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REVIEW

Recurrent *Clostridium difficile* infections: The importance of the intestinal microbiota

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Abstract

Clostridium difficile infections (CDI) are a leading cause of antibiotic-associated and nosocomial diarrhea. Despite effective antibiotic treatments, recurrent infections are common. With the recent emergence of hypervirulent isolates of *C. difficile*, CDI is a growing epidemic with higher rates of recurrence, increasing severity and mortality. Fecal microbiota transplantation (FMT) is an alternative treatment for recurrent CDI. A better understanding of intestinal microbiota and its role in CDI has opened the door to this promising therapeutic approach. FMT is thought to resolve dysbiosis by restoring gut microbiota diversity thereby breaking the cycle of recurrent CDI. Since the first reported use of FMT for recurrent CDI in 1958, systematic reviews of case series and case report have shown its effectiveness with high resolution rates compared to standard antibiotic treatment. This article focuses on current guidelines for CDI treatment, the role of intestinal microbiota in CDI recurrence and current evidence about FMT efficacy, adverse effects and acceptability.

Key words: *Clostridium difficile* infection; *Clostridium difficile* recurrence; Fecal microbiota transplantation; Stool transplantation; Microbiota

Core tip: Despite current antibiotic treatments, *Clostridium difficile* infection (CDI) is a growing epidemic with increasing rates of recurrence, severity and mortality. The treatment of recurrent CDI thus represents a real challenge. This article simultaneously focuses on current guidelines for CDI treatment, the role of gut microbiota in CDI recurrence and current evidence about fecal microbiota transplantation (FMT) efficacy, adverse effects and acceptability. According to studies published to date, FMT use for recurrent CDI is associated with high resolution rates compared with standard antibiotic treatment. Further studies are needed to confirm FMT effectiveness, and to determine the long-term consequences and good administration practices.

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INTRODUCTION

Clostridium difficile (*C. difficile*) infection (CDI) are the leading cause of nosocomial diarrhea, representing 20%-30% of diarrhea caused by antibiotics, and mortality is estimated at 2%[1,2]. Recent data from 28 community hospitals in the United States suggest that *C. difficile* has become the leading cause of healthcare-associated infection ahead of methicillin-resistant *Staphylococcus aureus*[3]. The increasing incidence of CDI among hospitalized and outpatients is

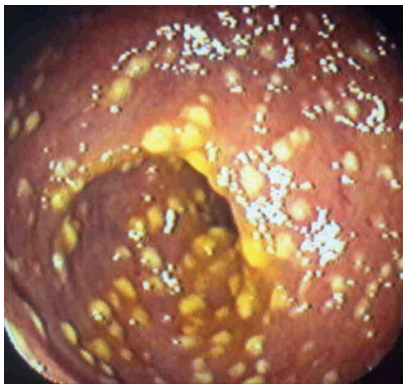

Figure 1 Colonoscopy showing typical yellow pseudomembranes that cover superficial mucosal ulcerations.

a real public health challenge with an increasing incidence from 30 per 100000 in 1996 to 84 per 100000 in 2005 in American acute care hospitals[4]. Indeed this is associated with an annual cost in the United States of an estimated 1 billion dollars[5]. Recent occurrence of severe *C. difficile* disease and higher mortality rates have been associated with the emergence of strains with increased virulence, the so-called "hypervirulent" isolates that belong to the BI/NAP1/027 category and which are fluoroquinolone-resistant[6].

The main *C. difficile* virulence factors are two exotoxins, the enterotoxin TcdA and the cytotoxin TcdB: their actions on the cytoskeleton and tight junctions result in decreased transepithelial resistance, fluid accumulation, and destruction of the intestinal epithelium. They also cause the release of inflammatory cytokines and promote neutrophil chemotaxis, thereby contributing to the mucosal injury[7].

Regardless of the treatment provided, and despite its effectiveness, more than 25% of patients will have a recurrence within 1 to 3 mo[8]. Taken together, CDI treatment today represents a therapeutic challenge because of the high prevalence of CDI, a significant rate of recurrence, and the recent emergence of the hypervirulent strain BI/NAP1/027. Considering the recent better understanding of gut microbiota and the importance of dysbiosis in the pathophysiology of recurrent CDI, there is growing interest in alternative therapeutic approaches, such as fecal microbiota transplantation (FMT) for patients in whom standard antibiotic therapy has failed. In this article we will review the current guidelines for CDI treatment, the importance of gut microbiota and its imbalance in CDI, and current evidence about FMT use in CDI treatment.

