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Successful treatment of Clostridioides difficile infection with single-donor faecal microbiota transplantation capsules

Frederik Cold[1, 2, 3], Camilla Kara Svensson[4], Alice Højer Christensen[2], Stig Günther[2], Andreas Munk Petersen[1, 5], Lars Hestbjerg Hansen[3] & Morten Helms[4]

1) Gastrounit, Medical Division, Copenhagen University Hospital – Hvidovre Hospital, 2) Department of Gastroenterology, Aleris-Hamlet Hospitals, Copenhagen, 3) Department of Plant and Environmental Sciences, Copenhagen University, 4) Department of Infectious Diseases, Copenhagen University Hospital – Hvidovre Hospital, 5) Department of Clinical Microbiology, Copenhagen University Hospital – Hvidovre Hospital, Denmark

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ABSTRACT

Introduction. Treatment of recurrent *Clostridioides difficile* infection with faecal microbiota transplantation (FMT) is highly effective and is the recommended treatment following a second recurrence. The cure rates of capsule treatment are high (82%-88%). Whether using multi-donor or single-donor FMT capsules affects cure rates remains incompletely understood.

Methods. A retrospective case series of patients with recurrent, refractory or fulminant *C. difficile* infection treated for three days with single-donor FMT capsules from October to December 2020 was conducted. The aim of the study was to investigate the clinical efficacy (cure rate) of the treatment and to compare cure rates with previously reported cure rates of treatment with multi-donor FMT capsules produced at the same stool bank. Clinical cure was defined as absence of diarrhoea or diarrhoea with a *C. difficile* negative stool sample eight weeks after treatment.

Results. Clinical cure was observed in 15 of the 18 (83.3%) patients following three days of FMT capsule treatment. Cure rates were comparable (p = 1.0) to previously reported cure rates (88.9%) of multi-donor FMT capsule treatment of recurrent *C. difficile* infection.

Conclusions. Three days of single-donor FMT capsule treatment was effective and safe in the treatment of recurrent, refractory and fulminant *C. difficile* infection with cure rates comparable to those of multi-donor FMT capsule treatment.

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Trial registration. not relevant.

Clostridioides (formerly *Clostridium*) *difficile* is a spore-forming bacterium that, under certain conditions, releases toxins resulting in infectious diarrhoea [1]. The primary treatment of *Clostridioides difficile* infection is antibiotics (vancomycin or fidaxomicin) [2]. In case of recurrent *C. difficile* infection, treatment with faecal microbiota transplantation (FMT) from healthy faecal donors may be considered. FMT treatment has shown superiority to antibiotics and is currently the recommended treatment option following a second recurrence of *C. difficile* infection [3, 4].

FMT treatment of recurrent *C. difficile* infection is delivered through upper or lower endoscopy, enemas or capsules. The highest cure rates have been reported when using repeat FMT delivered through lower endoscopy (88%-100%), whereas the efficacy of a single treatment regimen with FMT capsules treatment is 82%-88% [5, 6]. The exact mechanisms behind the successful effect of FMT in the treatment of recurrent *C. difficile* infection are not fully understood. The transfer of beneficial bacteria, virus and metabolites from the donor to the recipient is believed to cause the beneficial effects [7].

The incomplete understanding of the mechanisms of action and the transfer of a still not fully characterised living microbial community has made safety following FMT treatment a natural concern among clinicians and regulatory institutions [8]. Continuous efforts are being made to improve the understanding of the treatment and increase its safety, while keeping efficacy high [9]. This is done through ongoing improvements in the screening of donors and transferred material, but also by maintaining registries assessing the long-term risks of FMT.

When guidelines for screening of donors and faecal material are followed, FMT treatment is generally regarded safe both in the short- and the long term, although rare cases of transfer of multi-drug resistant bacteria have been reported [8, 10, 11].

Based on the hypothesis that multi-donor treatment with FMT capsules increases the chance of transferring beneficial microorganisms, this approach has previously been reported with high cure rates in the treatment of recurrent *C. difficile* infection by our study group [12]. However, multi-donor FMT treatment potentially increases the risk of transferring harmful microorganisms. Furthermore, as traceability might be impaired when using multi-donor FMT, the current state-of-the-art FMT treatment of recurrent *C. difficile* infection is to use single donors [13].

The aim of this study was to evaluate the effects of three-day single-donor FMT capsule treatment of recurrent *C. difficile* infection and to compare the efficacy to previous data on multi-donor treatment provided from the same FMT stool bank.

