Original article

Title

Incidence of bloodstream infections, length of hospital stay and survival in patients with recurrent *Clostridioides difficile* infection treated with fecal microbiota transplantation or antibiotics: a prospective cohort study.

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ABSTRACT

Background: *Clostridioides difficile* infection (CDI) is a risk factor for bloodstream infections (BSI). Fecal microbiota transplantation (FMT) is more effective than antibiotics in treating recurrent CDI, but its efficacy in preventing CDI-related BSI is uncertain.

Objective: To assess incidence of primary BSI in patients with recurrent CDI treated with FMT compared with patients treated with antibiotics.

Design: Prospective cohort study. FMT and antibiotic treated patients were matched using propensity score.

Setting: Single academic medical center.

Patients: 290 inpatients with recurrent CDI; 57 patients per treatment in matched cohort.

Interventions: FMT or antibiotics.

Measurements: Our primary outcome was the development of primary BSI within a 90-day follow-up. Secondary outcomes were length of hospitalization, and overall survival (OS) at 90 days.

Results: 109 patients were treated with FMT, and 181 received antibiotics. Five patients in the FMT group and 40 in the antibiotic group developed BSI. Due to differences in the patients treated with FMT and antibiotics in a number of baseline characteristics including the number of recurrences and CDI severity, comparative analyses were limited to the matched cohort. Subjects in the FMT group experienced a 23% lower risk of developing BSI (95% confidence interval 10-35%), 14 fewer days of hospitalization (95% confidence interval 9-20 days), and a 32% increase in OS (95% confidence interval 16-47%) compared with the antibiotic group.

Limitations: Non-randomized study with potential for unmeasured/residual confounding. Limited generalizability of the propensity score-matched cohort.

Conclusion: In a propensity score-matched cohort, patients with recurrent CDI treated with FMT were less likely to develop primary BSI.

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INTRODUCTION

Clostridioides difficile (previously called *Clostridium difficile*) infection (CDI) has become a major burden for healthcare systems, accounting for nearly 29,000 deaths/year in the United States (US) (1–3). The recent burden of CDI can be mostly explained by its rise in incidence, severity, mortality, and increased likelihood of recurrence (3-4). After a first episode of CDI, almost 20% of patients are likely to recur, and the risk of a further recurrence can be even higher (5). Recurrences contribute to the nearly $5 billion/year costs of CDI in the US, which includes longer hospitalization, and increased morbidity and mortality (3).

As recurrent CDI (rCDI) is often refractory to antibiotics, it is more likely than primary infection to be associated with life-threatening complications, including pseudomembranous colitis, toxic megacolon, need for colectomy, shock, perforation, bloodstream infection (BSI), and death (6). A substantial proportion of patients with CDI are likely to develop BSIs, which are mostly caused by intestinal microbes, and could lead to death in more than 50% of patients (7). The pathogenesis of this complication could be related to *C. difficile* itself, which may promote microbial translocation (8). Some evidence suggests that vancomycin therapy, which suppresses most anaerobic bacteria, could also be associated with this complication (7,8). This favours intestinal colonization by healthcare-associated pathogens, including vancomycin-resistant *Enterococci* (VRE) and *Candida* spp. (9,10). In support of this, independent risk factors for BSI secondary to CDI include ribotype 027, recurrent or severe disease, and vancomycin regimens higher than 500mg/day (7,8).

Fecal microbiota transplantation (FMT) aims to restore the normal composition of gut microbiota. FMT is superior to vancomycin in treating CDI (11–13) and is recommended to manage recurrences (14–16). FMT could also be a promising approach for severe CDI (17–20) and *C. difficile* ribotype 027 (21). In theory, the restoration of healthy microbiota through FMT might also prevent CDI-associated BSI via several mechanisms, including cure of the infection, avoiding need

for vancomycin, and decrease in antibiotic resistance gene expression in the gut resistome of the patient. This could decrease the incidence of BSI related to multi-drug resistant (MDR) bacteria (22). To date, however, there are neither clinical nor pre-clinical data to support this hypothesis. Our aim was therefore to assess the incidence of primary BSIs in a cohort of patients with rCDI treated with either FMT or antibiotics.

METHODS

Study design and patients

This was a single-center, prospective cohort study, reported following STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines (23). All patients with rCDI who were hospitalized from July 2013 to May 2018 at the Fondazione Policlinico Universitario "A. Gemelli", a tertiary care center based in Rome, Italy, were considered for inclusion.

Patients admitted to our hospital with a diagnosis of rCDI, following treatment of their first infection at another center, and those who developed a CDI recurrence during hospitalization at our center were eligible for inclusion. Thus, only patients who experienced their first observable episode of rCDI at our hospital were considered, while patients with a second recurrence following treatment for an initial CDI recurrence provided at our hospital were excluded. Other exclusion criteria were: refusal of informed consent; patients aged <18 years; and patients who developed secondary BSI from a known source. We excluded patients with a first episode of CDI, as FMT is not recommended in this situation (16), and those considered unfit for FMT to avoid selection bias.

The following data were recorded for each patient: age; gender; comorbidities assessed with the Charlson comorbidity index (24); development of bacterial, fungal, or polymicrobial BSI; date of death; length of hospital stay; number of previous CDI recurrences; severity of CDI (i.e., mild, severe, and fulminant as defined in Appendix); hospitalization or stay in a long-term care unit in the previous 90 days; admission to an intensive care unit (ICU) in the previous 90 days; MDR infection in the previous 90 days; antibiotic therapy in the previous 30 days; treatment provided for CDI (FMT or antibiotics); and insertion of any of a central venous catether (CVC), urinary catheter, or percutaneous endoscopic gastrostomy during hospitalization and before treatment.