CLOSTRIDIUM DIFFICILE INFECTION

Risk factors

A retrospective study published in 2003 identified independent risk factors for CDI occurrence, as listed in Table 1[9]. Among them, the use of antibiotics was identified as the most important. Indeed, over 90% of patients with CDI received an antibiotic within 14 d prior to infection.

Table 1 Independent risk factors for *Clostridium difficile* infections (adapted from[9])

Antibiotic use (especially 3rd generation cephalosporins, fluoroquinolones)
Patients older than 60 yr
Admission in previous 60 d
Use of proton pump inhibitors and histamine-2 blockers
Use of anti-motility agent
Mechanical ventilation
Hypoalbuminemia

Table 2 Clinical presentation of *Clostridium difficile* infections (adapted from[44])

	Clinical manifestations	Laboratory and imaging studies
C. difficile diarrhea	Diarrhea Abdominal pain +/- fever	Colonoscopy: unremarkable
C. difficile colitis	Diarrhea Abdominal pain Fever	Leukocytosis Colonoscopy: patchy or diffuse erythematous colitis without pseudomembranes
Pseudomembranous colitis	Diarrhea Abdominal pain Fever	Leukocytosis Colonoscopy: pathognomonic pseudomembranes (yellow plaques 2-20 mm)
Fulminant colitis	Profuse diarrhea or ileus Abdominal pain Fever +/- signs of shock	Leukocytosis (sometimes white blood cell count > 4×10^9/L) Elevated serum lactate Sigmoidoscopy: pseudomembranes Abdominal computed tomography scanner: megacolon, +/- bowel perforation

C. difficile: *Clostridium difficile*.

Fluoroquinolones and beta-lactams are particularly associated with CDI, and the risk increases with antibiotic duration and dose[9].

Clinical presentation of CDI

The diagnosis of CDI is based on (1) clinical evidence (presence of moderate to severe diarrhea or ileus); and (2) microbiological detection of *C. difficile* in stool [*C. difficile* toxin detection by PCR (sensitivity 90%; specificity 96%) or stool culture] or compatible endoscopic appearance or histopathologic evidence[1] (Figure 1). The presence of diarrhea should raise suspicion of CDI and further investigations should only be undertaken in the case of strong clinical suspicion owing to the high prevalence of asymptomatic carriers among hospitalized patients (7%-20%)[2].

C. difficile infections include a broad spectrum of clinical presentations. Assessing the severity of an episode is of particular importance because it will determine the choice of treatment (Tables 2 and 3). There is no consensus about the definition of a severe episode, but American and European experts agree that a severe CDI is associated with one or more of the following features:

Table 3 Severity of *Clostridium difficile* (adapted from[1,10,11])

Severity criteria according to American experts	Non severe CDI	White blood cell count < 15 × 10^9/L and creatinine level < 1.5 × baseline
	Severe CDI	White blood cell count > 15 × 10^9/L or creatinine level > 1.5 × baseline
	Severe and complicated CDI	Hypotension or shock or ileus or megacolon
Severity criteria according to European experts	Severe CDI	Age > 65 yr or severe comorbidities or intensive care admission or immunodeficiency or Presence of ⩾ 1 of the following criteria: Fever ⩾ 38.5 ℃ Shivering Hemodynamic instability Signs of peritonitis Signs of ileus White blood cell count > 15 × 10^9/L Creatinine level > 1.5 × baseline Elevated serum lactate Pseudomembranous colitis (colonoscopy) Distension of large intestine (computed tomography, CT scan) Colonic wall thickening (CT scan) Pericolonic fat stranding (CT scan) Ascites not explained by other causes

CDI: *Clostridium difficile* infection; CT: Computed tomography.

