1 Original article

2 **Title**

3 Incidence of bloodstream infections, length of hospital stay and survival in patients with recurrent

4 *Clostridioides difficile* infection treated with fecal microbiota transplantation or antibiotics: a

5 prospective cohort study.

6

7 **Authors**

8 Gianluca Ianiro[1], MD; Rita Murri[2], MD; Giusi Desirè Sciumè[1], MD; Michele Impagnatiello[1], MD;

9 Luca Masucci[3], MD; Alexander C. Ford[4,5], MB ChB, MD, FRCP; Graham R. Law[6], PhD; Herbert

10 Tilg[7], MD; Maurizio Sanguinetti[3], MD; Roberto Cauda[2], MD, PhD; Antonio Gasbarrini[1], MD;

11 Massimo Fantoni[2], MD; Giovanni Cammarota[1], MD.

12

13 **Affiliations**

14 [1]Digestive Disease Center, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome,

15 Italy

16 [2]Department of Infectious Disease, Fondazione Policlinico Universitario Agostino Gemelli IRCCS,

17 Rome, Italy

18 [3]Institute of Microbiology, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome,

19 Italy

20 [4]Leeds Institute of Medical Research at St. James's, University of Leeds, Leeds, UK

21 [5]Leeds Gastroenterology Institute, St. James's University Hospital, Leeds, UK

22 [6]Community & Health Research Unit, University of Lincoln, Lincoln, UK

23 [7]Department of Internal Medicine I, Gastroenterology, Endocrinology & Metabolism, Medical

24 University Innsbruck, Innsbruck, Austria

25

26 **Word count:** 3496

27

Key Words

29 Fecal microbiota transplantation; *Clostridium difficile* infection; colonoscopy; bloodstream

30 infections; sepsis; propensity score

Corresponding author

32 Dr. Gianluca Ianiro, MD; Digestive Disease Center, Fondazione Policlinico Universitario Agostino

33 Gemelli IRCCS, Università Cattolica del Sacro Cuore, Largo A. Gemelli 8, 00168 – Rome, Italy.

34 Tel/fax numbers: +39 06 30156265/+39 06 30157023; e-mail address: gianluca.ianiro@hotmail.it

35

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.

Funding Source: None.

61 **INTRODUCTION**

62

63   *Clostridioides difficile* (previously called *Clostridium difficile*) infection (CDI) has become a

64 major burden for healthcare systems, accounting for nearly 29,000 deaths/year in the United States

65 (US) (1–3). The recent burden of CDI can be mostly explained by its rise in incidence, severity,

66 mortality, and increased likelihood of recurrence (3-4). After a first episode of CDI, almost 20% of

67 patients are likely to recur, and the risk of a further recurrence can be even higher (5). Recurrences

68 contribute to the nearly $5 billion/year costs of CDI in the US, which includes longer

69 hospitalization, and increased morbidity and mortality (3).

70   As recurrent CDI (rCDI) is often refractory to antibiotics, it is more likely than primary

71 infection to be associated with life-threatening complications, including pseudomembranous colitis,

72 toxic megacolon, need for colectomy, shock, perforation, bloodstream infection (BSI), and death

73 (6). A substantial proportion of patients with CDI are likely to develop BSIs, which are mostly

74 caused by intestinal microbes, and could lead to death in more than 50% of patients (7). The

75 pathogenesis of this complication could be related to *C. difficile* itself, which may promote

76 microbial translocation (8). Some evidence suggests that vancomycin therapy, which suppresses

77 most anaerobic bacteria, could also be associated with this complication (7,8). This favours

78 intestinal colonization by healthcare-associated pathogens, including vancomycin-resistant

79 *Enterococci* (VRE) and *Candida* spp. (9,10). In support of this, independent risk factors for BSI

80 secondary to CDI include ribotype 027, recurrent or severe disease, and vancomycin regimens

81 higher than 500mg/day (7,8).

82   Fecal microbiota transplantation (FMT) aims to restore the normal composition of gut

83 microbiota. FMT is superior to vancomycin in treating CDI (11–13) and is recommended to manage

84 recurrences (14–16). FMT could also be a promising approach for severe CDI (17–20) and *C.*

85 *difficile* ribotype 027 (21). In theory, the restoration of healthy microbiota through FMT might also

86 prevent CDI-associated BSI via several mechanisms, including cure of the infection, avoiding need

87   for vancomycin, and decrease in antibiotic resistance gene expression in the gut resistome of the

88   patient. This could decrease the incidence of BSI related to multi-drug resistant (MDR) bacteria

89   (22). To date, however, there are neither clinical nor pre-clinical data to support this hypothesis.