Antibiotic regimens were categorized by class, administration route, duration, and dosages. Responsible physicians chose antibiotic regimens based on recommendations from the European

Society of Clinical Microbiology and Infectious Disease for the management of CDI (15). FMT, including donor selection, manufacturing of fecal infusates, and fecal delivery, was performed according to international guidelines (16). Blood cultures were performed if patients developed pyrexia (body temperature >38°C) or other signs or symptoms considered potentially attributable to sepsis. Participants were followed up with weekly visits or telephone calls from study enrollment until 90 days after the end of treatment. Definitions of CDI recurrence and BSI used in the study, and description of microbiological analyses and of our FMT protocol, are provided in the Appendix.

Outcomes

Our primary outcome was the development of primary BSI from any organism after treatment for rCDI, and within a 90-day follow-up period. Secondary outcomes were length of hospitalization and overall survival (OS) at 90 days.

Data analysis and statistics

For continuous variables, data were expressed as means with a standard deviation (SD) and median and interquartile range. For categorical variables, data were expressed as numbers and percentages. The difference in means or proportions in the demographic and clinical characteristics was examined using the standardized difference Cohen's d (25). There is no universally acknowledged value that demonstrates balanced data, but 10% is recommended as a reasonable cutoff for unbalance (26). The difference in means or proportions in the outcome data was examined using the difference and 95% confidence interval (CI).

We used propensity score matching to match comparison and intervention respondents to create a more valid and counterfactual comparison group (27). The propensity score was the probability of treatment with FMT based on the person's age, gender, number of CDI recurrences, severity of CDI, Charlson index, receipt of hospital or long-term care in the last 90 days, receipt of

antibiotics in the last 30 days, and insertion of any CVC or urinary catheter, using probit regression. Common support was used by dropping FMT cases whose propensity score was higher than the maximum or less than the minimum propensity score of the control group. The one-to-one nearest neighbor matching was done without caliper and without replacement, and ties did not exist. Although differences remained between the FMT and antibiotic patients in the matched sample, we did not repeat the matching process due to the already decreased number of patients who could be included (57/109 (52%) FMT patients and 57/181 (31%) antibiotic patients). After matching, the analysis was restricted to matched participants in the control and FMT groups. OS was evaluated using a Kaplan-Meier survivor function.

Statistical analyses were performed with SPSS version 20.0 (SPSS Inc., Chicago, IL., USA) and Stata Statistical Software: Release 14 (StataCorp LP, College Station, TX., USA). The function psmatch2 was used for propensity matching.

Role of the funding source

None

RESULTS

Demographic and clinical characteristics of patients at baseline

Three-hundred and twenty-eight subjects with rCDI, who where hospitalized from July 2013 through May 2018 at our hospital, were evalutated for recruitment. Most had been treated with either oral vancomycin or metronidazole, and only a minority with fidaxomicin, at their first episode of CDI. Thirty-eight patients were excluded for the following reasons: 25 subjects developed a secondary BSI from a known source (urinary sepsis: 12 patients; sepsis from CVC: eight patients; pulmonary sepsis: five patients); seven subjects were <18 years old; and six subjects were considered unfit for FMT. Data from the remaining 290 subjects (F=180, M=110, mean age 75 years) were analyzed. Of them, 109 patients (38%) were treated with FMT and 181 (62%) received antibiotic therapy.

In the antibiotic group, the following drugs and regimens were used for 10 days: pulsed oral vancomycin 125 mg q.i.d. (n=63); pulsed oral vancomycin 250 mg q.i.d. (n=41); oral metronidazole 500 mg t.i.d. plus pulsed oral vancomycin 125 mg q.i.d. (n= 25); oral metronidazole 500 mg t.i.d. (n= 24); fidaxomicin 200 mg b.i.d. (n= 11); pulsed oral vancomycin 500 mg q.i.d. (n=9); tapered oral vancomycin regimen (n=8).

There was a large difference in the number of previous CDI recurrences between the two groups (mean 2.82 in FMT group compared with 1.23 in the antibiotic group). Eighty percent of those treated with antibiotics had one recurrence of CDI, compared with 19% of the FMT group. The distribution of the number of recurrences in the two populations is detailed in Table 1. Thirty-five (32%) patients in the FMT group presented with severe CDI, compared with 39 (22%) patients in the antibiotic group. Thirteen patients in both the FMT group (12%) and antibiotic group (7%) had fulminant disease. Seventeen (16%) and eleven (6%) patients had a CVC at enrollment in the FMT and antibiotic groups respectively. Twenty-six (24%) patients had a urinary catheter at

enrollment in the FMT group and seventy-four (41%) in the antibiotic group. There were also differences in gender and Charlson comorbidity index at baseline between the two groups (Table 1).

A higher rate of patients in the FMT group (n=106) experienced sustained cure of CDI after treatment than patients in the antibiotic group (n=69) (97% vs 38%), similar to the results in another study from our group (90% vs 26%).[12] All patients whose CDI recurred once again after treatment were offered rescue therapy with FMT, fidaxomicin, or tapered vancomycin, based on the responsible physician's decision. No patients in the FMT group underwent surgery (colectomy or loop ileostomy) for overwhelming CDI, compared with 14 in the antibiotic group (0% vs 8%).

In the propensity score matching group, matched on a one-to-one basis, there were 57 pairs with no ties. Fifty-two patients treated with FMT (48%) were excluded as they exceeded the common support criteria. Using the standardized difference and a 10% cut-off, the FMT group patients were younger, had a lower Charlson comorbidity index, a greater proportion of patients with a mild clinical picture of CDI, a lower proportion with only one CDI recurrence, and a higher proportion with CVC at enrolment. Figure 1 shows the distribution of the frequency of propensity scores in the two groups.

