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ORIGINAL ARTICLE OPEN ACCESS

Impact of Clinical and Pharmacological Parameters on Faecal Microbiota Transplantation Outcome in *Clostridioides difficile* Infections: Results of a 5-Year French National Survey

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ABSTRACT

Background: Detailed comparative assessment of procedure-related factors associated with faecal microbiota transplantation (FMT) efficacy in *Clostridioides difficile* infection (CDI) is limited.

Aims: We took advantage of the differences in procedures at the various French FMT centres to determine clinical and procedure-related factors associated with FMT success in CDI.

Methods: We performed a nationwide retrospective multicentre cohort study. All FMTs performed within The French Faecal Transplant Group for CDI from 2018 to 2022 were included. Clinical data were collected retrospectively from recipient medical files, characteristics of stool transplant preparations were prospectively collected by each Pharmacy involved. Univariate and multivariate analyses were performed using Fisher's test and multiple logistic regression.

Results: Six hundred fifty-eight FMTs were performed for 617 patients in 17 centres. The overall efficacy of FMT was 84.3% (520/617), with 0.5% of severe adverse events possibly related to FMT (3/658). Forty-seven patients were treated at the first recurrence of CDI with a similar success rate (85.1%). Severe chronic kidney disease (CKD; OR: 2.18, 95%CI [1.20–3.88]), non-severe refractory CDI (OR: 15.35, [1.94–318.2]), the use of $\geq 80\%$ glycerol (OR: 2.52, [1.11–5.67]), insufficient bowel cleansing (OR: 5.47, [1.57–20.03]) and partial FMT retention (OR: 9.97, [2.62–48.49]) were associated with CDI recurrence within 8 weeks.

Conclusions: Conditions of transplant manufacturing, bowel cleansing, and a route of delivery tailored to the patient's characteristics are key factors in optimising FMT efficacy. FMT at first recurrence showed high success in real-life practice, whereas it had lower efficacy in severe CDI and non-severe refractory CDI.

1 | Introduction

Faecal microbiota transplantation (FMT) is a standard of care for multiple recurrent *Clostridioides difficile* infections (rCDI) [1, 2]. It consists of the transfer within the patient's gastrointestinal

tract of preparation from the stools of a healthy donor, following a minimum of 4 days of *C. difficile*-targeting antibiotics to prevent further CDI recurrence. With a reported efficacy ranging from 69% to 90%, it is the most efficient treatment available for multiple rCDI [3–5]. Moreover, FMT has a good safety profile,

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with less than 2% of serious adverse events reported [6]. FMT has also been described as a therapeutic option in severe or complicated CDI, non-severe refractory CDI, or, ultimately, after a first or second episode of CDI [7, 8]. Despite its high efficacy rate, particularly in comparison with the recommended regimen using antibiotics, some patients do not respond to FMT. Predictors of failure after FMT are not consensual and mainly rely on single-centre cohorts [3, 9]. The development of FMT was implemented in various ways over the past decades with different organisations in different countries, from centralised national FMT centres to private stool banks. International guidelines have been published to describe stool preparation, donor selection, and FMT procedures to standardise these procedures [10–13]. However, the level of evidence regarding the proposed procedures is limited, and their impact on FMT efficacy remains poorly evaluated, especially the interest in parameters such as the condition of thawing, the addition of glycerol, or the use of different anti-CDI antibiotics prior to FMT [14]. Yet in France, FMT has been performed in different regional centres for about 10 years under the coordination of the French Faecal Transplant Group (GFTF), a national non-profit organisation gathering all French FMT experts. This organisation has allowed the development of distinct processes of stool preparations from one centre to another while still complying with the recommended framework of FMT good practice guidelines [12]. We report here on the last 5 years (2018–2022) experience of the GFTF, taking advantage of the differences in terms of procedures and preparation between the various centres to assess in detail the clinical and technical parameters associated with FMT failure.

2 | Materials and Methods

2.1 | Definitions

Multiple recurrent CDI, severe, severe-complicated, or refractory CDI, was defined according to the ESCMID criteria issued in 2021 [1]. Multiple recurrent CDI was defined as ≥ 2 recurrences of CDI, with one recurrence defined as a CDI occurring within 8 weeks after a previous episode, provided the symptoms from the previous episode resolved after completion of initial treatment. Severe CDI was characterised by one of the following factors at presentation: fever, i.e., core body temperature $> 38.5^{\circ}\text{C}$, marked leukocytosis, i.e., leukocyte count $> 15 \times 10^9/\text{L}$, and rise in serum creatinine, that is, $> 50\%$ above the baseline. Severe complicated CDI was defined by the presence of one of the following factors that need to be attributed to CDI: hypotension, septic shock, elevated serum lactate, ileus, toxic megacolon, bowel perforation, or any fulminant course of disease (i.e., rapid deterioration of the patient). Refractory CDI is CDI not responding to recommended CDI antibiotic treatment, i.e., no response after 3–5 days of therapy. Refractory CDI can be part of either non-complicated or complicated CDI [1]. FMT success was defined by the absence of recurrence of CDI within the 8 weeks following FMT. The recurrence of CDI was defined by positive stool testing for *C. difficile* combined with the decision to treat it with anti-CDI antibiotics. Recurrence of CDI or persistence of refractory CDI was also considered as FMT failure, in addition to death or loss to follow-up at 8 weeks. Immunodeficiency was defined by the use of immunosuppressive therapy, corticosteroids $> 20\text{mg/day}$, chemotherapy, HIV with a CD4 count $< 200/$

mm^3 . Severe chronic kidney disease (CKD) was defined by a glomerular filtration rate $< 30\text{mL min}^{-1}$ using MDRD calculation or chronic dialysis.