90   Our aim was therefore to assess the incidence of primary BSIs in a cohort of patients with rCDI

91   treated with either FMT or antibiotics.

92

93 **METHODS**

94

95 **Study design and patients**

96   This was a single-center, prospective cohort study, reported following STrengthening the

97 Reporting of OBservational studies in Epidemiology (STROBE) guidelines (23). All patients with

98 rCDI who were hospitalized from July 2013 to May 2018 at the Fondazione Policlinico

99 Universitario "A. Gemelli", a tertiary care center based in Rome, Italy, were considered for

100 inclusion.

101   Patients admitted to our hospital with a diagnosis of rCDI, following treatment of their first

102 infection at another center, and those who developed a CDI recurrence during hospitalization at our

103 center were eligible for inclusion. Thus, only patients who experienced their first observable

104 episode of rCDI at our hospital were considered, while patients with a second recurrence following

105 treatment for an initial CDI recurrence provided at our hospital were excluded. Other exclusion

106 criteria were: refusal of informed consent; patients aged <18 years; and patients who developed

107 secondary BSI from a known source. We excluded patients with a first episode of CDI, as FMT is

108 not recommended in this situation (16), and those considered unfit for FMT to avoid selection bias.

109   The following data were recorded for each patient: age; gender; comorbidities assessed with

110 the Charlson comorbidity index (24); development of bacterial, fungal, or polymicrobial BSI; date

111 of death; length of hospital stay; number of previous CDI recurrences; severity of CDI (i.e., mild,

112 severe, and fulminant as defined in Appendix); hospitalization or stay in a long-term care unit in the

113 previous 90 days; admission to an intensive care unit (ICU) in the previous 90 days; MDR infection

114 in the previous 90 days; antibiotic therapy in the previous 30 days; treatment provided for CDI

115 (FMT or antibiotics); and insertion of any of a central venous catether (CVC), urinary catheter, or

116 percutaneous endoscopic gastrostomy during hospitalization and before treatment.

117   Antibiotic regimens were categorized by class, administration route, duration, and dosages.

118 Responsible physicians chose antibiotic regimens based on recommendations from the European

119 Society of Clinical Microbiology and Infectious Disease for the management of CDI (15). FMT,

120 including donor selection, manufacturing of fecal infusates, and fecal delivery, was performed

121 according to international guidelines (16). Blood cultures were performed if patients developed

122 pyrexia (body temperature >38°C) or other signs or symptoms considered potentially attributable to

123 sepsis. Participants were followed up with weekly visits or telephone calls from study enrollment

124 until 90 days after the end of treatment. Definitions of CDI recurrence and BSI used in the study,

125 and description of microbiological analyses and of our FMT protocol, are provided in the

126 Appendix.

127

128 **Outcomes**

129 Our primary outcome was the development of primary BSI from any organism after

130 treatment for rCDI, and within a 90-day follow-up period. Secondary outcomes were length of

131 hospitalization and overall survival (OS) at 90 days.

132

133 **Data analysis and statistics**

134 For continuous variables, data were expressed as means with a standard deviation (SD) and

135 median and interquartile range. For categorical variables, data were expressed as numbers and

136 percentages. The difference in means or proportions in the demographic and clinical characteristics

137 was examined using the standardized difference Cohen's d (25). There is no universally

138 acknowledged value that demonstrates balanced data, but 10% is recommended as a reasonable

139 cutoff for unbalance (26). The difference in means or proportions in the outcome data was

140 examined using the difference and 95% confidence interval (CI).

141 We used propensity score matching to match comparison and intervention respondents to

142 create a more valid and counterfactual comparison group (27). The propensity score was the

143 probability of treatment with FMT based on the person's age, gender, number of CDI recurrences,

144 severity of CDI, Charlson index, receipt of hospital or long-term care in the last 90 days, receipt of

145 antibiotics in the last 30 days, and insertion of any CVC or urinary catheter, using probit regression.

146 Common support was used by dropping FMT cases whose propensity score was higher than the

147 maximum or less than the minimum propensity score of the control group. The one-to-one nearest

148 neighbor matching was done without caliper and without replacement, and ties did not exist.

149 Although differences remained between the FMT and antibiotic patients in the matched sample, we

150 did not repeat the matching process due to the already decreased number of patients who could be

151 included (57/109 (52%) FMT patients and 57/181 (31%) antibiotic patients). After matching, the

152 analysis was restricted to matched participants in the control and FMT groups. OS was evaluated

153 using a Kaplan-Meier survivor function.