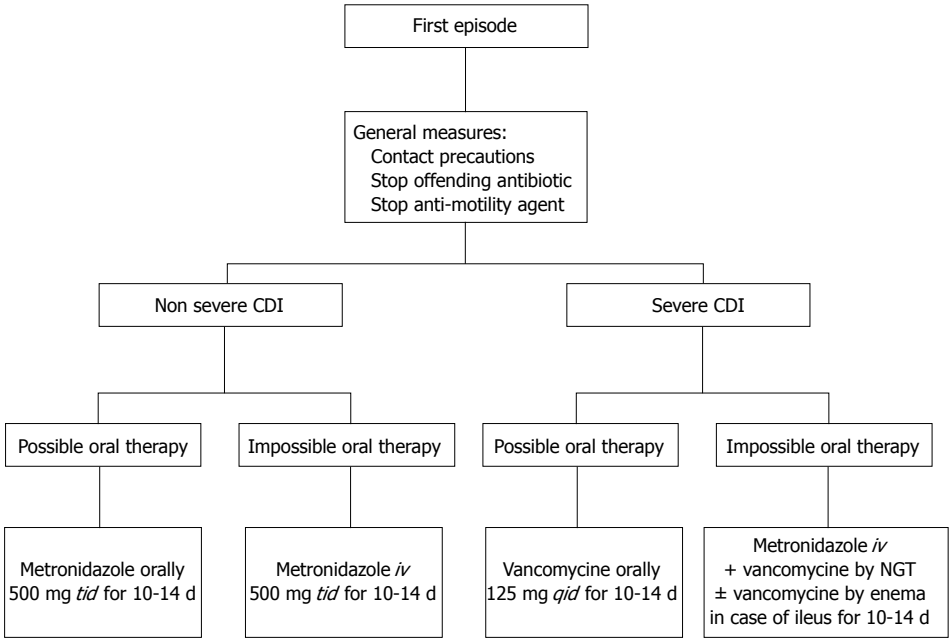

Figure 2 Algorithm for treatment of *Clostridium difficile* infections (adapted from[1,10,11]). *iv*: Intravenously; NGT: Nasogastric tube; CDI: *Clostridium difficile* infection.

clinical signs of severe colitis, an increase in serum creatinine of more than 50% of baseline, leukocytosis greater than 15 × 10^9/L, advanced age (⩾ 65 years old) and serious comorbidities[1,10].

Treatment response is present when either stool frequency decreases or stool consistency improves, parameters of disease severity improve and no new signs of severe disease develop; treatment response should be evaluated after at least three days[11]. After clinical response, it may take weeks for stool consistency and frequency to become entirely normal. After resolution of an episode, CDI recurs in about 25% of cases, regardless of the treatment provided (metronidazole or vancomycine) and its effectiveness[8]. Recurrence is defined as the return of symptoms within 8 wk after successful treatment[1]. Some factors are associated with a high risk of recurrence: patient's age (older than 65 years), further use of antibiotics, a low rate of anti-toxin A IgG, and a prior episode of CDI (the risk increases with the number of recurrences: 45% following the second episode and 65% after the third)[8].

Current treatment

The European Society of Clinical Microbiology and Infectious Diseases and the Infectious Disease Society of America have proposed recommendations for CDI treatment[1,10,11]. First, they offer some general measures such as stopping any offending antibiotic and anti-motility

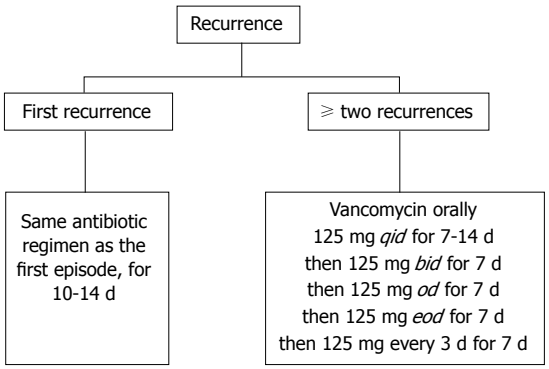

Figure 3 Algorithm for treatment of recurrent *Clostridium difficile* infections (adapted from[1,10,11]). eod: Every other day.

agents and establishing contact precautions[1,10]. For the treatment of a first non-severe episode, metronidazole is the first-line treatment. Indeed, randomized controlled trials have shown that metronidazole is as effective as vancomycin, and its use would prevent the appearance of vancomycin-resistant *Enterococcus*[12,13] (Figure 2). For the treatment of a first severe episode of CDI, vancomycin is used[14].