2.2 | Data Collection

All centres involved in the GFTF that performed FMT for CDI have been proposed to participate in the ongoing study. All FMT performed in France, within the GFTF framework, from the 1st of January 2018 to the 31st of December 2022 were included. Clinical data were collected retrospectively from recipient medical files using a centralised anonymous online form. Pharmaceutical procedures and characteristics of stool transplant preparations were collected from each centre's pharmacy database. These databases were systematically and prospectively completed for each patient to ensure the traceability and safety of transplant preparations in line with good manufacturing practice guidelines.

2.3 | Ethics

The study protocol was approved by a local Ethical Committee (DC 2014/148-bis). All patients have given written informed consent for the realisation of FMT and the use of their clinical data.

2.4 | Statistical Analysis

To identify the parameters associated with FMT failure at 8 weeks, univariate and multivariate analyses were performed. The univariate analysis was performed using Fisher's test for binary variables using R 4.2.0 (R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL) and logistic regression for continuous variables. The multivariate analysis was performed using multiple logistic regression using GraphPad Prism software v10.1.1 for MacOs (GraphPad Software, Boston, Massachusetts USA, www.graphpad.com). In the multivariate analysis, two models were generated using parameters that showed a statistically significant association with FMT outcomes in the univariate analysis. In the multivariate analysis, multiple recurrent CDI, no glycerol and thawing at room temperature were set as a reference for the category 'indication', 'glycerol', and 'thawing method', respectively.

3 | Results

3.1 | Population Characteristics and FMT Procedures

During the study period, 658 FMTs were performed for CDI in France (GFTF framework), involving 617 patients. Over the same period, the number of centres preparing FMT transplants has decreased from 17 in 2018 to 8 in 2022, with about 70% of all FMTs prepared in 3 centres (Table 1). Characteristics of the population are described in Table 1. Eight patients (1.2%) and 30 patients (4.6%) were, respectively, dead or lost to follow-up at the primary endpoint (8 weeks after FMT). Multiple rCDI

TABLE 1 | Population characteristics.

Population characteristics	N = 658 FMT
Patient age (years)—Mean (SD)	66.4 (17.7)
Male—n (%)	248 (37.7)
FMT centres—n (%)	
Saint-Antoine Hospital (Paris)	223 (33.9)
Marseille	173 (26.3)
Lyon	62 (9.4)
Clermont-Ferrand	58 (8.8)
Toulouse	50 (7.6)
Nantes	33 (5.0)
Pitié- Salpêtrière Hospital (Paris)	28 (4.3)
Others	31 (4.7)
Patient comorbidities	
Immunodeficiency	142 (21.6)
Severe chronic kidney failure*	76 (11.6)
Cirrhosis	30 (4.6)
Diabetes	83 (12.6)
IBD	20 (3)
UC	11 (1.7)
CD	9 (1.3)
Solid cancer	102 (15.5)
Haematological malignancy	56 (8.5)
Specific treatment	
PPI use at FMT	115 (17.5)
Antibiotic for an indication other than CDI	166 (25.2)
FMT indication—n (%)	
Multiple recurrent	546 (83.0)
Severe ongoing	48 (7.3)
Severe complicated ongoing	12 (1.8)
Refractory ongoing	5 (0.8)
Recurrent (<2 recurrences)	47 (7.1)
Number of CDI recurrence at FMT—median (IQR)	4 (2)
Number of FMTs/ patient—n (%)	
Only 1 FMT	580 (88.1)
2 FMT	33 (5.0)
3 FMT	4 (0.6)

Abbreviations: CD, Crohn's disease; CDI, *Clostridioides difficile* infection; FMT, faecal microbiota transplantation; IBD, inflammatory bowel disease; PPI, proton pump inhibitor; UC, ulcerative colitis.

*Glomerular filtration rate <30 mL/min and/or dialysis.

was the main indication for FMT (546/658; 83.0%), with a median number of recurrences of 4 (IQR=2). FMT procedures and stool preparations are described in Table 2. Most FMTs were performed using previously frozen faeces (621/658, 94.4%) through different concentrations of glycerol ranging from no cryopreservative (178/621; 28.7%) to glycerol dilution $\geq 80\%$ (140/621; 22.5%). When no cryopreservative was used, faeces were directly frozen within 6 h of emission, without processing or dilution, and then prepared for administration on the day of FMT (174/658; 26.4%). FMT procedures were mostly performed for inpatients (525/658; 79.8%) by upper route through gastro-duodenal infusion (393/658; 59.7%). Gastro-duodenal infusions were performed either through a nasogastric tube (354/658; 53.8%), a naso-duodenal tube (30/658; 4.6%), or upper endoscopy (9/658; 1.4%). The weight of faeces used for an FMT ranged from 12 to 194 g (median = 40 g, IQR = 20 g).

3.2 | FMT Outcomes

According to the number of FMTs performed per patient, the success rate of FMT was of 79.4% after one FMT (490/617) and 84.3% after 2 or more FMTs (520/617). No major difference was observed according to FMT indication (Figure S1) except for non-severe refractory CDI (success rate of 20%). Interestingly, patients who received FMT at first recurrence had a high success rate (85.1%; 40/47), similar to those who underwent FMT for multiple recurrences (79.6%; 435/546). Six patients were aged under 18 years and received a stool preparation from an adult donor with ultimately 5/6 successes (83.3%) and no adverse events reported.

