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Original article

The presence of *Clostridioides difficile* in faeces before and after faecal microbiota transplantation and its relation with recurrent *C. difficile* infection and the gut microbiota in a Dutch cohort

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A B S T R A C T

Objectives: The objectives of this study are to investigate the presence of *Clostridioides difficile* in faeces of patients with recurrent *C. difficile* infection (rCDI) before and after faecal microbiota transplantation (FMT) and to identify risk factors for faecal *C. difficile* and *C. difficile* infection (CDI) recurrence.
Methods: $n = 83$ faecal sample triads (pre-FMT [~1 day], post-FMT [~3 weeks], and a corresponding FMT donor sample), and $n = 22$ long-term (~1–3 years) follow-up faecal samples were collected from FMT-treated patients. The presence of *C. difficile* in faeces was assessed by enrichment broth culture and PCR (*tcdB* gene) and associated with patient characteristics, FMT outcome, duration of pre-FMT vancomycin, FMT donor, post-FMT antibiotic use, and faecal microbiota composition (shotgun metagenomics).
Results: The FMT cure rate for rCDI was 92.8% (77/83), with six early CDI recurrences (<2 months post-FMT). Toxigenic *C. difficile* was cultured in 27.7% (23/83) of all patients post-FMT, 23.4% (18/77) of patients cured 2 months post-FMT, and 13.6% (3/22) at long-term follow-up. Early CDI recurrence ($n = 6$) was associated with positive *C. difficile* culture post-FMT (21.7% [5/23] vs. 1.7% [1/60], p 0.01), post-FMT antibiotics (30.0% [3/10] vs. 4.6% [3/65], p 0.03), and a short course of pre-FMT vancomycin (median 6.0 days, IQR [5–12] vs. 18 days, IQR [10.8–29], $p < 0.05$). Additionally, positive *C. difficile* culture directly pre-FMT was associated with a short course of pre-FMT vancomycin (median 9 days IQR [5–18] vs. 17 days, IQR [10–29.2], p 0.04). Gut microbiota analyses did not reveal signatures associated with *C. difficile* culture result, despite statistically non-significant trends in relative abundances of the *Enterobacteriaceae* family, and *Dorea*, *Roseburia*, and *Clostridiales* species.
Discussion: Although eradication of *C. difficile* is not required for clinical cure of rCDI by FMT, it is associated with reduced prevalence of early CDI recurrence, as are the full completion of pre-FMT vancomycin (at least 10 days) and avoiding post-FMT antibiotics. **Bas Groenewegen, Clin Microbiol Infect 2025;31:568**

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Introduction

Faecal microbiota transplantation (FMT) has been globally implemented as an effective and safe therapy for patients with multiple recurrent *Clostridioides difficile* infection (rCDI), with cure rates of approximately 85% [1–4]. The clinical success of FMT for rCDI likely lies in the reconstitution of a diverse gut microbiota, providing colonisation resistance against *C. difficile* through mechanisms including nutrient competition, secretion of bioactive bacterial metabolites, and immune activation, among others [5,6].

It is currently unknown whether FMT eradicates *C. difficile* and its spores from the gut, and if this is required for clinical success. One study in patients with rCDI found low asymptomatic *C. difficile* carriage rates (~2–3%) within 4 weeks post-FMT using stool PCR; however, data on this subject remain limited [7].

The detection of *C. difficile* in faeces does not necessarily imply infection [8], and current *C. difficile* infection (CDI) treatment guidelines do not recommend testing for *C. difficile* to evaluate treatment response when clinical symptoms are absent [9]. Nonetheless, asymptomatic *C. difficile* colonisation may elevate the risk of progression to CDI, especially in those with a history of recurrent disease [10,11]. Furthermore, asymptomatic carriers may shed *C. difficile* spores into the environment, contributing to transmission [12,13]. Conversely, the risk of transmission or progression to CDI may depend on strain or host characteristics, as low transmission rates and lack of progression to CDI have been reported in endemic settings of *C. difficile* colonisation [14].