Development of BSI at 90 days

Table 2 summarizes outcome data. Overall, 45 patients developed BSI (16%) during the 90-day follow-up; five (5%) treated with FMT, and 40 (22%) with antibiotics. Five (5%) patients in the FMT group and 28 (16%) in the antibiotic group developed a bacterial BSI. No patients in the FMT group and 7 (12%) in the antibiotic group developed a fungal BSI. One patient in the FMT group and one in the antibiotic group developed a polymicrobial BSI (1% in each). Pathogens responsible for BSIs are shown in Table 3. Of the 11 patients, who received fidaxomycin to treat CDI, four developed BSI. In the propensity score matched cohort, two patients in the FMT group and 15 in the antibiotic group developed BSI during follow-up (4% vs 26%, difference of 23%, 95% CI 10-35%).

Length of hospitalization

In the whole cohort, mean length of hospitalization was 13.3 days in patients treated with FMT and 29.7 days in those treated with antibiotics. In the propensity score matched cohort, patients in the FMT group experienced a significantly shorter mean length of hospitalization (mean 13.4 days vs 27.8 days, difference 14 days 95% CI 9-20 days).

Overall survival at 90 days

In the whole cohort, 79 of 290 patients (27%) died during the 90-day follow-up period, 21 (7%) of them due to BSI (all in the antibiotic cohort). Causes of death in the remaining 58 patients were: deterioration of clinical condition due to overwhelming CDI (n=15, three in the FMT cohort and 12 in the antibiotic cohort); complications after surgery for CDI (n=11, all in the antibiotic cohort); ischemic heart disease (n=10, five in the FMT cohort and five in the antibiotic cohort); ischemic cerebrovascular disease (n=9, one in the FMT cohort and eight in the antibiotic cohort); cancer (n=3, one in the FMT cohort and two in the antibiotic cohort); pulmonary failure (n=3, all in the antibiotic cohort); acute-on-chronic kidney failure (n=3, all in the antibiotic cohort); variceal bleeding (n=2, one in the FMT cohort and one in the antibiotic cohort); and acute-on-chronic liver failure (n=2, all in the antibiotic cohort).

Fifty-eight patients (73%) died within 30 days from the end of treatment. Five of these had been treated with FMT, and 53 with antibiotics. All were inpatients at the time of death. None of the 11 patients who died due to complications of surgery for overwhelming CDI had developed BSI. The other 21 patients (27%) died between 31 and 90 days after the end of treatment; four were in the FMT group and 17 in the antibiotic group. Ninety-day OS for the whole cohort was 73%, with 100 (92%) patients alive in the FMT group, and 111 (61%) in the antibiotic group.

In the propensity-matched cohort, six (11%) patients in the FMT group and 24 (42%) in the antibiotic group died during the 90-day follow-up period, eight (7%) of them because of BSI and seven (12%) due to fungal infection. Most of these patients died within 30 days from the end of

238 treatment, three in the FMT group versus 15 in the antibiotic group. All were inpatients at the time
239 of death. The other 10 patients died between 31 and 90 days after the end of treatment, three in the
240 FMT group versus seven in the antibiotic group. Ninety-day OS for the propensity-matched cohort
241 was higher in the FMT group compared with the antibiotic group (n=51 (89%) vs n=33 (58%),
242 difference 32% (95% CI 16-47%)). Figure 2 shows OS in the propensity-matched cohort.
243

DISCUSSION

A considerable proportion of patients with CDI can develop a primary BSI, which is caused mainly by intestinal microbes (7,8). Some evidence suggests that this complication could contribute to the global burden of CDI by increasing length of hospital stay and mortality (7). Moreover, primary BSI is known to be the second most burdensome hospital-associated infection in Europe (28), due to both inpatient costs, and also needs that arise after discharge (29). Antibiotics commonly used to treat CDI, such as vancomycin, may not prevent the risk of BSI in these patients (9,10,30,31). In our study, patients with rCDI who received FMT were less likely to develop a primary BSI, and related complications, including death. There were notable differences between the FMT and antibiotic treated groups in the original cohort, particularly in relation to the number of CDI recurrences. We used propensity scores matched on nearest neighbor to reduce the differences between groups. However, there was not a perfect overlap in propensity scores, and imbalance in several baseline characteristics persisted between the propensity-score matched groups. This leaves the possibility that the two groups of patients are different, due to confounding, and we must be cautious in interpreting the findings.

In the propensity-matched cohort, with 57 patients per treatment, of the 17 patients who developed BSI from any organism, two were treated with FMT and 15 with antibiotics, with a significantly lower incidence in the FMT group. Patients from the matched cohort treated with FMT also experienced a significantly shorter mean hospitalization than those treated with antibiotics. Although we did not perform a cost-effectiveness analysis, this could have implications for the cost of managing patients with rCDI, already evaluated in dedicated health economics studies (32,33). Another noteworthy finding observed in the propensity matched cohort was the significantly higher OS at 90 days in patients treated with FMT. Most patients died within 30 days from the end of treatment, many because of CDI complications, and all were hospitalized at the time of death. There were small differences in death rates between the two groups between 30 and 90 days, when several

patients were discharged. These findings suggest that the longer 90-day OS experienced by patients in the FMT group is attributable to cure of CDI, leading to an improvement in their clinical condition. The higher number of post-surgical deaths in the antibiotic group could have increased the difference in death rates between groups. However, all surgical patients underwent surgery for overwhelming CDI, and not for other indications, so these events were always dependent on the clinical severity of CDI.