154 Statistical analyses were performed with SPSS version 20.0 (SPSS Inc., Chicago, IL., USA)

155 and Stata Statistical Software: Release 14 (StataCorp LP, College Station, TX., USA). The function

156 psmatch2 was used for propensity matching.

157

158 **Role of the funding source**

159 None

160

161   **RESULTS**

162

163   **Demographic and clinical characteristics of patients at baseline**

164   Three-hundred and twenty-eight subjects with rCDI, who where hospitalized from July 2013

165   through May 2018 at our hospital, were evalutated for recruitment. Most had been treated with

166   either oral vancomycin or metronidazole, and only a minority with fidaxomicin, at their first

167   episode of CDI. Thirty-eight patients were excluded for the following reasons: 25 subjects

168   developed a secondary BSI from a known source (urinary sepsis: 12 patients; sepsis from CVC:

169   eight patients; pulmonary sepsis: five patients); seven subjects were <18 years old; and six subjects

170   were considered unfit for FMT. Data from the remaining 290 subjects (F=180, M=110, mean age

171   75 years) were analyzed. Of them, 109 patients (38%) were treated with FMT and 181 (62%)

172   received antibiotic therapy.

173   In the antibiotic group, the following drugs and regimens were used for 10 days: pulsed oral

174   vancomycin 125 mg q.i.d. (n=63); pulsed oral vancomycin 250 mg q.i.d. (n=41); oral metronidazole

175   500 mg t.i.d. plus pulsed oral vancomycin 125 mg q.i.d. (n= 25); oral metronidazole 500 mg t.i.d.

176   (n= 24); fidaxomicin 200 mg b.i.d. (n= 11); pulsed oral vancomycin 500 mg q.i.d. (n=9); tapered

177   oral vancomycin regimen (n=8).

178   There was a large difference in the number of previous CDI recurrences between the two

179   groups (mean 2.82 in FMT group compared with 1.23 in the antibiotic group). Eighty percent of

180   those treated with antibiotics had one recurrence of CDI, compared with 19% of the FMT group.

181   The distribution of the number of recurrences in the two populations is detailed in Table 1. Thirty-

182   five (32%) patients in the FMT group presented with severe CDI, compared with 39 (22%) patients

183   in the antibiotic group. Thirteen patients in both the FMT group (12%) and antibiotic group (7%)

184   had fulminant disease. Seventeen (16%) and eleven (6%) patients had a CVC at enrollment in the

185   FMT and antibiotic groups respectively. Twenty-six (24%) patients had a urinary catheter at

186 enrollment in the FMT group and seventy-four (41%) in the antibiotic group. There were also

187 differences in gender and Charlson comorbidity index at baseline between the two groups (Table 1).

188   A higher rate of patients in the FMT group (n=106) experienced sustained cure of CDI after

189 treatment than patients in the antibiotic group (n=69) (97% vs 38%), similar to the results in another

190 study from our group (90% vs 26%).[12] All patients whose CDI recurred once again after treatment

191 were offered rescue therapy with FMT, fidaxomicin, or tapered vancomycin, based on the

192 responsible physician's decision. No patients in the FMT group underwent surgery (colectomy or

193 loop ileostomy) for overwhelming CDI, compared with 14 in the antibiotic group (0% vs 8%).

194   In the propensity score matching group, matched on a one-to-one basis, there were 57 pairs

195 with no ties. Fifty-two patients treated with FMT (48%) were excluded as they exceeded the

196 common support criteria. Using the standardized difference and a 10% cut-off, the FMT group

197 patients were younger, had a lower Charlson comorbidity index, a greater proportion of patients

198 with a mild clinical picture of CDI, a lower proportion with only one CDI recurrence, and a higher

199 proportion with CVC at enrolment. Figure 1 shows the distribution of the frequency of propensity

200 scores in the two groups.

201

202 **Development of BSI at 90 days**

203   Table 2 summarizes outcome data. Overall, 45 patients developed BSI (16%) during the 90-

204 day follow-up; five (5%) treated with FMT, and 40 (22%) with antibiotics. Five (5%) patients in the

205 FMT group and 28 (16%) in the antibiotic group developed a bacterial BSI. No patients in the FMT

206 group and 7 (12%) in the antibiotic group developed a fungal BSI. One patient in the FMT group

207 and one in the antibiotic group developed a polymicrobial BSI (1% in each). Pathogens responsible

208 for BSIs are shown in Table 3. Of the 11 patients, who received fidaxomycin to treat CDI, four

209 developed BSI. In the propensity score matched cohort, two patients in the FMT group and 15 in

210 the antibiotic group developed BSI during follow-up (4% vs 26%, difference of 23%, 95% CI 10-

211 35%).