While the use of non-*C. difficile*-targeted antibiotics post-FMT is a known risk factor for CDI recurrence [15], insufficient information exists regarding the role of anti-*C. difficile* antibiotic pre-treatment in the eradication of *C. difficile* and achieving FMT success. Although pre-FMT vancomycin has been associated with increased engraftment of donor-derived bacteria in healthy volunteers [16], the optimal duration of pre-treatment and its effects on the presence of *C. difficile* in faeces pre/post-FMT, gut microbiota composition, and clinical outcome in FMT-treated patients with rCDI remains poorly understood. Finally, although FMT donor selection has been reported to affect FMT outcome in rCDI [17], its impact on *C. difficile* eradication remains unexplored.

To further elucidate the effects of FMT in rCDI, we assessed the presence of *C. difficile* in faeces pre- and post-FMT in a cohort of Dutch FMT-treated patients with rCDI. We investigated the eradication of *C. difficile* by FMT and its potential associations with post-FMT CDI recurrence, the duration of pre-FMT anti-*C. difficile* antibiotics, FMT donor, post-FMT antibiotic use, and gut microbiota composition.

Methods

Cohort characteristics

We obtained faecal samples from patients with rCDI treated with donor faecal suspensions provided by the Netherlands Donor Feces Bank (NDFB) between May 2016 and May 2021. Sample triads were collected, comprising samples taken pre-FMT (~1 day [during/after pre-FMT anti-*C. difficile* antibiotics]), post-FMT (requested approximately 3 weeks post-FMT), and corresponding donor faeces samples used for the FMT product. Patients were contacted for long-term follow-up faeces samples (LTFU) 1–3 years post-FMT. Patient characteristics and FMT outcome were retrieved from our NDFB database. Written informed consent was obtained for using patient and donor faecal samples and clinical data, and approved by the Medical Ethics Committee at Leiden University Medical Center (P15.145, B21.049). This study conforms to the 1975 Declaration of Helsinki.

Patient selection and treatment

All FMT requests from Dutch treating physicians for rCDI treatment were evaluated by the NDFB multidisciplinary FMT-expert panel [4,18]. Patients with at least two rCDI episodes or severe therapy refractory CDI were eligible for FMT, irrespective of hospitalisation [4]. CDI was defined according to treatment guidance documents [9]. A two-stage testing algorithm for *C. difficile* was recommended. Detection of free *C. difficile* toxins was required in cases of gastrointestinal comorbidity [4,18]. Upon approval, patients received anti-*C. difficile* antibiotic pre-treatment for at least 4 days, and subsequently bowel lavage and FMT treatment at their local health care facility [4,18]. Faecal suspensions were infused via duodenal tube unless contraindicated, in which case colonoscopy was advised (Table S1) [4]. Pre- and post-FMT faeces samples were requested via the treating physician or directly from patients. Early CDI recurrence was defined as a CDI episode within 2 months post-FMT, whereas late CDI recurrence occurred later than 2 months post-FMT [4,9]. FMT cure was defined as the full resolution of CDI symptoms without early CDI recurrence [4].

Supplementary methods

Detailed methods on *C. difficile* identification (enrichment culture and *C. difficile tcdB* gene PCR) and characterisation, gut microbiota analysis via metagenomic shotgun sequencing, and statistical analyses are provided in Supplement 1. Metagenomic shotgun sequencing data were obtained from European Nucleotide Archive projects: PRJEB44737 and PRJEB64621 [19] (Table S2).

Results

Cohort characteristics

In total, 190 patients with rCDI were treated with FMT between May 2016 and May 2021 [19]. In 43.7% (83/190) of FMT-treated patients with rCDI, faeces sample triads were available for *C. difficile* testing (Fig. 1), including samples collected pre-FMT (median 1 day, IQR [1-2]), post-FMT (median 25 days, IQR [20–40]), and corresponding donor samples. Four of 83 pre-FMT samples were taken before starting pre-FMT antibiotics and excluded from relevant analyses (Fig. 1). The 83 patients received donor faecal suspensions derived from 71 donations, provided by 14 different donors. LTFU faeces samples (median 822 days, IQR [452–1113]) were available for 22 patients. The clinical cure rate at 2 months post-FMT was 92.8% (77/83), with six patients suffering an early CDI recurrence within this period. Additionally, 14 patients experienced late CDI recurrence post-FMT (16.9%, 14/83), one of whom had also suffered an early CDI recurrence. Selected relevant patient characteristics were similar to the complete cohort ($n = 190$) (Table S1), and summarised in Table 1 [4].