Forty-eight adverse events were reported (7.3%), including 3 qualified as severe (0.5%), involving one colonic perforation in the context of a severe-complicated CDI with an FMT through gastric infusion leading to patient death, one bacteraemia caused by *Streptococcus gallolyticus* in the days following FMT, leading to secondary arthritis, and one sepsis of uncertain relationship to FMT.

Among the eight patients who died during the 8-week follow-up, four deaths occurred in the context of CDI (two from refractory multi-germ septic shock, one from colonic perforation as mentioned above, and one in the context of recurrent CDI). Two patients died of unspecified causes in the context of multiple comorbidities, one patient died from a stroke, and one from severe COVID-19.

3.3 | Parameters Associated With FMT Failure

Univariate and multivariate analyses were performed to identify predictors of FMT failure (Figures 1 and 2; Table S1). Regarding FMT protocol, the use of frozen faeces ($p=0.01$), donor age ($p=0.01$) and a thawing protocol using a water bath at 37°C for 2 h for frozen faeces ($p=0.007$) were found to be significantly associated with CDI recurrence within 8 weeks only in the univariate analysis. Reported absence of efficient bowel cleansing ($p=0.003$) or partial FMT retention (vomiting per procedure for upper route FMT or enema retention <1 h)

TABLE 2 | Characteristics of stool transplants and faecal microbiota transplantation (FMT) procedure for CDI patients in France between 2018 and 2022.

Characteristics of FMT transplants	<i>n</i> = 658
Origin— <i>n</i> (%)	
Frozen	621 (94.4)
Fresh	37 (5.6)
Weight of faeces (g)—Mean (SD)	43.1 (20.67)
Among frozen preparation— <i>n</i> (%)	621
Glycerol	443 (71.3)
Glycerol 10%	303 (48.8)
Glycerol 80%	86 (13.8)
Glycerol 90%	54 (8.7)
Thawing method	
Over-night at 4°C	195 (31.4)
Room-temperature < 6 h	238 (38.3)
Water-bath	135 (21.8)
NC	53 (8.5)
Donor age (years)—Median (IQR)	33 (13)
Gender match between recipient and donor— <i>n</i> (%)	316 (48.7)
Route of administration— <i>n</i> (%)	
Gastro/duodenal infusion	393 (59.7)
Frozen capsule	90 (13.7)
Enema	77 (11.7)
Colonoscopy	42 (6.4)
NC	56 (8.5)
Procedure— <i>n</i> (%)	
Bowel preparation	644 (97.9)
Anti-CDI antibiotic preparation	619 (94.1)
vancomycine	399 (60.6)
metronidazole	1 (0.2)
fidaxomicine monotherapy	64 (9.7)
combined antibiotic	15 (2.3)
NC	140 (21.3)
Immediate failure of the procedure— <i>n</i> (%)	25 (3.8)
Failure of enema retention or vomiting of upper route FMT	11 (1.7)
Insufficient bowel cleansing	14 (2.1)
Others	1 (0.01)

Abbreviation: NC, not communicated.

($p=0.0003$) were found to be significantly associated with FMT failure in both the univariate analysis and multivariate analysis ($p=0.007$ and $p=0.001$, respectively). Of note, most of the FMT involving insufficient bowel cleansing were performed through the upper route (12/14; 85.7%). No specific route of administration was associated with FMT outcome. Neither a weight of stools below 30 g ($n=140/658$, 21.3%; $p=0.28$) nor ≤ 25 g ($n=118/658$, 17.9%; $p=0.07$) were associated with FMT failure in the univariate analysis (Figure 1; Table S1). Interestingly, an antibiotic pretreatment with fidaxomicin monotherapy before FMT ($n=64/658$, 9.7%; $p=0.87$) did not impact FMT outcome (Figure 1; Table S1). Among frozen preparations, the use of high-dose glycerol ($\geq 80\%$) was associated with a risk of failure in both univariate ($p < 10^{-4}$) and multivariate analyses ($p=0.026$) with an overall efficacy of 64.5% (80/124, Figure S2). The use of transplants prepared from native faeces directly frozen within 6 h of emission, without adding glycerol or other processing, was not associated with impaired FMT efficacy: 84.9% (90/106, Figure 1; Table S1).

Among recipient-related predictors, univariate analysis highlighted that male gender ($p=0.01$), severe CKD ($p=0.0009$), immunodeficiency ($p=0.03$), cirrhosis ($p=0.007$), current non-CDI antibiotic treatment at FMT ($p=0.002$) and non-severe refractory CDI ($p=0.008$) were associated with a higher risk of FMT failure. However, in the multivariate analysis, only severe CKD ($p=0.008$) and non-severe refractory ongoing CDI ($p=0.02$) remained statistically significant. Noteworthy, non-CDI antibiotic treatment at FMT almost reached statistical significance in the multivariate analysis ($p=0.058$).

4 | Discussion

We report here a 5-year nationwide French experience of FMT performed for treating CDI. We identified specific patient and procedure-related predictors associated with FMT failure. Indeed, our results highlighted that severe CKD, non-severe refractory CDI, the use of high-dose glycerol, and technical failure of the procedure (either insufficient bowel cleansing or insufficient FMT retention) were associated with CDI recurrence within 8 weeks after FMT.