METHODS

All patients from the capital region of Denmark presenting with recurrent or refractory *C. difficile* infection where FMT is considered a treatment option are referred to Copenhagen University Hospital – Hvidovre Hospital (CUHH), Department of Infectious Diseases.

The classifications of *C. difficile* infection severity and the definition of clinical cure used at the CUHH are based on current Danish and international recommendations [2, 4]. Recurrent *C. difficile* infection was defined as diarrhoea with the passage of ≥ 3 loose or liquid stools daily and a faecal sample positive for *C. difficile* (a positive polymerase chain reaction test result for *C. difficile* toxin A, toxin B or binary toxin) within 90 days after a former episode of *C. difficile* infection treated with vancomycin, metronidazole or fidaxomicin.

Refractory *C. difficile* infection was defined as persistent diarrhoea with the passage of ≥ 3 loose or liquid stools daily despite at least five days of ongoing treatment with vancomycin or fidaxomicin.

Severe *C. difficile* infection was defined as *C. difficile* infection with one or more of the following: abdominal pain, hypoalbuminaemia, leukocytosis, leukopenia or renal failure.

Fulminant *C. difficile* infection was defined as severe *C. difficile* infection with one or more of the following: altered mental status, fever (> 38.5 °C), hypotension, septic shock, multi-organ failure, treatment at an intensive care unit, ileus, toxic megacolon or pseudomembranous colitis.

Besides FMT capsules, FMT delivered through enemas and endoscopy, rectal bacteriotherapy (RBT) and

antibiotics are used in the treatment of recurrent, refractory or severe *C. difficile* infection [14]. The choice of treatment at the CUHH for an episode of *C. difficile* infection is based on patient preferences, the clinical decision of a physician and the current status of available treatments.

Treatment

Single-donor FMT capsules from the Aleris-Hamlet FMT Stool Bank were used in the treatment of recurrent, refractory and fulminant *C. difficile* infection from 1 October to 31 December 2020. Prior to FMT treatment, patients were pre-treated with oral vancomycin 125 mg (recurrent *C. difficile* infection) or 500 mg (refractory or fulminant *C. difficile* infection) × 4 daily for 7-10 days until two days before FMT treatment.

Regardless of previous treatment, all patients receiving FMT capsules from the Aleris-Hamlet FMT Stool Bank were treated with 25 FMT capsules daily on three consecutive days, taken in the morning following 6 h of fasting.

Donor selection and screening

Potential donors were screened vigorously through medical interviews and through blood and faecal samples in accordance with international guidelines [15]. Faecal samples were additionally screened every second week of the donation period **(see supplementary material: https://ugeskriftet.dk/files/a09210712_-_supplementary.pdf)**.

In addition to the international recommendations, a donor with a healthy lifestyle including daily physical activity, a healthy diet, low alcohol intake and no smoking was prioritised. A single male donor meeting all these criteria was used in the production of the FMT capsules.

Faecal microbiota transplantation capsule production

The donor was equipped with 500 ml bottles of oxygen-reduced sterile saline (0.9% NaCl). Immediately after producing the sample, the donor was instructed to cover the sample with the oxygen-reduced saline and to deliver it to the laboratory within 1 h. Hereafter, the sample was stored at 5 °C and processed in the laboratory no longer than 3 h from the time of delivery. Sample and saline were homogenised, filtered and centrifuged. The supernatant was discarded and the pellet mixed with 99.9% glycerol as a cryoprotectant to a final concentration of 33%. Most of the process was done in an anaerobic environment using argon gas to protect the sample and thereby minimising the time the faecal material was exposed to oxygen in atmospheric concentrations to less than one minute. Finally, the samples were frozen at –20 °C for 24 h, then at –50 °C for 24 h, and finally frozen at –80 °C for storage until use. Once the donor had passed the screening after eight weeks, the material was double encapsulated using Capsugel DR Caps size 0 and 00. Each capsule contained 0.6 ml of material; hereof 0.4 ml faecal material and 0.2 ml glycerol. A daily dosage of 25 capsules provides approximately 10 g of concentrated faecal matter derived from approximately 50 g of faeces. Capsules were stored at –80 °C until use.

Follow-up

All patients were followed for treatment response for at least eight weeks through telephone interviews by a medical doctor. The primary outcome of clinical resolution (cure) was defined as resolution of diarrhoea or diarrhoea with a negative stool test for *C. difficile* at least eight weeks after treatment. Only patients with ongoing diarrhoea after eight weeks were systematically tested for *C. difficile*. Microbiological recurrence of *C. difficile* and cases of multi-drug resistant bacterial infections or carriage were evaluated for up to six months following FMT capsule treatment through access to the national Danish microbiological database including all analysed microbiological samples from all clinical microbiological departments in Denmark.