Presence of C. difficile in patients with rCDI pre- and post-FMT: short- and long-term follow-up

In patients with rCDI, we cultured toxigenic *C. difficile* in 16.5% (13/79) of pre-FMT faeces samples, despite pre-FMT anti-*C. difficile* antibiotics. Post-FMT, 27.7% (23/83) of samples were culture positive for toxigenic *C. difficile* (Fig. 2 and Table 1). Non-toxigenic *C. difficile* was cultured in two patients (2.4%). Interestingly, 78.3%

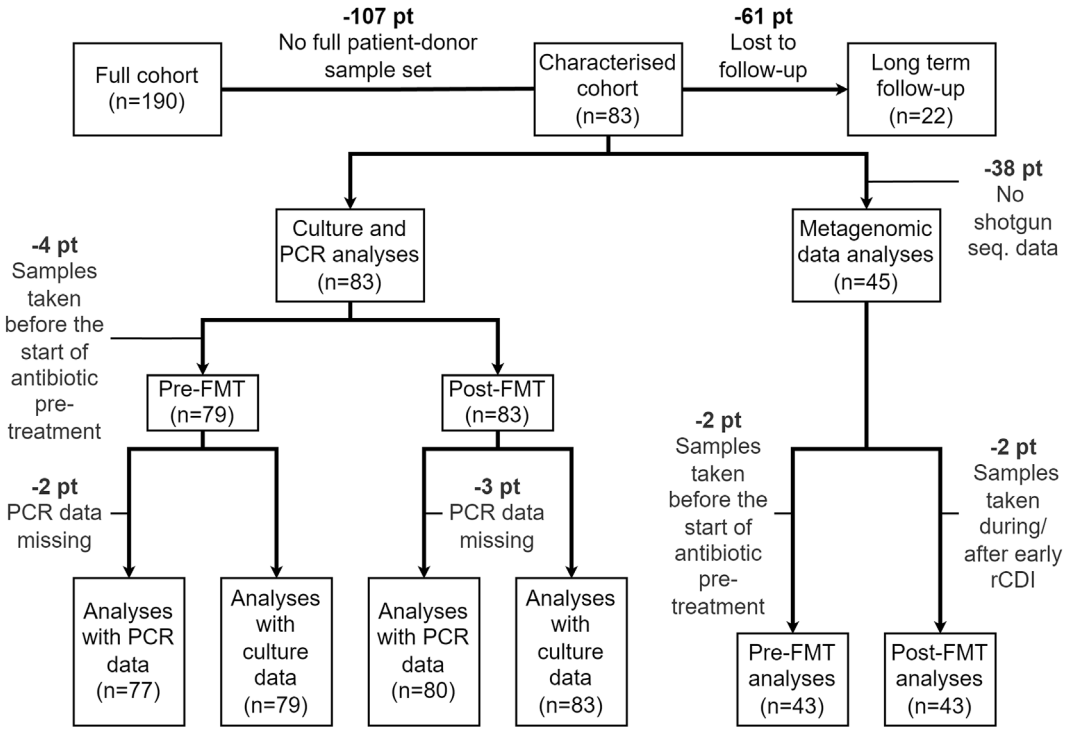

Fig. 1. Overview of data available for analyses. Included patients suffered from *C. difficile* infection and were treated with donor faecal suspensions provided by the Netherlands Donor Feces Bank between May 2016 and May 2021. Complete sample triads, consisting of a sample taken pre-FMT (~1 day), post-FMT (~3 weeks), and a corresponding donor faeces sample were available for 83 patients. For 22 patients, long-term follow-up samples (~1–3 years) were available. The presence of *C. difficile* in faeces was assessed using either PCR for the *C. difficile tcdB* gene (encoding *C. difficile* toxin B) or enrichment culture. Metagenomic shotgun sequencing data were available for triads of 45 patients and used to characterise the gut microbiota. FMT, faecal microbiota transplantation; PCR, polymerase chain reaction; early rCDI, recurrence of *Clostridioides difficile* infection within 2 months post-FMT.

(18/23) of patients with culturable toxigenic *C. difficile* 3 weeks post-FMT were clinically cured at 2 months. Overall, 92.8% (77/83) of patients were clinically cured 2 months post-FMT, indicating the presence of *C. difficile* in 23.4% (18/77) of asymptomatic, FMT-cured patients. No toxigenic or non-toxigenic *C. difficile* was cultured from donor faeces.