According to guidelines, the first recurrence should be treated with the same antibiotic as for the first episode[15] (Figure 3). For the second and any subsequent recurrences, metronidazole should not be used because of its potential neurotoxicity, so a taper regimen of vancomycin is employed for 35 to 42 d. This later recommendation is based primarily on the results of an observational study of 163 patients with recurrent CDI which compared metronidazole and different regimens of vancomycin. The highest success rate was achieved with a taper regimen of vancomycin (69% *vs* 30%-57% for other treatments)[16].

Recently, the FDA approved the use of fidaxomycin for the treatment of recurrent CDI. Fidaxomycin is a macrocyclic antibiotic characterized by little or no systemic absorption after oral administration and a narrow spectrum of activity against Gram-positive aerobic and anaerobic bacteria. This treatment is comparable to vancomycin in terms of resolution (88% *vs* 86%, respectively), but is associated with a lower risk of recurrence 4 wk after cessation of treatment (13%-15% *vs* 25%-27%)[17]. However, there is no prospective randomized controlled trial that investigated fidaxomicin's efficacy in patients with multiple recurrences of CDI; vancomycin is preferably administered using tapered regimen[11].

Concerning the use of probiotics, a meta-analysis concluded that probiotics composed of *Saccharomyces boulardii* or *Lactobacilli* could be used to prevent antibiotic-associated diarrhea[18]. A Cochrane systematic review concluded that even if the efficacy of using probiotics together with antibiotics seems to be superior for CDI treatment, there is not yet sufficient evidence to systematically recommend their use[19].

The importance of microbiota in CDI recurrence

The pathophysiologic features of recurrent CDI are not fully understood but likely involve two mechanisms: the resistance of *C. difficile* to metronidazole and vancomycin, and most importantly, the phenomenon of dysbiosis. The risk of recurrence is approximately 25% after a first CDI episode and dramatically increases with subsequent CDI recurrences[8]. Half of cases is considered as a relapse (*C. difficile* spores are not destroyed by antibiotics and can germinate to vegetative forms after antibiotic therapy), and the other half as a re-infection (infection by a new strain)[8].

Until recently, the lack of resistance of *C. difficile* to vancomycin and metronidazole seemed to be well demonstrated. However, recent studies have shown some resistance mechanisms of *C. difficile* thanks to new analytic methods able to stabilize and study *C. difficile* taken out of the gut[20].

Gut microbiota and its imbalance, called dysbiosis, has a crucial role in the pathophysiology of CDI recurrence. Over the last decade, an emerging consensus has formed about the importance of the intestinal microbiota, which has been considered similar to an organ. Gut microbiota is composed of more than 100 to 1000 microbial species that live in a host-microbe symbiotic relationship[21]. Among all gut bacterial phyla, *Bacteroides* and *Firmicutes* predominate[22]. The main functions of the microbiota can be classified as protective (commensal bacteria offer a resistance to colonization by enteric pathogens), metabolic (*e.g.*, some bacteria contribute to the catabolism of carbohydrates and to the synthesis of some vitamins), and immunologic (*e.g.*, some bacteria can activate regulatory T cells and induce a tolerance to innocuous antigens)[21,23,24].

Dysbiosis is associated with a number of diseases such as antibiotic-associated diarrhea, irritable bowel syndrome, inflammatory bowel diseases (IBDs)[25] and CDI recurrence.

Concerning CDI recurrence, antibiotics generate dysbiosis that is characterized by a reduced diversity of the microbiota, development of opportunistic species (*e.g.*, *Escherichia coli, Proteus mirabilis,* and *Enterococcus faecalis*), loss of resistance to colonization and increased synthesis of pro-inflammatory cytokines[26]. These disturbances promote colonization and infection with *C. difficile*, which further contributes to the dysbiosis (Figure 4)[27]. Standard antibiotic treatments and recurrent episodes contribute to the development of a vicious cycle.