As an explanation for our findings, FMT could act through several therapeutic pathways to prevent BSI, and related complications, in patients with CDI. FMT may directly reduce this risk, by curing CDI. *C. difficile* toxins are known to damage the gut barrier and increase intestinal permeability (34–36), thus favouring translocation of pathogens into the bloodstream. Moreover, severe CDI has been identified as a risk factor for BSI (7,8), suggesting that severe inflammation could further increase gut permeability. FMT is recognized as a highly effective therapy for sustained cure of rCDI (11), and increasing evidence suggests that it could also be a reliable treatment for severe CDI (12,20,37,38). In our study, the significantly higher rate of patients cured by FMT, rather than by antibiotics, may have contributed to decrease intestinal permeability and to prevent translocation of microbes, and therefore the development of BSI. Additionally, CDI is known to promote colonization of the gut by *Candida* (39). By curing CDI, FMT may not only decrease the rate of bacterial BSI, but also of *Candida*-dependent systemic infections.

Another possible explanation for our findings is the avoidance of repeated cycles of vancomycin, due to cure of CDI by FMT. High-dose (>500 mg/day) oral vancomycin has been previously identified as a risk factor for BSI development, (7) and this finding could be explained by its effect on gut microbiota. Oral vancomycin was shown to decrease Firmicutes (including *C. difficile*) and to increase Proteobacteria in humans (40). The increase of Proteobacteria phylum could lead to the overgrowth of bacteria commonly responsible for primary BSI, including *K. pneumoniae* or *E. coli*. Moreover, the sustained use of vancomycin could foster the development of resistant bacteria, such as VRE. In our study, both *K. pneumoniae* and VRE were found to be

296 commonly responsible for BSI, confirming this hypothesis. Oral vancomycin has also been shown
297 to promote intestinal colonization by *Candida* species (41), which could explain the reduction in
298 fungal BSIs seen in the FMT group.

299 Finally, the restoration of healthy microbiota through FMT could decrease the gut resistome,
300 which is the expression of antibiotic resistance genes by the gut microbiota, thus increasing the
301 likelihood of developing a BSI from MDR bacteria (42). FMT has been shown to be associated with
302 a decrease in antibiotic-resistant bacteria in a prospective pilot study (22). Intensive antibiotic
303 therapies could promote both the development of CDI, and the selection of antibiotic-resistant
304 bacteria, which are more difficult to eradicate, and are more likely to lead to BSI.

305 Our study has some limitations, due to its observational nature, which prevent us from
306 drawing definitive conclusions. On average, patients treated with FMT presented with worse
307 clinical conditions than those with antibiotics. We aimed to reduce this bias by performing
308 propensity score matching. However, differences between the propensity score matched comparison
309 groups remain with respect to several baseline characteristics. The propensity-matching method
310 removed 52 (48%) of the FMT-treated and 124 (69%) of the antibiotic-treated patients from the
311 analysis, which may limit the generalizability of this analyzed sub-cohort to the population of
312 people with rCDI.

313 We did not collect stool samples from patients before and after the treatments to assess
314 shifts in gut microbiota composition, nor did we assess the cost-effectiveness of using FMT in
315 rCDI. Additionally, we did not include in the analysis some baseline parameters, including
316 immunosuppression, liver or kidney disease, or previous chemotherapy or radiotherapy, which
317 might have influenced the development of BSI. Another limitation could be that fidaxomicin was
318 used in only a minority of patients in the antibiotic group; it has been suggested to be less likely
319 than vancomycin to promote colonization by Candida *species* and VRE (41). This can be explained
320 by the high costs of fidaxomicin, its lack of availability in Italy at the start of our study, and the
321 availability of FMT as a reliable therapeutic alternative, which has also been recently shown to be

322 superior to fidaxomicin in patients with rCDI (43). Finally, the observational study design could
323 have introduced bias into our analysis, due to other differences among patients that we did not
324 measure, and which may have driven the choice of treatment by responsible physicians. Future
325 studies that use a randomized controlled design, with microbial analysis, and that collect economic
326 data are required to confirm the efficacy and cost-effectiveness of FMT in preventing CDI and its
327 related complications.

328 In conclusion, in our propensity-score matched cohort, patients with rCDI treated with FMT
329 were less likely to develop primary BSI, experienced a shorter duration of hospitalization, and had a
330 reduction in the risk of overall mortality within 90 days, compared with those treated with
331 antibiotics. Should our results be confirmed by larger, randomized studies, FMT could be
332 considered an effective treatment option to both rCDI and prevent some of its related complications,
333 including BSI.

334

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539 **Table 1. Demographic and clinical characteristics of the patients in the original cohort and in the propensity score matched cohort.**

	Original cohort			**After propensity score matching**		
Variable	**Treated with FMT**	**Treated with antibiotics**	**Standardized difference**	**Treated with FMT**	**Treated with antibiotics**	**Standardized difference**
Number	109	181		57	57	
Mean age in years (SD)	75.3 (12.2)	74.7 (13.1)	-5%	73.7 (14.7)	76.3 (11.3)	20%
Gender						
Male (%)	37 (34)	75 (41)	19%	23 (40)	21 (37)	7%
Female (%)	72 (66)	106 (59)		34 (60)	36 (63)	
Mean Charlson Comorbidity Index (SD)	4.3 (1.9)	3.9 (1.7)	-19%	3.9 (1.9)	4.2 (1.5)	21%
Mean number of CDI recurrences (SD)	2.82 (1.34)	1.23 (0.48)	178%	1.88 (0.78)	1.61 (0.65)	-37%
Median number of CDI recurrences (IQR)	3 (2-4)	1 (1-1)		2 (1-2)	2 (1-2)	
Number of CDI recurrences (%)						
1	21 (19)	145 (80)		21 (37)	27 (47)	
2	25 (23)	31 (17)		22 (39)	25 (44)	
3	30 (28)	5 (3)		14 (25)	5 (9)	
4	23 (21)	0 (0)		0 (0)	0 (0)	
5+	10 (9)	0 (0)		0 (0)	0 (0)	
Clinical picture of CDI						
Mild (%)	61 (56)	129 (71)	32%	34 (60)	28 (49)	22%