At LTFU, 13.6% (3/22) of asymptomatic patients were culture positive for *C. difficile* (Fig. 2), with all pre- and short-term post-FMT samples culture negative. Each of these patients suffered late CDI recurrence between FMT and LTFU. Two were treated successfully with a second FMT, the third with vancomycin. Additionally, two patients culture negative at LTFU had experienced late CDI recurrence, both successfully treated with fidaxomicin.

Assessing interplay between the presence and type of C. difficile, CDI recurrence and post-FMT antibiotic use

Early CDI recurrence was associated with positive *C. difficile* culture post-FMT (p 0.01); however, this was non-significant after excluding those sampled at or after early CDI recurrence ($n = 4$) (p 0.43) (Table 1) [4]. No association was found between late CDI recurrence and culture results pre-FMT (p 0.11) or post-FMT (p 0.75) (Table 1) [4]. The distribution of 42 identified *C. difficile* ribotypes was not associated with *C. difficile* presence or CDI recurrence, and resembled Dutch national infection data (Fig. S1 and Tables S3 and S4) [20]. Post-FMT administration of non-*C. difficile*-targeted antibiotics was not significantly associated with post-FMT culture positivity (p 0.06) (Table 1) [4], but was associated with early CDI recurrence (30.0% [3/10] vs. 4.6% [3/65], OR 8.41, 95% CI: 0.95–76.09, p 0.03). Antibiotics administered within 3 weeks post-FMT included meropenem, ciprofloxacin, nitrofurantoin, amoxicillin, cefotaxime and amoxicillin-clavulanic acid, cefazolin, or unknown antibiotics ($n = 4$).

Impact of pre-FMT vancomycin duration and FMT donor selection on the presence of C. difficile and CDI recurrence

Most patients received pre-FMT anti-*C. difficile* treatment with vancomycin (83.1%, 69/83), whereas a small proportion (16.9%, 14/83) received other antibiotics (Table S5). To investigate the duration of pre-FMT anti-*C. difficile* antibiotics in relation to the presence of *C. difficile* and CDI recurrence, we focused on vancomycin-treated patients. Patients with a positive *C. difficile* culture pre-FMT had received shorter vancomycin pre-treatment (p 0.04) (Table 1) [4]. Patients with early CDI recurrence had also received shorter vancomycin pre-treatment (median 6.0 days, IQR [5–12] vs. 18.0 days, IQR [10.8–29], OR 0.8, 95% CI: 0.64–1, $p < 0.05$). No association was found between late CDI recurrence and pre-FMT vancomycin duration (median 12 days, IQR [9–19] vs. 18 days, IQR [10.3–28.8], OR 0.96, 95% CI: 0.9–1.02, p 0.19). Equivalent results were observed when repeating these analyses in our full dataset ($n = 190$) (Tables S1 and S6). FMT donor selection showed no associations with the presence of *C. difficile* and early or late CDI recurrence (Tables S7 and S8).

Enrichment culture vs. PCR and metagenomic shotgun sequencing to detect the presence of C. difficile in faeces

Compared with enrichment culture, PCR targeting the *C. difficile tcdB* gene showed low sensitivity for detecting the presence of toxigenic *C. difficile* (71% pre-FMT, 40% post-FMT), with only 3.3% of samples PCR positive where *C. difficile* could not be cultured (Tables S9 and S10). To further explore *C. difficile* detection

Table 1
Patient characteristics related to toxigenic *Clostridioides difficile* culture result pre- and post-faecal microbiota transplantation