Although there is an association, but no clear cause-effect relationship between dysbiosis and some gastrointestinal diseases, there is great interest in therapeutic approaches that could restore the equilibrium of the gut microbiota and improve these conditions. Probiotics are defined as "live micro-organisms which, when administered in adequate amounts as part of food, confer a health benefit on the host" (Joint FAO/WHO Expert consultation 2001)[28]. A meta-analysis concluded that the use of probiotics in combination with antibiotics in CDI treatment had no clear benefit in terms of recurrence risk compared to antibiotics alone[18]. However, according to preliminary results of an ongoing randomized placebo-

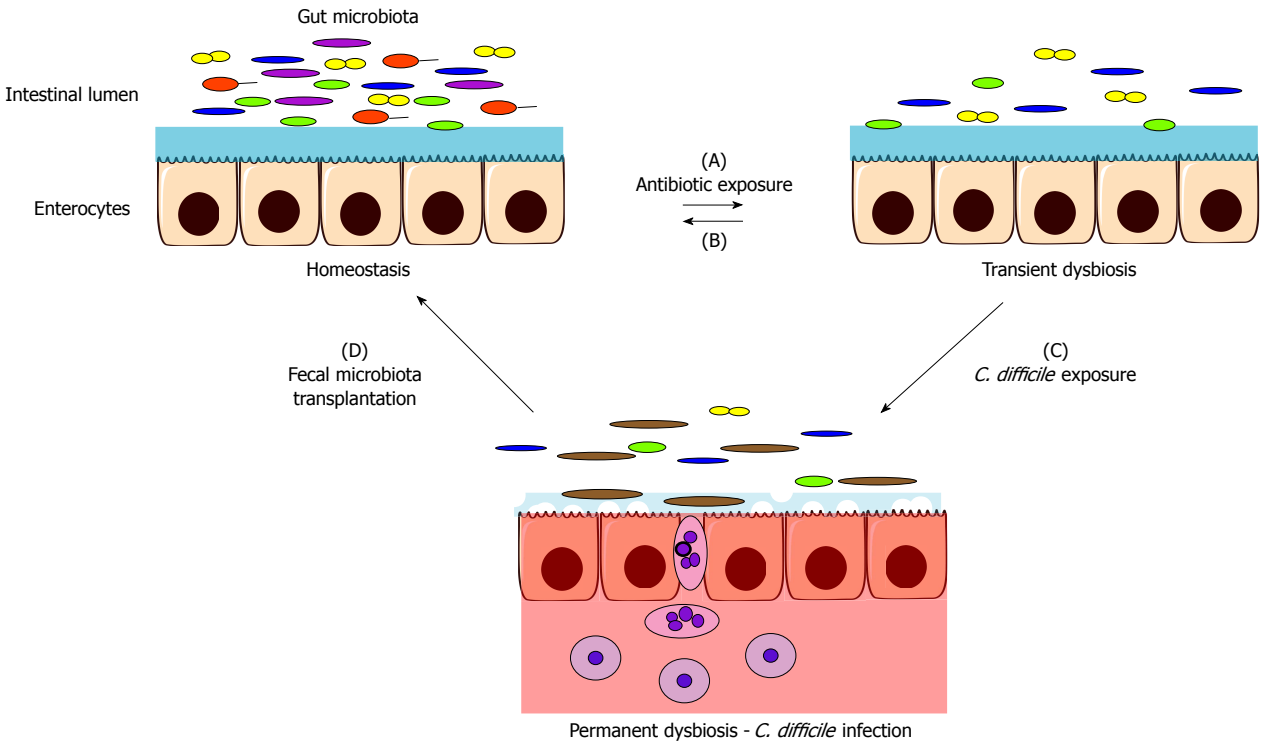

Figure 4 Perturbation of intestinal microbiota by antibiotics allowing *Clostridium difficile* infection and fecal microbiota transplantation effect (adapted from[27]). A: Antibiotic use destroys some sensitive bacteria and reduces the microbiota diversity and resistance to colonization by opportunistic pathogens; B: In the absence of opportunistic infection, microbiota usually recover its homeostasis; C: *Clostridium difficile* (*C. difficile*) infection can lead to persistent dysbiosis; D: Fecal microbiota transplantation restores microbiota diversity and colonization resistance and allows the elimination of *C. difficile*.

controlled trial, patients do appear to have less recurrent *C. difficile* diarrhea and early symptomatic improvement when using the probiotic *Lactobacillus* GG in combination with standard antiobiotics[29]. As for probiotics, the purpose of FMT is to resolve dysbiosis by restoring the phylogenetic diversity of intestinal flora and the resistance to colonization by *C. difficile*, thus allowing a return to normal colonic function (Figure 4). Unlike probiotics, which are only associated with a short-term change of the microbiota (10-14 d), FMT is able to significantly modify the recipient microbiota for at least 24 wk[30].