Serious adverse events were defined as death, hospitalisation or prolongation of hospitalisation, disability or permanent damage in the six months following treatment with FMT capsules and were assessed through the patients' health records.

Data extraction was performed independently by two investigators (FC and CKS). In the event of any dispute, the key decision was made by a third investigator (MH).

Statistics

Fisher's exact test was used to compare difference in proportions of clinical cure following primary and secondary treatment with results from a previous publication using multi-donor FMT capsules also from the Aleris-Hamlet Stool Bank [12].

Ethics

The present retrospective study was conducted as part of an internal quality assessment and approved by the Hospital Directory Board on 21 January 2021.

Trial registration: not relevant.

RESULTS

A total of 18 patients were treated for *C. difficile* infection with single-donor FMT capsules produced at the Aleris-Hamlet FMT Stool Bank (**Table 1**). Patients were aged from 22 to 87 years and had a median of two recurrences of *C. difficile* infection. A single patient was treated with FMT capsules as treatment of the first episode of *C. difficile* infection due to fulminant *C. difficile* infection. Nine patients were treated with FMT capsules as primary treatment of recurrent *C. difficile* infection (**Figure 1**) and another nine patients previously unsuccessfully treated with RBT or FMT received the FMT capsules as secondary treatment (**Figure 2**). Clinical cure was observed in 15 of the 18 (83.3%) patients following a single three-day treatment with FMT capsules. Eleven patients had resolution of diarrhoea and were not tested for *C. difficile* again, two patients had resolution of diarrhoea and a negative stool test for *C. difficile* and two patients had ongoing diarrhoea and a negative stool test for *C. difficile*. One patient without clinical cure was successfully re-treated with serial FMT capsules also from the Aleris-Hamlet Stool Bank for six days, bringing the total of patients with clinical cure up to 16 patients (88.9%) following re-treatment.

TABLE 1 Patients receiving faecal microbiota transplantation capsules in treatment of *Clostridioides difficile* infection.

	As primary treatment of recurrent, refractory or fulminant C. difficile infection	As secondary treatment of recurrent, refractory or fulminant C. difficile infection following unsuccessful FMT or RBT treatment
N	9	9
Age, median (range), yrs	55 (22-77)	77 (31-87)
C. difficile infection recurrences, median (range), n	2 (1-3)	3 (2-6)
C. difficile infection level, n		
Recurrent	7	8
Refractory	2	0
Fulminant	0	1[a]
Charlson Comorbidity Index, median (range)	3 (0-5)	5 (0-10)
Males/females, n	4/5	2/7
C. difficile subtype, n		
Non CD027	4	2
Non CD027: cdtA+	2	0
Non CD027: toxin A + toxin B	0	3
Non CD027: toxin A + toxin B and binary toxin	0	1
Not specified	3	3

cdtA$^+$ = *C. difficile* binary toxin A; FMT = faecal microbiota transplantation; RBT = rectal bacteriotherapy.
a) The patient's episode of infection was the first episode of *C. difficile* infection.

FIGURE 1 Clinical resolution following faecal microbiota transplantation (FMT) capsules as primary treatment of recurrent *Clostridioides difficile* infection following antibiotics.