Characteristic	Pre-FMT ($n = 79$[a])			Post-FMT ($n = 83$)		
	Culture negative ($n = 66$[a])	Culture positive ($n = 13$[a])	Statistics (OR [95% CI], p value)	Culture negative ($n = 60$)	Culture positive ($n = 23$)	Statistics (OR [95% CI], p value)
Patient sex (female)	63.64% (42/66)	53.85% (7/13)	OR 0.67 (0.17–2.72), p 0.54	68.33% (41/60)	47.83% (11/23)	OR 0.43 (0.14–1.28), p 0.13
Age	Mean 72.14, SD 14.05	Mean 72.23, SD 15.94	OR 1 (0.96–1.04), p 0.98	Mean 70.8, SD 16.03	Mean 73.17, SD 11.59	OR 1.01 (0.98–1.05), p 0.51
Early CDI recurrence	4.55% (3/66)	7.69% (1/13)	OR 1.74 (0.03–23.84), p 0.52	1.67% (1/60)	21.74% (5/23)	OR 15.74 (1.62–784.06), **p 0.01**
Early CDI recurrence, excl. severe CDI	4.55% (3/66)	8.33% (1/12)	OR 1.89 (0.03–26.16), p 0.49	1.69% (1/59)	22.73% (5/22)	OR 16.34 (1.67–816.13), **p 0.01**
Early CDI recurrence, excl. post sample at/after recurrence	—	—	—	1.67% (1/60)	5.26% (1/19)	OR 3.22 (0.04–260.97), p 0.43
Late CDI recurrence	21.21% (14/66)	0% (0/13)	OR 0 (0–1.42), p 0.11	18.33% (11/60)	13.04% (3/23)	OR 0.67 (0.11–2.92), p 0.75
Duration of vancomycin pre-treatment[b]	Median 17, IQR (10–29.25)	Median 9, IQR (5–18)	OR 0.92 (0.85–1), **p 0.04**	Median 17, IQR (10.75–29)	Median 13, IQR (6–24.5)	OR 0.98 (0.94–1.02), p 0.33
Non-*C. difficile*-targeted antibiotics 3 weeks post-FMT	—	—	—	7.55% (4/53)	27.27% (6/22)	OR 4.48 (0.93–24.51), p 0.06
Severe CDI	0% (0/66)	7.69% (1/13)	OR Inf (0.13-Inf), p 0.16	1.67% (1/60)	4.35% (1/23)	OR 2.65 (0.03–213.62), p 0.48
Prior CDI episodes	Mean 4.14, SD 1.22	Mean 4.15, SD 1.14	OR 1.01 (0.61–1.58), p 0.97	Mean 4.18, SD 1.26	Mean 4, SD 1.35	OR 0.89 (0.59–1.3), p 0.56
PPI use (yes)	42.86% (27/63)	50% (6/12)	OR 1.33 (0.32–5.58), p 0.75	44.64% (25/56)	40.91% (9/22)	OR 0.86 (0.28–2.6), p 0.80
Immunocompromised[c]	22.73% (15/66)	23.08% (3/13)	OR 1.02 (0.16–4.69), p 1	25% (15/60)	13.64% (3/22)	OR 0.48 (0.08–1.98), p 0.37
Medical history of IBD	12.12% (8/66)	0% (0/12)	OR 0 (0–3.29), p 0.35	13.56% (8/59)	4.35% (1/23)	OR 0.29 (0.01–2.42), p 0.43
Medical history of kidney disease	22.73% (15/66)	23.08% (3/13)	OR 1.02 (0.16–4.69), p 1	21.67% (13/60)	26.09% (6/23)	OR 1.27 (0.34–4.33), p 0.77

One out of 83 patients was labelled severely immunocompromised. Associations with $p < 0.05$ are highlighted in bold.
CDI, *Clostridioides difficile* infection; FMT, faecal microbiota transplantation; IBD, inflammatory bowel disease; IQR, interquartile range; PPI, proton pump inhibitors; SD, standard deviation.
[a] Patients with samples taken before or at the start of antibiotic pre-treatment for FMT were excluded from pre-FMT analyses (4/83).
[b] Pre-FMT: the number of days between the start of antibiotic pre-treatment and pre-FMT faeces collection.
[c] Immunocompromised classified according to Terveer et al. [4].