FECAL MICROBIOTA TRANSPLANTATION

Definition

FMT consists in the instillation of a suspension of stool from a healthy donor *via* the upper gastrointestinal route (usually nasoduodenal or nasojejunal tube) or lower gastrointestinal route (colonoscopy or retention enema).

Indications

Current indications of FMT for CDI treatment are[31]: (1) recurrent CDI: at least 3 episodes of mild to moderate CDI and failure of a 6- to 8-wk taper regimen of vancomycin, with or without an alternative antibiotic (*e.g.*, rifaximin, nitazoxanide); or at least 2 episodes of severe CDI resulting in hospitalization and significant morbidity; (2) moderate CDI not responding to standard therapy (vancomycin) for at least a week; and (3) severe (and perhaps even fulminant *C. difficile* colitis) with no response to standard therapy after 48 h.

Fecal microbiota transplantation procedure

To date, there is no standardized protocol for microbiota transplantation although the Fecal Microbiota Transplantation Workgroup published some recommendations in 2011[31].

Donors are screened for exclusion criteria such as antibiotic use during the last 3 mo, intestinal infection, inflammatory bowel disease, a history of neoplasia and presence of some infectious diseases (in particular, stool testing for *C. difficile*, *Salmonella* and *Shigella* and serologic testing for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus)[31]. Donors are usually relatives or household members, as there is likely to be reduced risk of transmission of an infectious agent (since donors and recipients should share the same infectious risks). One systematic review showed that this strategy was associated with a higher resolution rate (93%) compared to the use of stools from an unrelated donor (84%)[32]. However, contrary to this report, a recent meta-analysis showed that there was no significant difference whether the donor was a relative or not[33].

Donor stools are collected within 6 h before transplantation; they are generally mixed with a saline solution and the supernatant is filtered. After having received a bowel lavage solution, the recipient receives *via* upper or lower gastrointestinal route 500 mL of the suspension (given in small amounts of 25-50 mL). Lower gastrointestinal delivery *via* colonoscopy or enema seems to be more ef-

Table 4 Characteristics of some recent studies concerning fecal microbiota transplantation in recurrent *Clostridium difficile* infection treatment

Ref.	Study type	Patients (*n*)	FMT delivery modality	Success rate	Follow-up
Garborg *et al*[35], 2010	Retrospective study	40	Gastroscope Colonoscope	73% after 1 instillation 83% after 2 instillations	10 wk
Burke *et al*[36], 2013	Review	115	Naso-enteric tube Gastroscope Colonoscope Retention enema	89.6%	2 mo to 5 yr
Gough *et al*[32], 2011	Review	317	Naso-enteric tube Gastroscope Colonoscope Retention enema	89% after 1 instillation 92% after ⩾ 2 instillations	3 d to 5 yr
Kassam *et al*[33], 2013	Meta-analysis	273	Naso-enteric tube Gastroscope Colonoscope Retention enema	89%	2 wk to 8 yr
Van Nood *et al*[38], 2013	Randomized controlled trial	43	Naso-duodenal tube	81.3% after 1 instillation 93.8% after 2 instillations	10 wk

FMT: Fecal microbiota transplantation.

fective[33]. The amount of stool has not been standardized. Transplantation of more than 50 g of stool seems to be associated with a higher resolution rate than transplantation of less than 50 g (86% and 82% respectively)[32]. Similarly, administration of more than 500 mL may also be associated with a higher resolution rate than administration of less than 200 mL (97% and 80% respectively)[32].

Current evidence about fecal microbiota transplantation

From 1958, when the use of FMT for treatment of pseudomembranous colitis was first described by Eiseman *et al*[34], until 2011, published studies on the effectiveness of FMT have largely consisted of case reports or reviews on case series[35]. These studies suggest that FMT is effective for treating relapsing CDI in adults and children[36,37].

A systematic review published in 2011 studied 317 patients treated with FMT for recurrent CDI between 1958 and 2011[32] (Table 4). This review showed that 85%-90% of patients treated with FMT did not develop recurrence during the follow-up period (which varied from 3 d to 5 years), again pointing to FMT as an effective treatment for recurrent CDI[32].