212 **Length of hospitalization**

213   In the whole cohort, mean length of hospitalization was 13.3 days in patients treated with

214 FMT and 29.7 days in those treated with antibiotics. In the propensity score matched cohort,

215 patients in the FMT group experienced a significantly shorter mean length of hospitalization (mean

216 13.4 days vs 27.8 days, difference 14 days 95% CI 9-20 days).

217

218 **Overall survival at 90 days**

219   In the whole cohort, 79 of 290 patients (27%) died during the 90-day follow-up period, 21

220 (7%) of them due to BSI (all in the antibiotic cohort). Causes of death in the remaining 58 patients

221 were: deterioration of clinical condition due to overwhelming CDI (n=15, three in the FMT cohort

222 and 12 in the antibiotic cohort); complications after surgery for CDI (n=11, all in the antibiotic

223 cohort); ischemic heart disease (n=10, five in the FMT cohort and five in the antibiotic cohort);

224 ischemic cerebrovascular disease (n=9, one in the FMT cohort and eight in the antibiotic cohort);

225 cancer (n=3, one in the FMT cohort and two in the antibiotic cohort); pulmonary failure (n=3, all in

226 the antibiotic cohort); acute-on-chronic kidney failure (n=3, all in the antibiotic cohort); variceal

227 bleeding (n=2, one in the FMT cohort and one in the antibiotic cohort); and acute-on-chronic liver

228 failure (n=2, all in the antibiotic cohort).

229   Fifty-eight patients (73%) died within 30 days from the end of treatment. Five of these had

230 been treated with FMT, and 53 with antibiotics. All were inpatients at the time of death. None of the

231 11 patients who died due to complications of surgery for overwhelming CDI had developed BSI.

232 The other 21 patients (27%) died between 31 and 90 days after the end of treatment; four were in

233 the FMT group and 17 in the antibiotic group. Ninety-day OS for the whole cohort was 73%, with

234 100 (92%) patients alive in the FMT group, and 111 (61%) in the antibiotic group.

235   In the propensity-matched cohort, six (11%) patients in the FMT group and 24 (42%) in the

236 antibiotic group died during the 90-day follow-up period, eight (7%) of them because of BSI and

237 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

244 **DISCUSSION**

245

246   A considerable proportion of patients with CDI can develop a primary BSI, which is caused

247 mainly by intestinal microbes (7,8). Some evidence suggests that this complication could contribute

248 to the global burden of CDI by increasing length of hospital stay and mortality (7). Moreover,

249 primary BSI is known to be the second most burdensome hospital-associated infection in Europe

250 (28), due to both inpatient costs, and also needs that arise after discharge (29). Antibiotics

251 commonly used to treat CDI, such as vancomycin, may not prevent the risk of BSI in these patients

252 (9,10,30,31). In our study, patients with rCDI who received FMT were less likely to develop a

253 primary BSI, and related complications, including death. There were notable differences between

254 the FMT and antibiotic treated groups in the original cohort, particularly in relation to the number

255 of CDI recurrences. We used propensity scores matched on nearest neighbor to reduce the

256 differences between groups. However, there was not a perfect overlap in propensity scores, and

257 imbalance in several baseline characteristics persisted between the propensity-score matched

258 groups. This leaves the possibility that the two groups of patients are different, due to confounding,

259 and we must be cautious in interpreting the findings.

260   In the propensity-matched cohort, with 57 patients per treatment, of the 17 patients who

261 developed BSI from any organism, two were treated with FMT and 15 with antibiotics, with a

262 significantly lower incidence in the FMT group. Patients from the matched cohort treated with FMT

263 also experienced a significantly shorter mean hospitalization than those treated with antibiotics.

264 Although we did not perform a cost-effectiveness analysis, this could have implications for the cost

265 of managing patients with rCDI, already evaluated in dedicated health economics studies (32,33).

266 Another noteworthy finding observed in the propensity matched cohort was the significantly higher

267 OS at 90 days in patients treated with FMT. Most patients died within 30 days from the end of

268 treatment, many because of CDI complications, and all were hospitalized at the time of death. There

269 were small differences in death rates between the two groups between 30 and 90 days, when several

270   patients were discharged. These findings suggest that the longer 90-day OS experienced by patients

271   in the FMT group is attributable to cure of CDI, leading to an improvement in their clinical

272   condition. The higher number of post-surgical deaths in the antibiotic group could have increased

273   the difference in death rates between groups. However, all surgical patients underwent surgery for

274   overwhelming CDI, and not for other indications, so these events were always dependent on the

275   clinical severity of CDI.