The overall success rate described in our cohort was similar to that described in routine care [3]. It was recently estimated in a meta-analysis from the Cochrane initiative (77% 95% CI: 54.5–100) [15], with an increased success rate after multiple FMTs as previously described [16]. Our results also confirm the relevance of FMT in severe or severe-complicated CDI [17–20]. Despite only a few patients being treated for non-severe refractory CDI in our cohort, this condition was associated with a significant risk of FMT failure in both univariate and multivariate analyses. The definition of refractory CDI is a subject of debate, as it may overlap with a differential diagnosis of prolonged diarrhoea associated with *C. difficile* colonisation [1]. This could explain the low response rate observed in our cohort in this clinical context. Microbiological failure of vancomycin or fidaxomicin is very limited [21], and

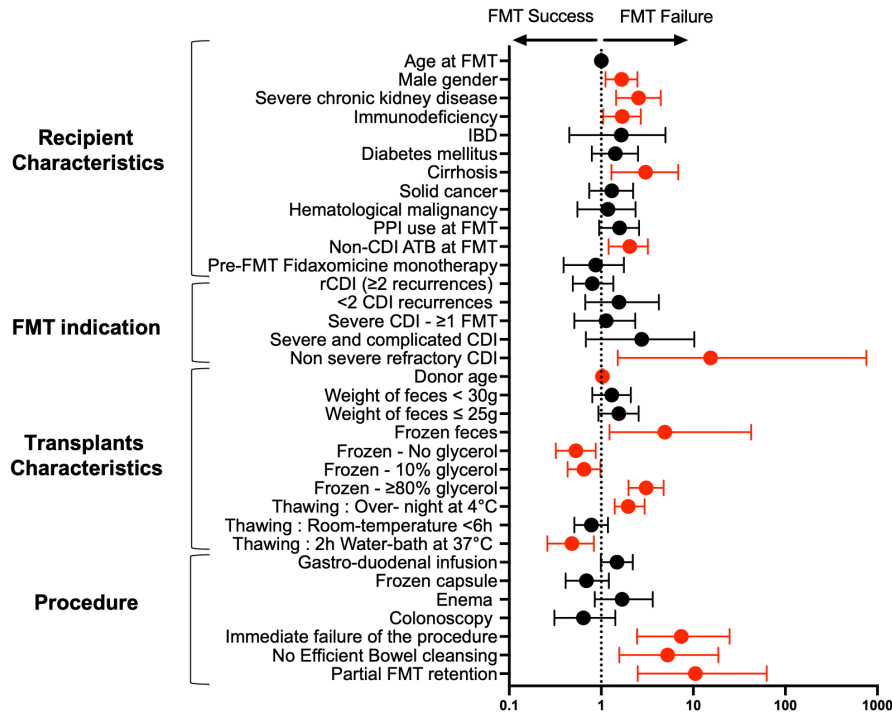


FIGURE 1 | Parameters associated with CDI recurrence at 8 weeks after FMT—Univariate analysis. Fisher’s test and logistic regression. ATB, antibiotics; CDI, *Clostridioides difficile* infection; FMT, faecal microbiota transplantation; IBD, inflammatory bowel disease; PPI, proton pump inhibitor.

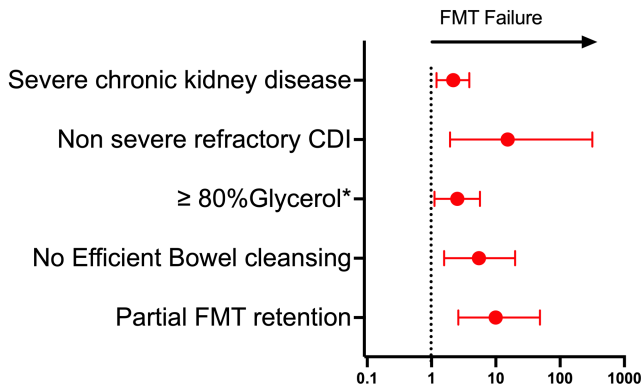


FIGURE 2 | Parameters associated with CDI recurrence at 8 weeks after FMT—Multivariate analysis. Logistic regression. FMT, faecal microbiota transplantation. *Calculated for frozen preparation.

clinicians should systematically consider additional differential diagnosis before using FMT in the context of persistent diarrhoea with microbiological positive testing for CDI [4]. In opposition, the use of FMT at first CDI recurrence was associated with a high efficacy in our population, in accordance with the results of a small controlled randomised trial from Hvas’group [8] and in line with recent guidelines from the American Gastroenterological Association that recommends considering FMT for patients with less than two CDI recurrences at high risk of either recurrent CDI or a morbid CDI recurrence [22].

Severe CKD is known to be associated with an increased risk of CDI, but its impact on FMT has been poorly described and

deserves to be clarified. Here, we observed a more than twice increased risk of FMT failure comparable to the 2-fold increased risk of developing CDI for CKD patients [23]. This result could be explained by the specific dysbiosis observed in severe CKD patients, partly due to the multiple medical interventions they are subjected to that can alter the gut microbiota and impair FMT effects (frequent hospitalisation, antibiotics exposure, altered bile acid intestinal metabolism etc.) [24, 25]. In particular, depletion of butyrate-producing bacteria (such as *Roseburia*), a common feature of CKD patients [26], has also been associated with FMT failure [27]. Strategies to improve FMT efficacy in this population should be developed involving possibly the combination of therapies targeting the microbiota and other treatments, such as the bezlotoxumab, an anti-toxin B antibody that has shown efficacy in CKD patients [28].