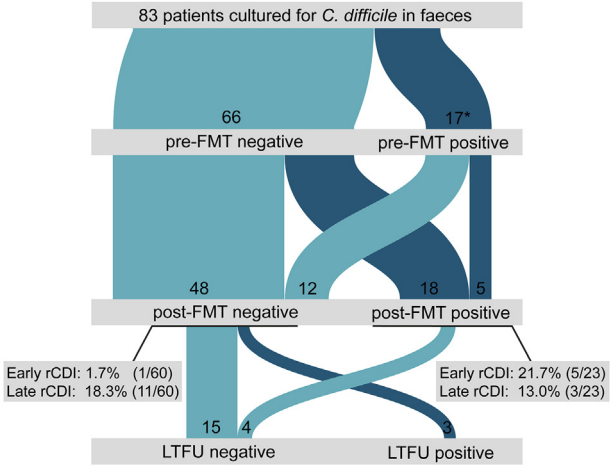

Fig. 2. Enrichment culture results for toxigenic *Clostridioides difficile* and CDI recurrence rates. Faeces samples were obtained approximately 1 day before FMT and 3 weeks after FMT. LTFU faecal samples were obtained approximately 1–3 years after FMT. *Four out of 83 pre-FMT samples were taken before the start of antibiotic pre-treatment for FMT. All were culture-positive for *C. difficile* (4/17) and excluded from relevant analyses (Fig. 1). FMT, faecal microbiota transplantation; LTFU, long-term follow-up; CDI, *C. difficile* infection; early rCDI, recurrence of *C. difficile* infection within 2 months post-FMT; late rCDI, recurrence of *C. difficile* infection later than 2 months post-FMT.

methods, we applied a previously described metagenomic sequencing-based approach to our shotgun sequencing data ($n = 45$) (Fig. 1) [21], yielding 48% sensitivity compared with enrichment culture (Table S11). Further comparison with PCR (*tcdB*) indicated an inverse association between metagenomic *C. difficile* (r_00721) relative abundance and PCR quantification cycle (Cq) values (Spearman $R = -0.78$, 95% CI: −0.93, −0.38, $p < 0.01$) with potential loss of metagenomic detection at PCR Cq > 32 (Fig. S2).

Gut microbiota composition relative to the presence of C. difficile

Post-FMT metagenomes were available for 43/83 patients (Fig. 1). In these data, no differences were observed in microbial alpha and beta diversity in relation to post-FMT *C. difficile* culture result (Fig. 3A–C). Visual inspection of microbiota composition (Fig. 3D) and specific species-level filtering, respectively, suggested increased relative abundance in *Enterobacteriaceae*, and decreased relative abundance in *Dorea longicatena* (r_03693), *Roseburia* species (m_12366) and *Clostridiales* species (m_12288) in *C. difficile* culture-positive patients post-FMT ($n = 10$), though none of the adjusted p values was significant (Fig. 3E and Table S12). Pre-FMT metagenomes were available for 43/83 patients (Fig. 1). Here, a disturbed microbiota composition was observed, with higher microbial diversity and richness in patients with positive *C. difficile* culture pre-FMT ($n = 9$), compared with culture-negative patients ($n = 34$) (Fig. S3A–C).