Severe (%)	35 (32)	39 (22)		18 (32)	22 (39)	
Fulminant (%)	13 (12)	13 (7)		5 (9)	7 (12))	
CVC at enrollment						
Yes (%)	17 (16)	11 (6)	31%	8 (14)	5 (9)	17%
No (%)	92 (84)	170 (94)		49 (86)	52 (91)	
Urinary catheter at enrollment						
Yes (%)	26 (24)	74 (41)	37%	14 (25)	16 (28)	8%
No (%)	83 (76)	107 (59)		43 (75)	41 (72)	
Hospitalization within 90 days before enrollment						
Yes (%)	66 (61)	108 (60)	2%	35 (61)	37 (65)	7%
No (%)	43 (39)	73 (40)		22 (39)	20 (35)	
Surgery within 30 days before enrollment						
Yes (%)	14 (13)	22 (12)	2%	9 (16)	7 (12)	10%
No (%)	95 (87)	159 (88)		48 (84)	50 (88)	
Antibiotics within 30 days before enrollment						
Yes (%)	60 (55)	95 (53)	5%	35 (61)	33 (58)	7%
No (%)	49 (45)	86 (47)		22 (61)	24 (42)	
MDR infection within 90 days before enrollment						

Yes (%)	13 (12)	13 (7)	16%	3 (5)	3 (5)	0%
No (%)	96 (88)	168 (93)		54 (95)	54 (95)	
ICU admission within 90 days before enrollment						
Yes (%)	6 (6)	10 (6)	0%	2 (4)	1 (2)	11%
No (%)	103 (94)	171 (94)		55 (96)	56 (98)	

540

541 CDI= *C. difficile* infection; CVC=central venous catheter; FMT= fecal microbiota transplantation; ICU= Intensive care unit; IQR = Interquartile

542 range; MDR= multi-drug resistant; SD= standard deviation.

543

544 **Table 2: Outcome data in the original cohort and in the propensity score matched cohort.**

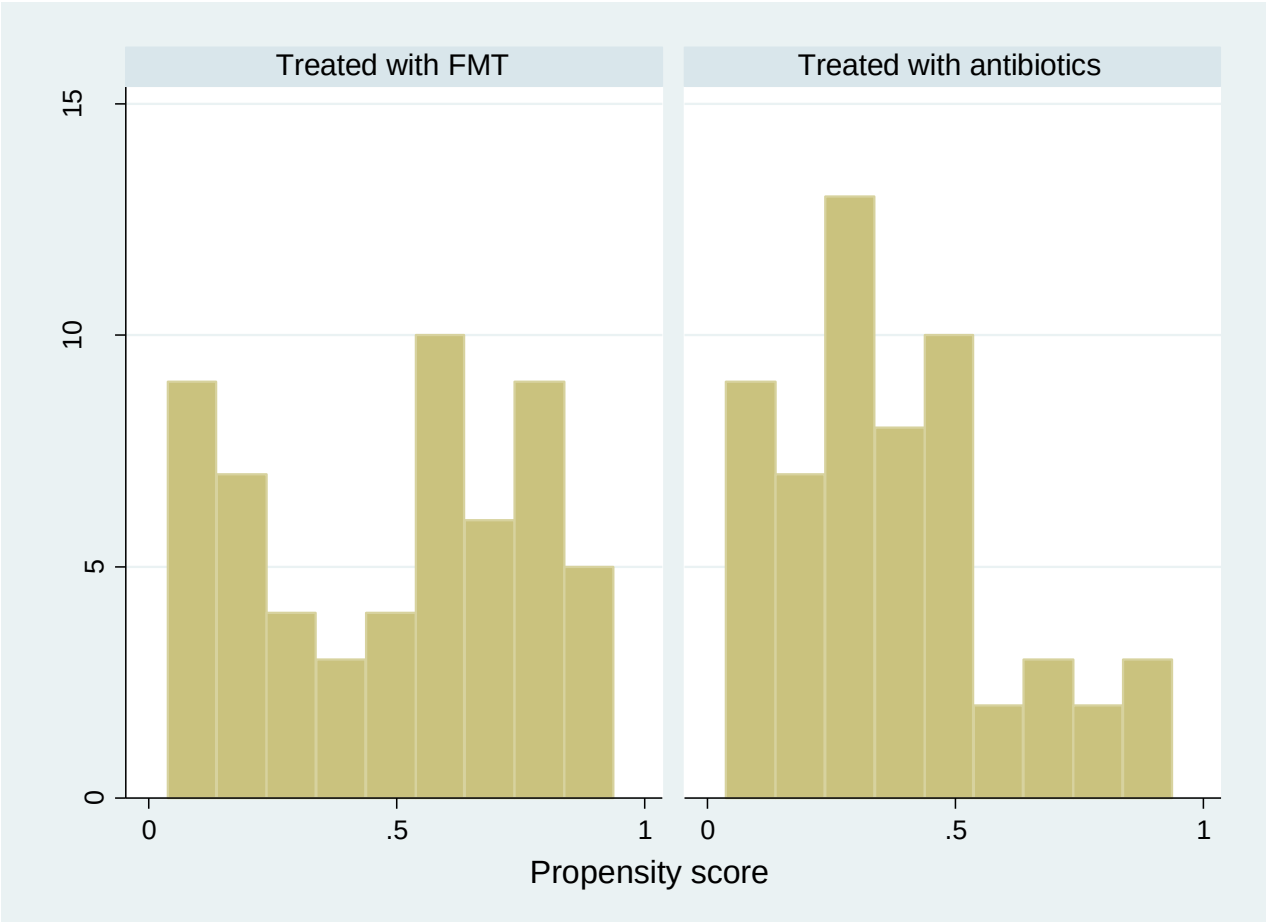
		Original cohort			**After propensity score matching**			
Outcome level	**Variable**	**Treated with FMT**	**Treated with antibiotics**	**Difference**	**Treated with FMT**	**Treated with antibiotics**	**Difference**	**95% confidence interval**
	Number	109	181		57	57		
Primary	Bloodstream infection (%)	5 (5)	40 (22)	16%	2 (4)	15 (26)	23%	10-35%
	Polymicrobial	1 (1)	1 (1)		0 (0)	0 (0)		
	Bacterial	5 (5)	28 (15)		2 (4)	8 (14)		
	Fungal	0 (0)	7 (12)		0 (0)	7 (12)		
Secondary	Length of hospitalization in days							
	Mean (SD)	13.3 (14.8)	29.7 (22.6)	24	13.4 (13.7)	27.8 (17.6)	14	9-20
	Median (interquartile range)	8 (2-20)	22 (14-39)		9 (2-21)	22 (14-40)		
	Alive after 90 days (%)	100 (92)	111 (61)	30%	51 (89)	33 (58)	32%	16-47%
	Total deaths within 0-90 days	9 (8)	70 (39)		6 (11)	24 (42)		
	Death within days 0-30	5	53		3	15		
	Death within days 31-90	4	17		3	7		