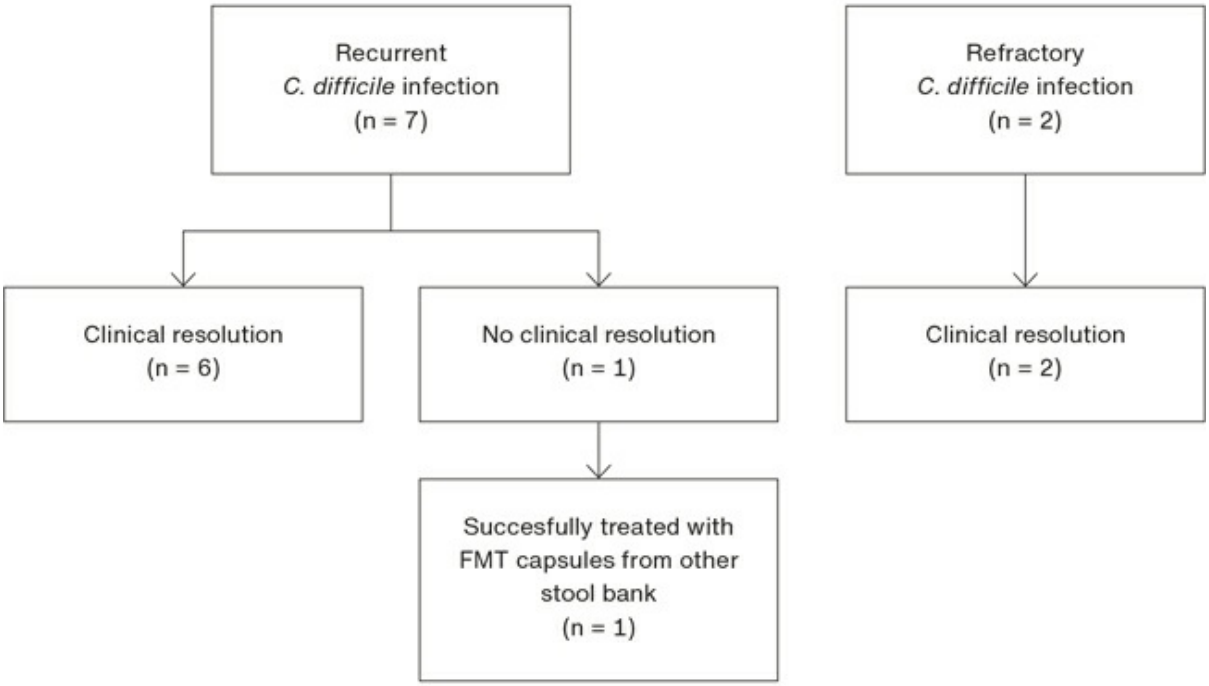
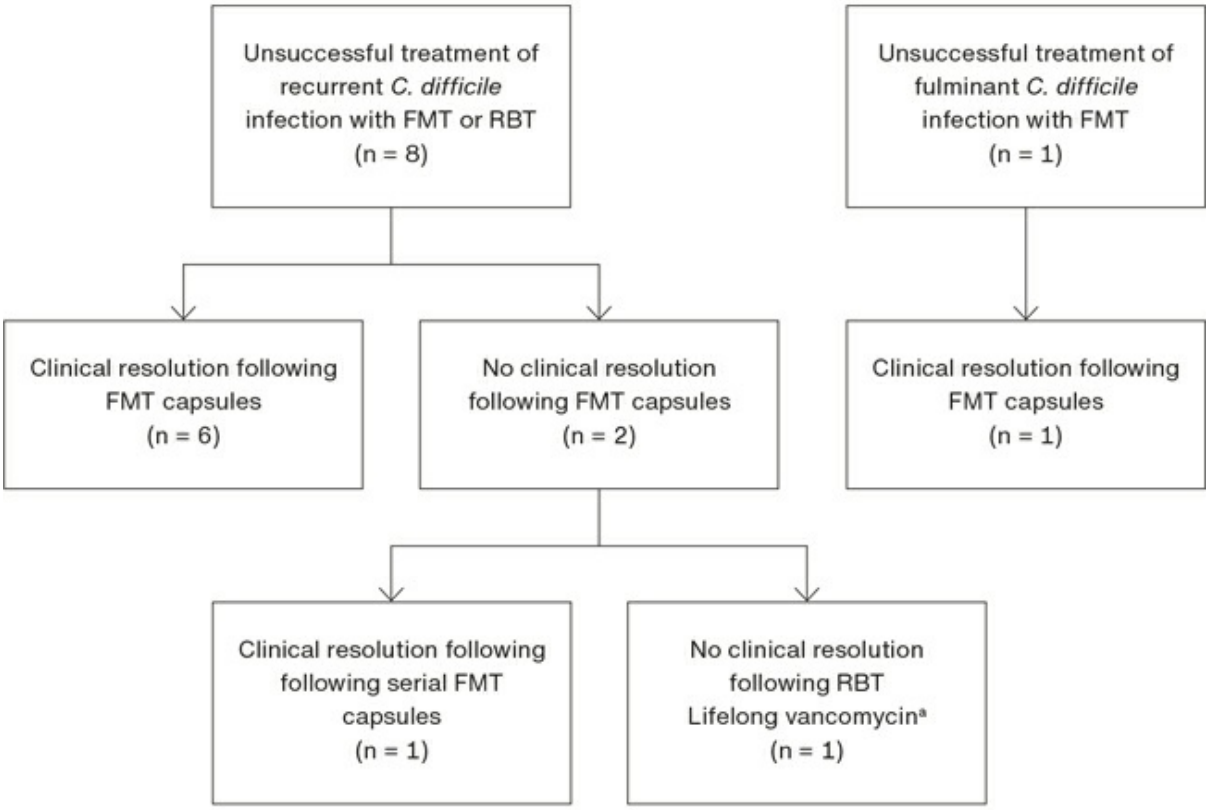


FIGURE 2 Clinical resolution following faecal microbiota transplantation (FMT) capsules as secondary treatment of recurrent *Clostridioides difficile* infection following antibiotics and unsuccessful treatment with FMT or rectal bacteriotherapy (RBT).



a) Patient received three types of antibiotics within the first eight weeks following FMT: penicillin, dicloxacillin and cephalosporine.