276   As an explanation for our findings, FMT could act through several therapeutic pathways to

277   prevent BSI, and related complications, in patients with CDI. FMT may directly reduce this risk, by

278   curing CDI. *C. difficile* toxins are known to damage the gut barrier and increase intestinal

279   permeability (34–36), thus favouring translocation of pathogens into the bloodstream. Moreover,

280   severe CDI has been identified as a risk factor for BSI (7,8), suggesting that severe inflammation

281   could further increase gut permeability. FMT is recognized as a highly effective therapy for

282   sustained cure of rCDI (11), and increasing evidence suggests that it could also be a reliable

283   treatment for severe CDI (12,20,37,38). In our study, the significantly higher rate of patients cured

284   by FMT, rather than by antibiotics, may have contributed to decrease intestinal permeability and to

285   prevent translocation of microbes, and therefore the development of BSI. Additionally, CDI is

286   known to promote colonization of the gut by *Candida* (39). By curing CDI, FMT may not only

287   decrease the rate of bacterial BSI, but also of *Candida*-dependent systemic infections.

288   Another possible explanation for our findings is the avoidance of repeated cycles of

289   vancomycin, due to cure of CDI by FMT. High-dose (>500 mg/day) oral vancomycin has been

290   previously identified as a risk factor for BSI development, (7) and this finding could be explained

291   by its effect on gut microbiota. Oral vancomycin was shown to decrease Firmicutes (including *C.*

292   *difficile*) and to increase Proteobacteria in humans (40). The increase of Proteobacteria phylum

293   could lead to the overgrowth of bacteria commonly responsible for primary BSI, including *K.*

294   *pneumoniae* or *E. coli*. Moreover, the sustained use of vancomycin could foster the development of

295   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

339

340

341

342

343

344

345

REFERENCES

1. Miller BA, Chen LF, Sexton DJ, Anderson DJ. Comparison of the Burdens of Hospital-Onset, Healthcare Facility-Associated Clostridium difficile Infection and of Healthcare-Associated Infection due to Methicillin-Resistant Staphylococcus aureus in Community Hospitals. Infect Control Hosp Epidemiol. 2011; 32:387-90. [PMID: 21460491] doi: 10.1086/659156.

2. Dubberke ER, Olsen MA. Burden of Clostridium difficile on the Healthcare System. Clin Infect Dis. 2012;55:S88–92. [PMID: 22752870] doi: 10.1093/cid/cis335.

3. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, et al. Burden of Clostridium difficile infection in the United States. N Engl J Med. 2015; 372:825-34. [PMID: 25714160] doi:10.1056/NEJMoa1408913

4. Ma GK, Brensinger CM, Wu Q, Lewis JD. Increasing Incidence of Multiply Recurrent *Clostridium difficile* Infection in the United States. Ann Intern Med. 2017;167:152. [PMID: 28672282] doi: 10.7326/M16-2733.

5. Kelly CP. Can we identify patients at high risk of recurrent Clostridium difficile infection? Clin Microbiol Infect. 2012;18: 21–27. [PMID: 23121551] doi: 10.1111/1469-0691.12046.

6. Olsen MA, Yan Y, Reske KA, Zilberberg MD, Dubberke ER. Recurrent Clostridium difficile infection is associated with increased mortality. Clin Microbiol Infect. 2015;21:164–70. [PMID: 25658560] doi: 10.1016/j.cmi.2014.08.017.

7. Falcone M, Russo A, Iraci F, Carfagna P, Goldoni P, Vullo V, et al. Risk Factors and Outcomes for Bloodstream Infections Secondary to Clostridium difficile Infection. Antimicrob Agents Chemother. 2016;60:252–7. [PMID: 26482315] doi:10.1128/AAC.01927-15.

8. Russo A, Falcone M, Fantoni M, Murri R, Masucci L, Carfagna P, et al. Risk factors and clinical outcomes of candidaemia in patients treated for Clostridium difficile infection. Clin

372   Microbiol Infect.. 2015;21:493.e1-493.e4. [PMID: 25698658] doi:

373   10.1016/j.cmi.2014.12.024.

374   9.   Donskey CJ, Chowdhry TK, Hecker MT, Hoyen CK, Hanrahan JA, Hujer AM, et al. Effect

375   of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of

376   colonized patients. N Engl J Med. 343:1925–32. [PMID: 11136263] doi:

377   10.1056/NEJM200012283432604

378   10. Pultz NJ, Stiefel U, Ghannoum M, Helfand MS, Donskey CJ. Effect of parenteral antibiotic

379   administration on establishment of intestinal colonization by Candida glabrata in adult mice.