Table 3. Involved organisms in bloodstream infections in the original study cohort.

Microbes	**Total number of patients**	**FMT group**	**Antibiotic group**
Bacteria			
XDR *Acinetobacter baumannii*	2	**0**	2
Escherichia coli	3	**0**	3
Enterococcus faecalis	3	**1**	2
VR *Enterococcus faecium*	3	**0**	3
Klebsiella oxytoca	1	**1**	0
Klebsiella pneumoniae	3	**1**	2
CRE *Klebsiella pneumoniae*	3	**0**	3
ESBL *Klebsiella pneumoniae*	1	**1**	0
Staphylococcus spp	7	**1**	6
Staphylococcus aureus	1	**0**	1
MR *Staphylococcus aureus*	1	**0**	1
Proteus mirabilis	1	**0**	1
XDR *Proteus mirabilis*	1	**0**	1
Corinebacterium spp	1	**0**	1
Total	**31**	**5**	**26**
Fungi			
Candida albicans	9	0	9
Candida parapsilosis	4	0	4
Candida tropicalis	1	0	1
Total	**14**	**0**	**14**
Overall	**45***	**5**	**40**

* 12 of 45 patients developed a polymicrobial bloodstream infection (bloodstream infections from multiple bacteria in 10 patients; bloodstream infections from fungal and bacterial organisms in 2 patients). CRE= carbapenem-resistant Enterobacteriaceae; ESBL= extended-spectrum beta-lactamase; FMT= fecal microbiota transplantation; MR= methicillin-resistant; XDR= extensively drug-resistant.

552 **Figure 1: Frequency distribution of propensity scores (probability of treatment with FMT)**
553 **for the propensity score matched cohort in patients treated with FMT and patients treated**
554 **with antibiotics.**



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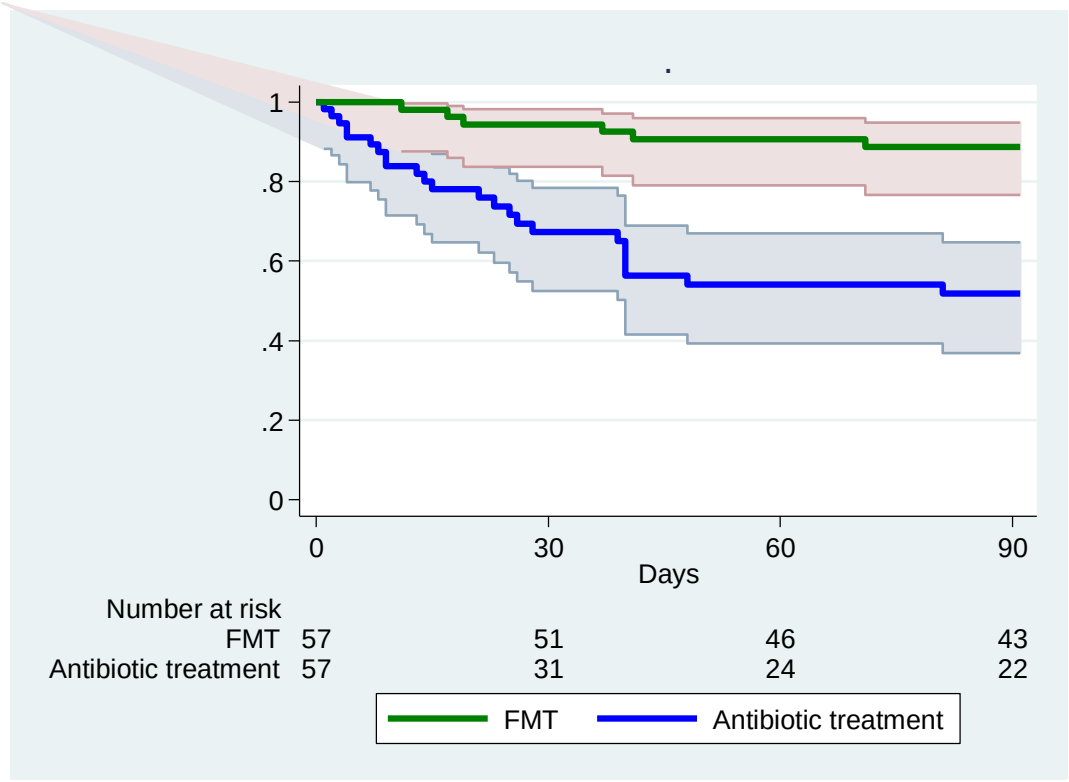
559 **Figure 2: Overall survival at 90 days in 57 patients treated with FMT compared with 57**