Recurrence of Clostridioides difficile in the follow-up period

Two of the 16 successfully treated patients were diagnosed with recurrence of *C. difficile* infection in the six months following FMT treatment.

Cases of multi-resistant bacterial infections

No cases of multi-resistant bacterial infections were recorded in the six months following FMT capsule treatment.

Serious adverse events

Seven patients were hospitalised in the six months following FMT capsule treatment. In one patient withworsening of ulcerative colitis following treatment, the hospital admission was considered possibly related to the treatment. The other hospital admissions were considered unrelated to treatment. Two patients died in the six months following FMT capsule treatment. Neither of these deaths was considered to be related to their treatment.

Clinical efficacy compared with multi-donor faecal microbiota transplantation capsules

In a previously published case series from our study group, clinical cure was observed in eight of nine (88.9%) patients with recurrent *C. difficile* infection treated with three days of multi-donor FMT capsules produced at the

Aleris-Hamlet FMT Stool Bank [12]. Clinical cure following re-treatment with FMT capsules was observed in the ninth patient. Comparing the cure rates of treatment with single-donor FMT capsules to the previous results of treatment with multi-donor FMT capsules, we observed no statistically significant difference in primary ($p = 1.0$) or secondary cure rates ($p = 0.54$).

DISCUSSION

The results from this case series show that a single-donor FMT capsule treatment was effective in the treatment of recurrent, refractory and fulminant *C. difficile* infection. The cure rate of 83.3% was comparable to previously published results from treatment with multi-donor FMT capsules from our study group. The reported cure rates are in line with those reported in a recently published meta-analysis, including all studies investigating the effects of FMT capsules in recurrent *C. difficile* infection reporting a primary cure of 85% (95% confidence interval: 82%-88%) across all studies [5].

The strength of this study was that we were able to compare cure rates between treatment with single- and multi-donor FMT capsules from the same FMT stool bank previously used in the same trial site giving us the opportunity to compare cure rates following treatment. Several limitations apply to the study why some of the conclusions should be considered with caution. The small sample size and the non-controlled set-up make it impossible to conclude whether single-donor capsules are truly equally effective to multi-donor capsules. Furthermore, we were unable to present microbiome analysis before and after treatment. The donor in this case series also provided faeces to the multi-donor FMT capsules. Therefore, the single donor might have accounted for the efficacy of the multi-donor capsules.

Many unanswered questions remain in relation to the treatment of recurrent *C. difficile* infection using FMT capsules. Based on current knowledge, procedural differences, including the use of multi-donor treatment, do not seem to affect clinical cure rates [5, 16]. Due to safety concerns of multi-donor treatment and the high cure rates following single-donor treatment, future use of multi-donor FMT treatment of recurrent *C. difficile* infection must be considered obsolete.

Our reported short-term safety of the treatment adds to the current understanding that FMT treatment is safe in the short term when screening of donors and faecal samples are performed in accordance with international guidelines [13, 17]. To improve the safety of FMT treatment of recurrent *C. difficile* infection, we urge researchers to further develop treatment regimens to increase the safety of the treatment, while keeping the efficacy of the treatment high. Two approaches to achieving this may be to determine the lowest effective treatment dose or to only transfer sterile faecal material without the bacterial fraction, called faecal virome transplantation, which has shown promising results [18, 19].

CONCLUSIONS

This case series on three-day single-donor FMT capsule treatment shows that the treatment was safe and effective in the treatment of recurrent, refractory and fulminant *C. difficile* infection. The treatment was as effective as previously published results using multi-donor FMT capsules. Further developments of FMT capsule treatment are needed to improve safety while keeping the efficacy of the treatment high.

Correspondence *Morten Helms*. E-mail: Morten.Helms@regionh.dk

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Conflicts of interest Potential conflicts of interest have been declared. Disclosure forms provided by the authors are available with the article at ugeskriftet.dk/dmj

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