A meta-analysis published in 2013 confirmed the efficacy of FMT for recurrent CDI, showing resolution in 89% of cases, while a subgroup analysis showed a trend towards significant higher resolution rate when FMT was provided *via* lower gastrointestinal route[33] (Table 4). Another retrospective study confirmed a high resolution rate after a follow-up of 90 d[26].

In 2013, Van Nood *et al*[38] published the first multicentric, prospective, open-label, randomized controlled trial that included 43 patients with CDI recurrence (Table 4). The primary outcome was resolution without recurrence within 10 wk after treatment. FMT *via* naso-duodenal tube, in association with a shortened treatment of vancomycin (5 d), was significantly more effective than vancomycin alone for 14 d (resolution rate 81% *vs* 31%). Three patients experienced recurrence despite one infusion and a second transplantation allowed resolution (increasing resolution rate to 94%)[38]. The diversity of recipients' gut microbiota after FMT was significantly improved, with an increase of *Bacteroides* and some *Clostridium* species and a decrease of *Proteobacteria*[38]. Note that there is an association between the modification of gut microbiota composition, the resolution of dysbiosis and the resolution of recurrent CDI.

Adverse effects, safety issues

Concerning short term adverse effects, Van Nood *et al*[38] observed diarrhea (94% of patients), cramping (31% of patients) and belching (19% of patients) immediately after FMT. During the subsequent weeks of follow-up, 19% of patients reported constipation. A recent case report described a flare of ulcerative colitis after treatment of recurrent CDI with FMT[39].

There are still unanswered questions regarding the short and long term consequences of FMT. The few published studies describe microbiota modifications after a short follow-up period (10 wk in Van Nood *et al* study[38], 24 wk in Grehan *et al*[30]). So far, we still do not know whether FMT could pose a risk for the development of some diseases from the donor. Even if there is no clear cause-effect relationship but only associations between gut microbiota composition and some diseases (cardiovascular diseases, IBDs, diabetes, non alcoholic fatty liver disease, obesity for example), to date no study has assessed the risk of developing one of these conditions after FMT. While donors are primarily screened for infectious diseases or digestive neoplasia, we still do not know whether they should also be screened for other diseases (immunologic or cardiovascular diseases for example).

Despite encouraging results of FMT in recurrent CDI, further studies are needed to confirm its efficacy and also to define "good practices" for donor selection,

stool preparation, the method of administration, and the indications of this treatment. Thus, because FMT meets the legal definition of a drug and a biological product, the FDA is attempting to regulate the multiple steps of FMT[40].

Future challenge and future directions

Given its effectiveness, 97% of patients who received FMT would repeat the treatment[26]. A recent survey conducted among 192 healthy patients confirmed that in a hypothetical case of recurrent CDI, 81% would choose FMT over antibiotics alone when informed of the effectiveness of each treatment. This rate rose to almost 90% if the administration of feces was odorless or given as a pill[41].

Despite this high acceptance rate, the development of an optimal formulation and pharmaceutical form is a current challenge. There is growing interest in fecal extracts or multistrain preparations. Petrof *et al*[42] developed a stool substitute preparation, containing 33 bacterial isolates, made from purified intestinal bacterial culture from a healthy donor. With this preparation, they successfully treated recurrent CDI in 2 patients. A recent retrospective study showed that use of a multistrain mixture of probiotics in combination with antibiotics could allow complete resolution of CDI[43]. The best composition of stool substitutes or multistrain mixture of probiotics and their efficacy still needs to be confirmed.

CONCLUSION

The incidence of CDIs and their recurrences are increasing despite effective treatment. Recurrence risk is about 25% after the first CDI episode and more than 45% after the first relapse. Metronidazole and vancomycin are recommended for the treatment of a first episode, and their efficacy has been well demonstrated in non severe and severe cases respectively. The recommended treatment of the second and subsequent recurrences is a taper regimen of vancomycin. Considering the high recurrence rate of CDI and the associated morbidity and mortality, there is growing interest in developing new therapeutic approaches. The association between gut microbiota imbalance, dysbiosis, and CDI recurrence has motivated the use of FMT to restore the microbiota equilibrium and resolve recurrent CDI. According to studies published to date, resolution rates of recurrent CDI seem to be higher when using FMT associated with antibiotics than antibiotics alone. The effectiveness of this treatment is promising, but further studies are needed to confirm these results, to define "good practices" of FMT and to identify any long term effects.

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