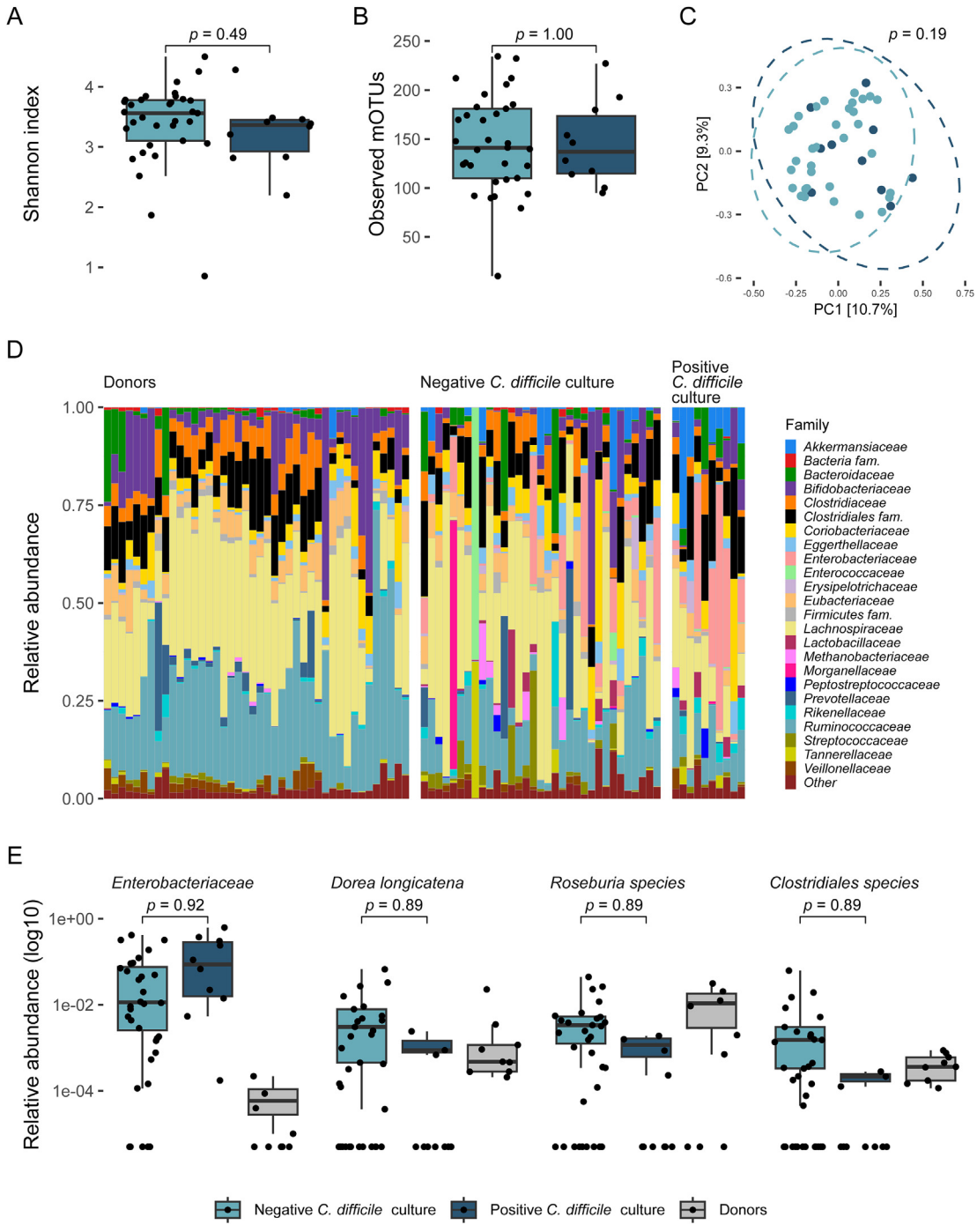

Fig. 3. Comparison of gut microbiota parameters between patients with negative versus positive culture results for *Clostridioides difficile* in faeces after faecal microbiota transplantation. (a) Microbial alpha-diversity (Shannon, mOTU level, rarefied). (b) Microbial richness, (mOTU level, rarefied). (c) Principal coordinate analysis (Bray-Curtis dissimilarity). (d) Microbial composition at family level of donor and patient samples. (e) Relative abundance of the top microbial family identified by differential abundance analysis (*Enterobacteriaceae*) and the top three (groups of) species with the largest negative generalised fold change: (*Dorea longicatena* [r_03693], *Roseburia* species incertae sedis [m_12366], Clostridiales species incertae sedis [m_12288]) (Table S12). Depicted p values from differential abundance analysis were adjusted for multiple comparisons. Donor samples were not included in this analysis and depicted for illustrative purposes. Where multiple FMT-treatments were produced by a single donor, median donor-specific relative abundance was calculated.

Discussion

To optimise and personalise FMT treatment, understanding *C. difficile* eradication and FMT outcomes in relation to patient characteristics, FMT treatment factors, and the gut microbiota is essential. In this study, we found *C. difficile* present in 23.4% of patients with rCDI clinically cured at 2 months post-FMT, and in 13.6% of patients 1–3 years post-FMT. Short duration of pre-FMT vancomycin and post-FMT administration of non-*C. difficile*-targeted antibiotics was identified as potential risk factors for early post-FMT CDI recurrence. Although microbial trends suggested associations with *C. difficile* culture results, no definitive microbiota

profiles linked with colonisation resistance against *C. difficile* were identified. These findings provide further insight into the effects of FMT and vancomycin pre-treatment on *C. difficile* eradication and CDI recurrence.

