeutic failure.

In addition, our study reported an incidence of adverse events of 54.5%. All adverse events observed were mild, early, and self-limiting. Regarding causality, the reported symptoms were likely related to FMT. No deaths, hospitalizations, or development of new diseases occurred during the follow-up period. However, it is not possible to attribute the occurrence of mild adverse events only to microbiota transplant, since the complaints were also observed after bowel preparation and colonoscopy.

In a systematic review, Wang et al[37] assessed the incidence of adverse events in 1,089 patients undergoing FMT. Among the patients, 831 were treated for refractory or recurrent CDI. The overall incidence of adverse events in the CDI group was 28.0%. In addition, the incidence of serious adverse events was 2.0% in the upper gastrointestinal tract and 6.1% in the lower gastrointestinal tract. The most common symptoms were abdominal discomfort, bloating, diarrhea, nausea, constipation, and transient fever. However, the actual incidence of adverse events may have been underestimated because transient or mild adverse events can be ignored by researchers. Meanwhile, two pioneering studies on FMT have reported a higher incidence rate of adverse events. Van Nood et al[38] reported an incidence rate of 93.1% (27/29). FMT was performed using a nasoduodenal probe, and the main reported symptoms were belching, nausea, abdominal cramps, diarrhea, abdominal pain, infection, and dizziness combined with diarrhea. In a randomized clinical trial of FMT using colonoscopy, Cammarota et al[39] reported an incidence of 94% (19/20). The main symptoms were diarrhea, bloating, and abdominal cramps that disappeared within 12 h. Serious adverse events were not observed.

Few studies have been conducted on circulating *C. difficile* ribotypes in Brazil. In the present study, ribotype 106, which is commonly reported worldwide and in previous Brazilian studies, was isolated from a patient who did not respond to FMT[40,41]. Ribotype 106 is known to produce more spores compared to other ribotypes, which justifies its potential for recurrence, increased geographic distribution, and a favorable antibiogram profile[40]. Interestingly, the so-called hypervirulent strains, including ribotypes 027 and 078, were not detected in the present study, similar to most studies in Brazil[5,41]. Notably, all strains isolated in the present study were susceptible to metronidazole and vancomycin, which are the main antimicrobials used in the treatment of CDI. This finding agrees with those of previous studies in Brazil, which suggested that the frequencies of resistance to these antimicrobials are low[42]. In contrast, one strain (ribotype 131) was resistant to macrolides and tetracyclines, which appears to be a frequent resistance pattern in *C. difficile* isolates from humans and animals[42]. Interestingly, after FMT, this strain was no longer isolated from the patient, indicating that FMT can also be a tool against antimicrobial resistance[43].

The main limitation of this study was its small sample size. However, this was a pilot study that implemented a new methodology in our institution and allowed access to a treatment unavailable until then. The clinical response and adverse events found in this study were similar to those described in other studies. Another advantage of our study is the methodological rigor, mainly in the selection of donors and patients. All patients had a confirmed diagnosis, and results were not influenced by bias in the selection of asymptomatic *C. difficile* carriers or those with diarrhea due to etiologies other than CDI.

CONCLUSIONS

In our small cohort, FMT was effective in the treatment of recurrent CDI. The primary resolution rate with a single FMT was 80%, and the overall resolution after the second FMT was 90%, even in patients with severe CDI and multiple comorbidities. In addition, the occurrence of adverse events was similar to those observed in other studies, with no serious adverse events or transmission of infectious diseases. FMT also appears to be safe in immunosuppressed patients. Even in emerging countries, where there are concerns about tropical and infectious diseases, FMT may be a good treatment strategy for recurrent CDI. Further prospective studies with larger numbers of participants are needed to conclusively determine its efficacy and safety in the Brazilian population.

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Conflict of interest

The authors declare no conflict of interest.

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