Detailed assessment of procedure-related factors associated with FMT efficacy is limited, either due to small populations in randomised clinical trials, a lack of comparative conditions in large single-centre cohorts, or limited available information in multicentric cohorts. Here, by taking advantage of the differences in procedures at the various French FMT centres, we have addressed poorly evaluated practical issues concerning FMT protocols.

Our study evaluated several questions raised by FMT that remained unanswered. First, as most available data report the use of FMT following anti-CDI antibiotic treatment with vancomycin, the impact on FMT efficacy of a previous treatment with fidaxomicin monotherapy was largely unknown. Although fidaxomicin is considered to have a limited impact on the gut microbiota compared to other antibiotics, we observed in our

study that a pre-treatment with monotherapy fidaxomicin has no impact on FMT efficacy.

Second, the weight of faeces required for FMT is still a matter of debate; although some studies have suggested that a faecal weight of 12.5 to 25 g may be sufficient [10], some retrospective cohorts have suggested a loss of efficacy below 50 g [29]. On the one hand, in our cohort, a faecal weight of less than 30 g was used in around 20% of our cohort and was not associated with a loss of FMT efficacy, irrespective of the route of administration. On the other hand, the strict success of the FMT procedure (bowel cleansing and transplant retention) appeared crucial for FMT efficacy. In line with this observation, enema should be used with caution after assessment of anal continence to avoid rapid transplant evacuation (<1 h), a limitation which may explain why gastro-duodenal infusion was the preferred route of administration in our cohort. Moreover, the fact that only two centres had access to FMT capsules during the study period, the need for the patient to be able to swallow a large quantity of high-volume capsules combined with the potential risk of serious adverse events related to colonoscopy, likely contributed to the high rate of upper-route FMT use in our population. Moreover, efficient bowel cleansing appeared to be a key determinant of FMT success in our cohort. As most of the patients with insufficient bowel cleansing received FMT by upper route in our cohort, the importance of bowel cleansing for FMT using enema still needs to be evaluated as the literature showed contradictory results with exceptionally low rates of FMT efficacy in trials with no bowel cleansing [30], while some authors reported similar results than described using bowel preparation [31].

Third, in our cohort, glycerol used, in particular at high concentrations, was associated with decreased FMT efficacy. Although associated with an increase in microbial viability after freezing [32], glycerol may impact bacterial fitness and metabolism [33, 34], which may affect bacterial strain engraftment and FMT efficacy. Furthermore, in the absence of glycerol, faeces were generally rapidly frozen with limited handling and directly prepared on the day of FMT. Of note, dry preservation of stools has also been described to preserve the viability of fragile microorganisms (particularly anaerobic), maintaining the overall microbial richness of stools sample from healthy volunteers in comparison with procedures using culture medium with or without cryopreservative [35].

Finally, the thawing protocol can significantly impact bacteria growth and FMT sample preparation [36]. However, nothing is known about the impact of this critical stage of transplant preparation on the efficacy of FMT. Our data suggest that thawing in a hot water bath at 37°C could be associated with an increase in the efficiency of FMT. Thawing using a water bath has been associated with increased bacterial load in the food industry compared to other cooler thawing methods [37]. As FMT has been proven to be associated with strains engraftment [38], this may explain the possible association we observed. More studies are needed to understand the microbiological impact of direct freezing and the different thawing protocols on FMT transplants.

Our work has intrinsic limitations due to its retrospective nature. Precise reasons for choosing FMT at first CDI recurrence were not systematically specified. Bowel cleansing was subjectively

described on the basis of the stool's aspect at FMT and the patient's ability to take a sufficient quantity of prescribed laxatives. The diagnostic methods for CDI were not systematically documented, and misdiagnosis may explain some FMT failures, particularly in cases of non-severe refractory CDI. Finally, the very low rate of declared adverse events compared to the 19% rate described previously in the largest systematic review may be due to the under-declaration of minor adverse events. However, the reported rate of serious adverse events we report is similar to what has been previously described (0.1%–1.3%) [6, 18]. In addition, loss of follow-up was limited (<5%) and data on faecal preparation and management were collected prospectively in compliance with Good Manufacturing Practices and the obligation to ensure traceability by the pharmacy in charge of the faecal transplant preparation. However, prospective nationwide cohorts are needed to ensure optimal follow-up of both donors and FMT recipients to uncover potential long-term and rare complications as it is now performed in France through the COSMIC-FMT cohort (NCT06311006).

In conclusion, this work gives new insights into specific clinical and pharmacological parameters that can impact FMT efficacy. Limiting the use of glycerol $\leq 10\%$ in FMT transplant preparation and use of 37°C water bath thawing may improve FMT efficacy, especially when considering patients at high risk of failure, such as in severe CKD or non-severe refractory CDI. Transplant retention and bowel cleansing should also be systematically optimised. These results may also contribute to the design and evaluation of new microbiota-derived therapies for CDI, particularly where product preparations are similar to those used for FMT [39].