Using culture-based methods, we detected *C. difficile* in a high percentage of patients clinically cured by FMT, compared with a previous PCR-based study (23.4% vs. ~3%) [7], likely partially because of lower sensitivity of the PCR-based approach as reported by us and others [22]. Although most patients who were culture positive for *C. difficile* 2 months post-FMT were clinically cured, post-FMT *C. difficile* presence and post-FMT antibiotic administration were associated with early CDI recurrence. This aligns with literature identifying non-*C. difficile*-targeted antibiotics as a risk factor for CDI recurrence [15]. Therefore, although routine *C. difficile* testing after CDI treatment is not recommended in asymptomatic patients, persistent presence of *C. difficile* in high-risk patients, particularly those treated with non-*C. difficile*-targeted antibiotics post-FMT, may warrant increased vigilance for CDI recurrence [4,9].

A recent study detected *C. difficile* in only 30% of patients with active CDI, using metagenomic shotgun sequencing [21], which was potentially attributed to the initiation of anti-*C. difficile* antibiotics during sampling. Using the same method, we detected *C. difficile* in only 12.2% of samples, compared with 25.6% using enrichment culture and subsequent selection of toxigenic isolates. Additionally, we observed an inverse association between metagenomic relative abundance and PCR Cq values, with no metagenomic detection at Cq values > 32, suggesting a potential detection limit. These results imply that metagenomic shotgun sequencing may not (yet) be comparable to enrichment culture or PCR in a clinical microbiology setting.

In patients with rCDI undergoing FMT, antibiotic pre-treatment serves to diminish and eradicate *C. difficile* and alleviate symptoms [9]. Early consensus recommended at least 4 days of pre-FMT antibiotic treatment [1,23,24]. In our study, a short pre-FMT vancomycin duration (~6 days) was associated with early CDI recurrence, compared with ~18 days in those cured at 2 months. These results imply that completing at least the standard 10-day vancomycin course, as recommended in CDI treatment guidelines, may be preferred pre-FMT [9].

This study found no association between the presence of *C. difficile* in faeces post-FMT and gut microbial diversity, contrasting with previous studies reporting decreased diversity in patients asymptomatically carrying *C. difficile* [21,25]. Further microbiota analysis showed non-significant trends related to the presence of *C. difficile* post-FMT: a potential increase in relative abundance of *Enterobacteriaceae* and decreases in *D. longicatena*, and groups of *Roseburia* and *Clostridiales* species. Although similar trends have been described [21,26,27], large metagenomic studies are required to accurately characterise potential microbial signatures associated with *C. difficile* post-FMT. In pre-FMT samples, *C. difficile* eradication was associated with reduced microbial diversity, possibly reflecting collateral damage caused by effective anti-*C. difficile* antibiotics on the microbiota [28].

This study has limitations. The sample size of our cohort lacked power for accurate analyses of FMT donor selection and microbial differential abundance, likely leading to (over)conservative results. Furthermore, the centralised role of the NDFB, facilitating countrywide treatment at local hospitals, introduced practical limitations in sample collection and variability in collection date. In some patients, the post-FMT sampling timepoint overlapped with early CDI recurrence, complicating the analysis of *C. difficile* presence as a risk factor for early CDI recurrence. Finally, the rCDI population is a complicated target group with many treatment-demanding pre-existent comorbidities, inducing confounding factors in the (microbiota) analyses [4].

Conclusions

In conclusion, *C. difficile* was present in the faeces in nearly a quarter of FMT-cured patients with rCDI, indicating that complete eradication is not essential for clinical cure. Nevertheless, our results imply that *C. difficile* eradication and completion of the standard 10-day vancomycin course pre-FMT may help prevent early CDI recurrence, besides avoiding post-FMT antibiotics. Standardised sample collection of patients with rCDI, combined with close monitoring of clinical history and follow-up in (inter)national FMT registries [29,30], will enable future studies to better characterise microbiota signatures specific to *C. difficile* eradication and CDI recurrence, aiding the development of improved treatment strategies.

Author contributions

BG and EMT were responsible for writing – original draft. EMT and JJK were responsible for conceptualisation. BG, IMJGS and EKLB were responsible for the investigation. BG, EMT, AK, AJvdB and QRD were responsible for formal analysis. BG, EMT, EvL, AJvdB, EKLB, EvN, JvP, AG, EJK and JJK were responsible for data curation. EMT, JJK, MW, WKS and QRD were responsible for supervision. All authors reviewed the manuscript and approved the final version.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2024.12.003.

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