380   Antimicrob Agents Chemother. 2005;49:438–40. [PMID: 15616330] doi:

381   10.1128/AAC.49.1.438-440.2005

382   11. Ianiro G, Maida M, Burisch J, Simonelli C, Hold G, Ventimiglia M, et al. Efficacy of

383   different faecal microbiota transplantation protocols for Clostridium difficile infection: A

384   systematic review and meta-analysis. United European Gastroenterol J. 2018;6:1232-1244.

385   [PMID: 30288286] doi: 10.1177/2050640618780762.

386   12. Cammarota G, Masucci L, Ianiro G, Bibbò S, Dinoi G, Costamagna G, et al. Randomised

387   clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the

388   treatment of recurrent *Clostridium difficile* infection. Aliment Pharmacol Ther. 2015;41:835–

389   43. PMID [25728808] doi: 10.1111/apt.13144.

390   13. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al.

391   Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*. N Engl J Med.

392   2013;368:407-15. [PMID: 23323867] doi:10.1056/NEJMoa1205037

393   14. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al.

394   Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J

395   Gastroenterol. 2013; 108:478-98. [PMID: 23439232] doi:10.1038/ajg.2013.4

396   15. Debast SB, Bauer MP, Kuijper EJ, European Society of Clinical Microbiology and Infectious

397   Diseases. European Society of Clinical Microbiology and Infectious Diseases: Update of the

398   Treatment Guidance Document for Clostridium difficile Infection. Clin Microbiol Infect.

399   2014;20:1–26. [PMID: 24118601] doi: 10.1111/1469-0691.12418.

400 16. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al. European

401   consensus conference on faecal microbiota transplantation in clinical practice. Gut.

402   2017;66:569–80. [PMID: 28087657]; doi: 10.1136/gutjnl-2016-313017.

403 17. Agrawal M, Aroniadis OC, Brandt LJ, Kelly C, Freeman S, Surawicz C, et al. The Long-

404   term Efficacy and Safety of Fecal Microbiota Transplant for Recurrent, Severe, and

405   Complicated Clostridium difficile Infection in 146 Elderly Individuals. J Clin Gastroenterol.

406   2015;50:1. [PMID: 26352106] doi: 10.1097/MCG.0000000000000410.

407 18. Cammarota G, Ianiro G, Magalini S, Gasbarrini A, Gui D. Decrease in Surgery for

408   *Clostridium difficile* Infection After Starting a Program to Transplant Fecal Microbiota. Ann

409   Intern Med. 2015;163:487. [PMID: 26370022] doi: 10.7326/L15-5139.

410 19. Ianiro G, Valerio L, Masucci L, Pecere S, Bibbò S, Quaranta G, et al. Predictors of failure

411   after single faecal microbiota transplantation in patients with recurrent Clostridium difficile

412   infection: results from a 3-year, single-centre cohort study. Clin Microbiol Infect.

413   2017;23:337.e1-337.e3. [PMID: 28057560] doi: 10.1016/j.cmi.2016.12.025

414 20. Fischer M, Sipe B, Cheng Y-W, Phelps E, Rogers N, Sagi S, et al. Fecal microbiota

415   transplant in severe and severe-complicated Clostridium difficile: A promising treatment

416   approach. Gut Microbes. 2017;8:289–302. [PMID: 28001467] doi:

417   10.1080/19490976.2016.1273998.

418 21. Lagier J-C, Delord M, Million M, Parola P, Stein A, Brouqui P, et al. Dramatic reduction in

419   Clostridium difficile ribotype 027-associated mortality with early fecal transplantation by the

420   nasogastric route: a preliminary report. Eur J Clin Microbiol Infect Dis. 2015;34:1597–

421   601.[PMID: 25947205] doi: 10.1007/s10096-015-2394-x.

422 22. Bilinski J, Grzesiowski P, Sorensen N, Madry K, Muszynski J, Robak K, et al. Fecal

423   Microbiota Transplantation in Patients With Blood Disorders Inhibits Gut Colonization With

424   Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. Clin Infect Dis.

425   2017;65:364–70. [PMID: 28369341] doi: 10.1093/cid/cix252.

426   23.   STROBE Statement-Checklist of items that should be included in reports of cohort studies.

427   Available from: http://www.epidem.com/

428   24.   Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic

429   comorbidity in longitudinal studies: development and validation. J Chronic Dis.

430   1987;40:373–83. [PMID: 3558716].

431   25.   Cohen, J. 1988. Statistical Power Analysis for the Behavioral Sciences 2nd ed. Hillsdale, NJ:

432   Erlbaum.

433   26.   Austin PC. Balance diagnostics for comparing the distribution of baseline covariates

434   between treatment groups in propensity-score matched samples. Stat Med. 2009;28:3083-107.