Author Contributions

Nicolas Benech: conceptualization, methodology, writing – review and editing, writing – original draft, formal analysis, investigation, validation, project administration, visualization, data curation. **Nadim Cassir:** writing – review and editing, investigation, data curation. **Laurent Alric:** investigation, writing – review and editing. **Frédéric Barbut:** writing – review and editing, resources. **Rui Batista:** writing – review and editing, resources. **Alexandre Bleibtreu:** investigation, writing – review and editing. **Thomas Briot:** writing – review and editing, resources. **Benjamin Davido:** investigation, writing – review and editing. **Tatiana Galperine:** writing – review and editing. **Anne-Christine Joly:** writing – review and editing, resources. **Nathalie Kapel:** writing – review and editing, resources. **Chloé Melchior:** investigation, writing – review and editing. **Alexis Mosca:** investigation, writing – review and editing. **Biba Nebbad:** writing – review and editing, resources. **Bénédicte Pigneur:** investigation, writing – review and editing. **Stéphane M. Schneider:** investigation, writing – review and editing. **Mathieu Wasiak:** writing – review and editing, resources. **Julien Scanzi:** investigation, writing – review and editing. **Harry Sokol:** conceptualization, investigation, writing – original draft, writing – review and editing, methodology, visualization, supervision.

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Conflicts of Interest

H.S. received consultancy or lecture fees, from Biocodex, Abbvie, Astellas, Danone, Ferring, Mayoly Spindler, MSD, Novartis, Roche, Janssen, Tillots, Enterome, Maat, Ipsen, MSD, BiomX, Galapagos, Biocodex, Biogen, Novartis, Lilly, Viatrix, Nestlé, Urigo, Adare, Fresenius, Celltrion, Sanofi, and Takeda, has stocks from Enterome and is a co-founder of Exeliom Bioscience. N.B. received lecture fees from Tillots Pharma and travel grants from Pfizer, participated on an advisory board for Ferring Pharmaceuticals with payment to his institution, is a PI of a clinical trial supported by Exeliom Biosciences. BD received travel grants from Pfizer, Gilead and Viiv healthcare. ACJ received congress fundings by Fresenius Kabi, Viatrix. C.M. has served as a consultant/advisory board member for Kyowa Kirin, Norgine, Biocodex, Mayoly Spindler, Tillots, Ipsen, Nestlé HealthScience and Viatrix. NK received a grant from Maat for a collaborative research study. TG received travel grants from Tillots Pharma. SMS received consultancy or lecture honoraria from Amgen, Axium mTech, Baxter, Fresenius-Kabi, Grand Fontaine, Nestlé Health Science, Nutricia, Pfizer, Takeda, VectivBio and Viatrix. A.M. received consultancy, or lecture fees, from Biocodex, Danone, Nestlé, Adare, Biogaia, Havea. J.S. received consultancy, or lecture fees, from Biocodex, Mayoly Spindler, Sanofi, Pileje, Janssen, Dynveo, Havea, Activia, Bioderma, and travel grants from Sandoz and Biocodex. L.A. received consultancy or lecture fees, travel grants from: Abbvie, Gilead, Janssen, BMS, MSD, Novartis, Almylan, Viatrix, Takeda. FB received consultancy, lecture fees or scientific grants from Abbott, Astellas, Biomérieux, Cepheid, Cubist, Da Volterra, Danone, Ferring, MSD, Pfizer, Quidel, Sanofi Pasteur, Summit, and Tillots. ACJ received congress fundings by Fresenius Kabi, Viatrix. BP received lecture fees from Biocodex and Pfizer. A.B. declares links of interest with Pfizer, Shionogi, Eumedica, Sanofi, Gilead, Astellas, Menarini, Janssen for congress fees, symposia, or being investigator in clinical studies. M.W., N.C., T.B., R.B., B.N. disclose no conflicts.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. J. van Prehn, E. Reigadas, E. H. Vogelzang, et al., "European Society of Clinical Microbiology and Infectious Diseases: 2021 Update on the Treatment Guidance Document for *Clostridioides difficile* Infection in Adults," *Clinical Microbiology and Infection* 27 (2021): S1–S21, <https://doi.org/10.1016/j.cmi.2021.09.038>.
2. S. Johnson, V. Lavergne, A. M. Skinner, et al., "Clinical Practice Guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA): 2021 Focused Update Guidelines on Management of *Clostridioides difficile* Infection in Adults," *Clinical Infectious Diseases* 73, no. 5 (2021): 755–757, <https://doi.org/10.1093/cid/ciab718>.
3. C. R. Kelly, E. F. Yen, A. M. Grinspan, et al., "Faecal Microbiota Transplantation is Highly Effective in Real-World Practice: Initial Results From the FMT National Registry," *Gastroenterology* 1 (2020): 183–192.e3, <https://doi.org/10.1053/j.gastro.2020.09.038>.
4. E. M. Terveer, K. E. Vendrik, R. E. Ooijevaar, et al., "Faecal Microbiota Transplantation for *Clostridioides difficile* Infection: Four Years' Experience of The Netherlands Donor Feces Bank," *United European Gastroenterology Journal* 8, no. 10 (2020): 1236–1247, <https://doi.org/10.1177/2050640620957765>.
5. C. L. Hvas, S. M. Dahl Jørgensen, S. P. Jørgensen, et al., "Faecal Microbiota Transplantation is Superior to Fidaxomicin for Treatment of Recurrent *Clostridium difficile* Infection," *Gastroenterology* 156, no. 5 (2019): 1324–1332.e3, <https://doi.org/10.1053/j.gastro.2018.12.019>.