560 **patients treated with antibiotics matched by propensity score.**



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563 FMT= fecal microbiota transplantation. Shaded area displays 95% confidence interval

564

APPENDIX

Definitions

RCDI was defined as diarrhea and positivity for the *C. difficile* toxin stool test within 8 weeks from the end of the previous antibiotic treatment (15). Patients were classified, based on their clinical picture, according to the latest recommendations from the Infectious Disease Society of America for the management of CDI (44). Criteria for defining non-severe CDI were leukocytosis with a white blood cell count of ≤15 000 cells/mL and a serum creatinine level <1.5 mg/dL. Severe CDI was defined by leukocytosis with a white blood cell count of ≥15 000 cells/mL or a serum creatinine level >1.5 mg/dL. Finally, fulminant CDI was diagnosed in the presence of hypotension or shock, ileus, or megacolon (44). Nosocomial CDI was defined as CDI diagnosed more than 48 hours after hospital admission.

An episode of BSI was defined, according to recommendations from the Centers for Disease Control and Prevention, as the presence of one or more recognized pathogens in at least one blood culture or, for potential skin contaminants, the positivity of two or more separate blood cultures within 48 hours, in the presence of at least two signs or symptoms of a systemic inflammatory response (45). Candidaemia was defined as one or more blood cultures positive for *Candida* genus, together with clinical signs and symptoms of infection (46).

A primary BSI was defined as one occurring without a known source of infection. A secondary BSI was defined as one arising from a recognized source. CVCs were accounted to be the likely source of infection if blood cultures from the catheter lumen, but not from a peripheral vein, were positive within 2 hours (47).

Microbiological analyses

589 Stool tests for *C. difficile* toxin were performed using Liaison® C.difficile GDH-Toxin A/B
590 kit (DiaSorin Inc. 1951 Northwestern Avenue Stillwater. MN, USA). All tests were performed in
591 the microbiology laboratory of the hospital.
592 To identify pathogens for the diagnosis of BSI, blood samples were collected for culture and
593 inoculated in BACTEC bottles (Becton Dickinson Instrument Systems, Sparks, Md.) or Bact/Alert
594 bottles (bioMérieux, Marcy l'Etoile, France) and incubated for up to 5 days in the BACTEC FX or
595 Bact/Alert VIRTUO automated blood culture (BC) instruments. When the growth index of a bottle
596 was positive, broth aliquots from each positive BC bottle were collected for standard method (Gram
597 staining, and culture-based method) and direct method using MALDI BioTyper (Bruker Daltonik
598 GmbH, Leipzig, Germany).
599
600 **Description of the FMT protocol.**
601 The FMT protocol included the following steps: 1) selection and screening of donors; 2)
602 manufacturing of fecal infusate; 3) fecal infusion procedure.
603
604 *Selection of donors*
605 The selection of donors was performed by two authors (G. C. and G. I.) following protocols
606 recommended by international guidelines (16). Healthy volunteers younger than 50 years of age
607 were initially screened through a specific questionnaire to exclude:
608 A) Possible risk factors for potentially transmittable diseases due to their medical history
609 and lifestyle habits, including: history of, or known exposure to, HIV, HBV, HCV, syphilis, human
610 T-lymphotropic virus I and II, malaria, trypanosomiasis, tuberculosis; systemic infections not
611 controlled at the time of donation; use of illegal drugs; risky sexual behavior; previous reception of
612 tissue/organ transplant; previous (less than 12 months) reception of blood products; recent (less than
613 6 months) needle stick accident; recent (less than 6 months) body tattoo, piercing, earring, or
614 acupuncture; recent medical treatment in poorly hygienic conditions; risk of transmission of

diseases caused by prions; recent parasitosis or infection from rotavirus, Giardia lamblia, or other microbes with gastrointestinal involvement; recent (less than 6 months) travel in tropical countries, countries at high risk of communicable diseases, or traveller's diarrhea; recent (less than 6 months) history of vaccination with a live attenuated virus; healthcare or animal workers.

B) Gastrointestinal, metabolic and neurological disordes, including: irritable bowel syndrome, inflammatory bowel disease, functional chronic constipation, celiac disease, or other chronic gastrointestinal diseases; chronic, systemic autoimmune disorders with gastrointestinal involvement; gastrointestinal cancer or polyposis (or high risk for these disorders); recent diarrhea or hematochezia; neurological disorders and/or psychiatric conditions; overweight or obesity (body mass index >25)

C) The use of drugs that could impair the composition of the intestinal microbiota, including: recent (<3 months) exposure to antibiotics, immunosuppressants, chemotherapeuthics, or chronic therapy with proton pump inhibitors.