435   [PMID: 19757444]. doi: 10.1002/sim.3697.

436   27.   Rosenbaum PR, Donald BR. The central role of the propensity score in observational studies

437   for causal effects. Biometrika. 1983;70: 41–55. doi: 10.1093/biomet/70.1.41

438   28.   Cassini A, Plachouras D, Eckmanns T, Abu Sin M, Blank H-P, Ducomble T, et al. Burden of

439   Six Healthcare-Associated Infections on European Population Health: Estimating Incidence-

440   Based Disability-Adjusted Life Years through a Population Prevalence-Based Modelling

441   Study. Harbarth S, editor. PLOS Med. 2016;13:e1002150. [PMID: 27755545] doi:

442   10.1371/journal.pmed.1002150.

443   29.   Tiru B, DiNino EK, Orenstein A, Mailloux PT, Pesaturo A, Gupta A, et al. The Economic

444   and Humanistic Burden of Severe Sepsis. Pharmacoeconomics. 2015;33:925–37. [PMID:

445   25935211] doi: 10.1007/s40273-015-0282-y.

446   30.   Edlund C, Barkholt L, Olsson-Liljequist B, Nord CE. Effect of vancomycin on intestinal

447   flora of patients who previously received antimicrobial therapy. Clin Infect Dis.

448   1997;25:729–32. [PMID: 9314469].

449   31.   Louie TJ, Emery J, Krulicki W, Byrne B, Mah M. OPT-80 eliminates Clostridium difficile

450   and is sparing of bacteroides species during treatment of C. difficile infection. Antimicrob

451   Agents Chemother. 2009;53:261–3. [PMID: 18955523] doi: 10.1128/AAC.01443-07.

452   32.   Reigadas Ramírez E, Bouza ES. Economic Burden of Clostridium difficile Infection in

453   European Countries. Adv Exp Med Biol. 2018;1050:1-12. [PMID: 29383660] doi:

454   10.1007/978-3-319-72799-8_1.

455   33.   Brain D, Yakob L, Barnett A, Riley T, Clements A, Halton K, et al. Economic evaluation of

456   interventions designed to reduce Clostridium difficile infection. PLoS One.

457   2018;13:e0190093. [PMID: 29298322] doi: 10.1371/journal.pone.0190093.

458   34.   Triadafilopoulos G, Pothoulakis C, O'Brien MJ, LaMont JT. Differential effects of

459   Clostridium difficile toxins A and B on rabbit ileum. Gastroenterology. 1987;93:273–9.

460   [PMID: 3596162].

461   35.   Hecht G, Pothoulakis C, LaMont JT, Madara JL. Clostridium difficile toxin A perturbs

462   cytoskeletal structure and tight junction permeability of cultured human intestinal epithelial

463   monolayers. J Clin Invest. 1988;82:1516–24. [PMID: 3141478].

464   36.   Heyman M, Corthier G, Lucas F, Meslin JC, Desjeux JF. Evolution of the caecal epithelial

465   barrier during Clostridium difficile infection in the mouse. Gut. 1989;30:1087–93. [PMID:

466   2504650].

467   37.   Ianiro G, Masucci L, Quaranta G, Simonelli C, Lopetuso LR, Sanguinetti M, et al.

468   Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin

469   for the treatment of severe refractory *Clostridium difficile* infection-single versus multiple

470   infusions. Aliment Pharmacol Ther. 2018;48:152–9. [PMID: 29851107] doi:

471   10.1111/apt.14816.

472   38.   Fischer M, Sipe BW, Rogers NA, Cook GK, Robb BW, Vuppalanchi R, et al. Faecal

473   microbiota transplantation plus selected use of vancomycin for severe-complicated

474   *Clostridium difficile* infection: description of a protocol with high success rate. Aliment

475   Pharmacol Ther. 2015;42:470–6. [PMID: 26096320] doi: 10.1111/apt.13290.

476   39.   Raponi G, Visconti V, Brunetti G, Ghezzi MC. Clostridium difficile Infection and Candida

477   Colonization of the Gut: Is There a Correlation? Clin Infect Dis. 2014;59:1648–9. [PMID:

478   25091308] doi: 10.1093/cid/ciu637.

479   40.   Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L.

480   Short-Term Antibiotic Treatment Has Differing Long-Term Impacts on the Human Throat

481   and Gut Microbiome. Ratner AJ, editor. PLoS One. 2010;5:e9836. [PMID: 20352091] doi:

482   10.1371/journal.pone.0009836.