6. C. Marcella, B. Cui, C. R. Kelly, G. Ianiro, G. Cammarota, and F. Zhang, "Systematic Review: The Global Incidence of Faecal Microbiota Transplantation-Related Adverse Events From 2000 to 2020," *Alimentary Pharmacology & Therapeutics* 53, no. 1 (2021): 33–42, <https://doi.org/10.1111/apt.16148>.
7. G. Cammarota, G. Ianiro, H. Tilg, et al., "European Consensus Conference on Faecal Microbiota Transplantation in Clinical Practice," *Gut* 66, no. 4 (2017): 569–580, <https://doi.org/10.1136/gutjnl-2016-313017>.
8. S. M. D. Baunwall, S. E. Andreasen, M. M. Hansen, et al., "Faecal Microbiota Transplantation for First or Second *Clostridioides difficile* Infection (EarlyFMT): A Randomised, Double-Blind, Placebo-Controlled Trial," *Lancet Gastroenterology & Hepatology* 7, no. 12 (2022): 1083–1091, [https://doi.org/10.1016/S2468-1253\(22\)00276-X](https://doi.org/10.1016/S2468-1253(22)00276-X).
9. M. Fischer, D. Kao, S. R. Mehta, et al., "Predictors of Early Failure After Faecal Microbiota Transplantation for the Therapy of Clostridium Difficile Infection: A Multicenter Study," *American Journal of Gastroenterology* 111, no. 7 (2016): 1024–1031, <https://doi.org/10.1038/ajg.2016.180>.
10. G. Cammarota, G. Ianiro, C. R. Kelly, et al., "International Consensus Conference on Stool Banking for Faecal Microbiota Transplantation in Clinical Practice," *Gut* 68, no. 12 (2019): 2111–2121, <https://doi.org/10.1136/gutjnl-2019-319548>.
11. K. Jj, O. Re, H. Cl, et al., "A Standardised Model for Stool Banking for Faecal Microbiota Transplantation: A Consensus Report From a Multidisciplinary UEG Working Group," *United European Gastroenterology Journal* 9, no. 2 (2021): 229–247, <https://doi.org/10.1177/2050640620967898>.
12. European Directorate for the Quality of Medicines & HealthCare, "Guide to the Quality and Safety of Tissues and Cells for Human Application—European Directorate for the Quality of Medicines & HealthCare—EDQM," accessed August 23, 2023, <https://www.edqm.eu/en/guide-to-the-quality-and-safety-of-tissues-and-cells-for-human-application1>.
13. E. M. Terveer, Y. H. van Beurden, A. Goorhuis, et al., "How to: Establish and Run a Stool Bank," *Clinical Microbiology and Infection* 23, no. 12 (2017): 924–930, <https://doi.org/10.1016/j.cmi.2017.05.015>.
14. G. Ianiro, M. Maida, J. Burisch, et al., "Efficacy of Different Faecal Microbiota Transplantation Protocols for *Clostridium difficile* Infection: A Systematic Review and Meta-Analysis," *United European Gastroenterology Journal* 6, no. 8 (2018): 1232–1244, <https://doi.org/10.1177/2050640618780762>.
15. N. Z. Minkoff, S. Aslam, M. Medina, et al., "Faecal Microbiota Transplantation for the Treatment of Recurrent *Clostridioides difficile* (*Clostridium difficile*)," *Cochrane Database of Systematic Reviews* 4, no. 4 (2023): CD013871, <https://doi.org/10.1002/14651858.CD013871.pub2>.
16. E. van Nood, A. Vrieze, M. Nieuwdorp, et al., "Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*," *New England Journal of Medicine* 368, no. 5 (2013): 407–415, <https://doi.org/10.1056/NEJMoA1205037>.
17. M. Fischer, B. W. Sipe, N. A. Rogers, et al., "Faecal Microbiota Transplantation Plus Selected Use of Vancomycin for Severe-Complicated *Clostridium difficile* Infection: Description of a Protocol With High Success Rate," *Alimentary Pharmacology & Therapeutics* 42, no. 4 (2015): 470–476, <https://doi.org/10.1111/apt.13290>.
18. M. Agrawal, O. C. Aroniadis, L. J. Brandt, et al., "The Long-Term Efficacy and Safety of Faecal Microbiota Transplant for Recurrent, Severe, and Complicated *Clostridium difficile* Infection in 146 Elderly Individuals," *Journal of Clinical Gastroenterology* 50, no. 5 (2016): 403–407, <https://doi.org/10.1097/MCG.0000000000000410>.
19. N. Benech, G. Leboucher, C. Monard, and T. Ferry, "Septic Shock due to Refractory Severe *Clostridioides Difficile* Colitis Rapidly Resolving After Faecal Microbiota Transplantation," *BML Case Reports* 13, no. 9 (2020): e234329, <https://doi.org/10.1136/bcr-2020-234329>.
20. M. Hocquart, J. C. Lagier, N. Cassir, et al., "Early Faecal Microbiota Transplantation Improves Survival in Severe *Clostridium difficile* Infections," *Clinical Infectious Diseases* 66, no. 5 (2018): 645–650, <https://doi.org/10.1093/cid/cix762>.
21. J. Freeman, J. Vernon, K. Morris, et al., "Pan-European Longitudinal Surveillance of Antibiotic Resistance Among Prevalent *Clostridium difficile* Ribotypes," *Clinical Microbiology and Infection* 21, no. 3 (2015): 248.e9–248.e16, <https://doi.org/10.1016/j.cmi.2014.09.017>.
22. A. F. Peery, C. R. Kelly, D. Kao, et al., "AGA Clinical Practice Guideline on Faecal Microbiota-Based Therapies for Select Gastrointestinal Diseases," *Gastroenterology* 166, no. 3 (2024): 409–434, <https://doi.org/10.1053/j.gastro.2024.01.008>.
23. S. Dudzicz, A. Wiecek, and M. Adamczak, "Clostridioides Difficile Infection in Chronic Kidney Disease—An Overview for Clinicians," *Journal of Clinical Medicine* 10, no. 2 (2021): E196, <https://doi.org/10.3390/jcm10020196>.
24. X. Wang, S. Yang, S. Li, et al., "Aberrant Gut Microbiota Alters Host Metabolome and Impacts Renal Failure in Humans and Rodents," *Gut* 69, no. 12 (2020): 2131–2142, <https://doi.org/10.1136/gutjnl-2019-319766>.
25. N. Benech and L. Koppe, "Is There a Place for Faecal Microbiota Transplantation in Chronic Kidney Disease?," *Nephrology, Dialysis, Transplantation* 37, no. 12 (2022): 2303–2306, <https://doi.org/10.1093/ndt/gfac277>.
26. J. Zhao, X. Ning, B. Liu, R. Dong, M. Bai, and S. Sun, "Specific Alterations in Gut Microbiota in Patients With Chronic Kidney Disease: An Updated Systematic Review," *Renal Failure* 43, no. 1 (2021): 102–112, <https://doi.org/10.1080/0886022X.2020.1864404>.
27. H. Tian, J. Cui, C. Ye, et al., "Depletion of Butyrate-Producing Microbes of the Firmicutes Predicts Nonresponse to FMT Therapy in Patients With Recurrent *Clostridium difficile* Infection," *Gut Microbes* 15, no. 1 (2023): 2236362, <https://doi.org/10.1080/19490976.2023.2236362>.
28. Y. Golan, H. L. DuPont, F. Aldomiro, E. H. Jensen, M. E. Hanson, and M. B. Dorr, "Renal Impairment, *C. difficile* Recurrence, and the Differential Effect of Bezlotoxumab: A Post Hoc Analysis of Pooled Data From 2 Randomized Clinical Trials," *Open Forum Infectious Diseases* 7, no. 7 (2020): ofaa248, <https://doi.org/10.1093/ofid/ofaa248>.
29. G. Ianiro, L. Valerio, L. Masucci, et al., "Predictors of Failure After Single Faecal Microbiota Transplantation in Patients With Recurrent *Clostridium difficile* Infection: Results From a 3-Year, Single-Centre Cohort Study," *Clinical Microbiology and Infection* 23, no. 5 (2017): 337.e1–337.e3, <https://doi.org/10.1016/j.cmi.2016.12.025>.
30. S. S. Hota, V. Sales, G. Tomlinson, et al., "Oral Vancomycin Followed by Faecal Transplantation Versus Tapering Oral Vancomycin Treatment for Recurrent *Clostridium difficile* Infection: An Open-Label, Randomized Controlled Trial," *Clinical Infectious Diseases* 64, no. 3 (2017): 265–271, <https://doi.org/10.1093/cid/ciw731>.
31. C. H. Lee, T. Steiner, E. O. Petrof, et al., "Frozen vs. Fresh Faecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent *Clostridium difficile* Infection: A Randomized Clinical Trial," *Journal of the American Medical Association* 315, no. 2 (2016): 142–149, <https://doi.org/10.1001/jama.2015.18098>.
32. S. P. Costello, M. A. Conlon, M. S. Vuaran, I. C. Roberts-Thomson, and J. M. Andrews, "Faecal Microbiota Transplant for Recurrent *Clostridium difficile* Infection Using Long-Term Frozen Stool Is Effective: Clinical Efficacy and Bacterial Viability Data," *Alimentary Pharmacology & Therapeutics* 42, no. 8 (2015): 1011–1018, <https://doi.org/10.1111/apt.13366>.
33. Y. Doi, "Glycerol Metabolism and Its Regulation in Lactic Acid Bacteria," *Applied Microbiology and Biotechnology* 103, no. 13 (2019): 5079–5093, <https://doi.org/10.1007/s00253-019-09830-y>.
34. I. Poblete-Castro, C. Wittmann, and P. I. Nikel, "Biochemistry, Genetics and Biotechnology of Glycerol Utilization in *Pseudomonas*