All potential donors who passed the questionnaire underwent blood exams (including: serology for cytomegalovirus, Epstein-Barr virus, hepatitis A, hepatitis B, hepatitis C, and hepatitis E viruses, syphilis, HIV-1 and HIV-2 viruses, *Entamoeba histolytica*; complete blood count, C-reactive protein, erythrocyte sedimentation rate, albumin, creatinine, electrolytes, aminotransferases, bilirubin, gamma-glutamyltransferase, alkaline phosphatase) and stool exams (including: culture and toxin for *C. difficile*; detection of enteric pathogens, including Salmonella, Shigella, Campylobacter, Escherichia coli O157 H7, Yersinia, vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus, or Gram-negative multidrug-resistant bacteria; Norovirus; antigens and/or acid fast staining for *Giardia lamblia* and *Criptosporidium parvum*; Protozoa (including *Blastocystis hominis*) and helminths; faecal occult blood testing) to exclude potentially transmittable diseases.

639 Finally, candidates underwent a further questionnaire the day of each donation to exclude
640 any recent acute gastrointestinal illness or symptom, newly contracted infections, use of new drugs,
641 or other potentially harmful situations for the patient.
642 The assignment of faecal infusates from healthy donors to patients was done randomly,
643 without any specific recipient-donor match, as suggested by international guidelines (16).
644
645 *Manufacturing of faecal infusate*
646 All faecal infusate samples were prepared in the microbiology laboratory of our hospital by
647 L.M., following manufacturing protocols recommended by international guidelines (16). We used
648 either fresh or frozen feces, using at least 50g of feces for each sample. Feces were collected by the
649 donor on the day of infusion and rapidly transported to our hospital. In the hospital's microbiology
650 laboratory, the feces were diluted with at least 250 mL of sterile saline (0.9%). The deriving
651 solution was blended, and the supernatant strained and poured into a sterile container. For frozen
652 samples, glycerol was added up to a final concentration of 10% before freezing, and the samples
653 were stored at -80°C. On the day of fecal infusion, frozen infusates were thawed in a warm (37°C)
654 water bath.
655
656 *Fecal infusion procedure*
657 The fecal infusion procedure included the following steps: a 3-day pre-treatment with oral
658 vancomycin (250 mg by mouth four times a day), followed by bowel cleansing with 2L of macrogol
659 per day for 2 days, and then fecal infusion from healthy donors by colonoscopy, as previously
660 described (12). All procedures were performed by two expert endoscopists (G. C., G. I), using
661 peediatric colonoscopes and carbon dioxide insufflation. The infusate was delivered within 6 hours
662 after donor supply (if fresh feces were used) or after thawing (if frozen feces were used), through
663 the operative channel of the scope after reaching the most proximal point of the colon, using 50mL

664 syringes filled with the infusate during colonoscopy. Finally, the patients were monitored in the
665 recovery room of the endoscopy center for 2 to 3 hours after the procedure.
666
667

668 ***Patients excluded from the propensity-matched cohort***

669 The propensity score matched cohort excluded a set of patients from the analysis. The
670 characteristics of this excluded cohort are shown in Supplementary Table 1. The main difference
671 between the two treatment groups, in the group excluded by the propensity matching, is that 95% of
672 the patients in the antibiotic-treated group had only one CDI recurrence, compared with the FMT
673 group, where 100% had two or more CDI recurrences.

674

675

676 **Supplementary Table 1: Baseline and outcome data for those excluded from the propensity**

677 **score matched cohort.**

Variable	Treated with FMT	Treated with antibiotics
Number	52	124
Mean age in years (SD)	77.1 (8.64)	74.1 (13.81)
Gender		
Male (%)	40 (77)	54 (44)
Female (%)	12 (23)	70 (56)
Mean Charlson Comorbidity Index (SD)	4.39 (1.77)	3.76 (1.81)
Number of CDI recurrences (%)		
1	0 (0)	118 (95)
2	3 (6)	6 (5)
3	16 (31)	0 (0)
4	23 (44)	0 (0)
5+	10 (19)	0 (0)
Clinical picture of CDI		
Mild (%)	27 (52)	101 (82)
Severe (%)	17 (24)	17 (14)
Fulminant (%)	8 (15)	6 (5)
CVC at enrollment		
Yes (%)	9 (17)	6 (5)
No (%)	43 (83)	118 (95)
Urinary catheter at enrollment		
Yes (%)	12 (23)	58 (47)
No (%)	40 (77)	66 (53)
Hospitalized within 90 days before enrollment		
Yes (%)	31 (60)	71 (57)
No (%)	21 (40)	53 (43)
Surgery within 30 days before enrollment		
Yes (%)	5 (10)	15 (12)

No (%)	47 (90)	109 (88)
Antibiotics within 30 days before enrollment		
Yes (%)	25 (48)	62 (50)
No (%)	27 (52)	62 (50)
MDR infection within 90 days before enrollment		
Yes (%)	10 (19)	10 (8)
No (%)	42 (81)	114 (92)
ICU admission within 90 days before enrollment		
Yes (%)	4 (8)	9 (7)
No (%)	48 (92)	115 (93)
Bloodstream infection (%)	3 (6)	25 (20)
Bacterial	3 (6)	20 (16)
Fungal	1 (2)	6 (5)
Length of hospitalization in days		
Mean (SD)	13.3 (16.1)	30.5 (24.6)
Median (interquartile range)	6 (2-19.5)	22.5 (14-38)
Alive after 90 days (%)		
Total deaths 0-90 days	3 (6)	78 (63)
Death within days 0-30	2 (4)	36 (29)
Death within days 31-90	1 (2)	42 (34)

678 CDI= *C. difficile* infection; CVC=central venous catheter; FMT= fecal microbiota transplantation;

679 ICU= Intensive care unit; MDR= multi-drug resistant; SD= standard deviation.

680

681

682