Species,” *Microbial Biotechnology* 13, no. 1 (2019): 32–53, <https://doi.org/10.1111/1751-7915.13400>.

35. A. Biclôt, G. R. B. Huys, R. Bacigalupe, et al., “Effect of Cryopreservation Medium Conditions on Growth and Isolation of Gut Anaerobes From Human Faecal Samples,” *Microbiome* 10, no. 1 (2022): 80, <https://doi.org/10.1186/s40168-022-01267-2>.

36. L. E. Papanicolas, J. M. Choo, Y. Wang, et al., “Bacterial Viability in Faecal Transplants: Which Bacteria Survive?,” *eBioMedicine* 41 (2019): 509–516, <https://doi.org/10.1016/j.ebiom.2019.02.023>.

37. Food Safety and Inspection Service, “The Big Thaw—Safe Defrosting Methods,” accessed February 6, 2024, <http://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/food-safety-basics/big-thaw-safe-defrosting-methods>.

38. G. Ianiro, M. Punčochář, N. Karcher, et al., “Variability of Strain Engraftment and Predictability of Microbiome Composition After Faecal Microbiota Transplantation Across Different Diseases,” *Nature Medicine* 28 (2022): 1913–1923.

39. N. Benech, F. Barbut, F. Fitzpatrick, et al., “Update on Microbiota-Derived Therapies for Recurrent *Clostridioides difficile* Infections, 2023,” *Clinical Microbiology and Infection* 30, no. 4 (2023): 462–468, <https://doi.org/10.1016/j.cmi.2023.12.007>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.