483   41.   Nerandzic MM, Mullane K, Miller MA, Babakhani F, Donskey CJ. Reduced Acquisition and

484   Overgrowth of Vancomycin-Resistant Enterococci and Candida Species in Patients Treated

485   With Fidaxomicin Versus Vancomycin for Clostridium difficile Infection. Clin Infect Dis.

486   2012;55:S121–6. [PMID: 22752860] doi: 10.1093/cid/cis440.

487   42.   van Schaik W. The human gut resistome. Philos Trans R Soc Lond B Biol Sci.

488   2015;370:20140087. doi: 10.1098/rstb.2014.0087.

489   43.   Hvas CL, Dahl Jørgensen SM, Jørgensen SP, Storgaard M, Lemming L, Hansen MM, et al.

490   Fecal Microbiota Transplantation Is Superior to Fidaxomicin for Treatment of Recurrent

491   Clostridium difficile Infection. Gastroenterology. 2019. pii: S0016-5085;35434-9. [PMID:

492   30610862] doi: 10.1053/j.gastro.2018.12.019.

493   44.   McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical

494   Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update

495   by the Infectious Diseases Society of America (IDSA) and Society for Healthcare

496   Epidemiology of America (SHEA). Clin Infect Dis. 2018;66:987–94. [PMID: 29562266] doi:

497   10.1093/cid/ciy149.

498   45.   Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–

499   associated infection and criteria for specific types of infections in the acute care setting. Am J

500   Infect Control. 2008;36:309–32. [PMID: 18538699] doi: 10.1016/j.ajic.2008.03.002.

501   46.   Pappas PG, Kauffman CA, Andes D, Benjamin, Jr. DK, Calandra TF, Edwards, Jr. JE, et al.

502   Clinical Practice Guidelines for the Management of Candidiasis: 2009 Update by the

503   Infectious Diseases Society of America. Clin Infect Dis. 2009;48:503–35. [PMID: 19191635]

504   doi: 10.1086/596757.

505   47.   O'Grady NP, Alexander M, Dellinger EP, Gerberding JL, Heard SO, Maki DG, et al.

506   Guidelines for the Prevention of Intravascular Catheter–Related Infections. Infect Control

507   Hosp Epidemiol. 2002;23:759–69. [PMID: 12517020] doi: 10.1086/502007.

508

509 **ACKNOWLEDGEMENTS**

514 **Current Author Addresses**

515 Gianluca Ianiro, Rita Murri, Giusi Desirè Sciumè, Michele Impagnatiello, Luca Masucci, Maurizio

516 Sanguinetti, Roberto Cauda, Antonio Gasbarrini, Massimo Fantoni, Giovanni Cammarota:

517 Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Largo A. Gemelli 8, 00168 Rome,

518 Italy.

519 Alexander C. Ford: Bexley Wing, St. James's University Hospital Leeds, LS9 7TF, United

520 Kingdom.

521 Graham R. Law: Sarah Swift Building, University of Lincoln, Lincoln, LN6 7TS, United Kingdom

522 United Kingdom.

523 Herbert Tilg: Department of Internal Medicine I, Gastroenterology, Hepatology, Metabolism &

524 Endocrinology; Anichstrasse 35, 6020 Innsbruck, Austria.

525

526

527 **Author contributions**

528 • Study concept and design: Gianluca Ianiro, Rita Murri, Massimo Fantoni, Giovanni

529   Cammarota

530 • Acquisition of data: Gianluca Ianiro, Rita Murri, Michele Impagnatiello, Giusi Desirè

531   Sciumè, Luca Masucci, Massimo Fantoni, Giovanni Cammarota

532 • Analysis and interpretation of data: Gianluca Ianiro, Rita Murri, Alexander C. Ford, Graham

533   R. Law, Massimo Fantoni, Giovanni Cammarota

534   •   Drafting of the manuscript: Gianluca Ianiro, Rita Murri, Luca Masucci, Alexander C. Ford,

535   Graham R Law, Giovanni Cammarota

536   •   Critical revision of the manuscript for important intellectual content: All authors

537   All authors approved the final version of the manuscript

538

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

Variable	Original cohort			After propensity score matching		
	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.**

| Outcome level	Variable	Original cohort			After propensity score matching			
		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		

545 **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**

546

547 * 12 of 45 patients developed a polymicrobial bloodstream infection (bloodstream infections from
548 multiple bacteria in 10 patients; bloodstream infections from fungal and bacterial organisms in 2
549 patients). CRE= carbapenem-resistant Enterobacteriaceae; ESBL= extended-spectrum beta-
550 lactamase; FMT= fecal microbiota transplantation; MR= methicillin-resistant; XDR= extensively
551 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**
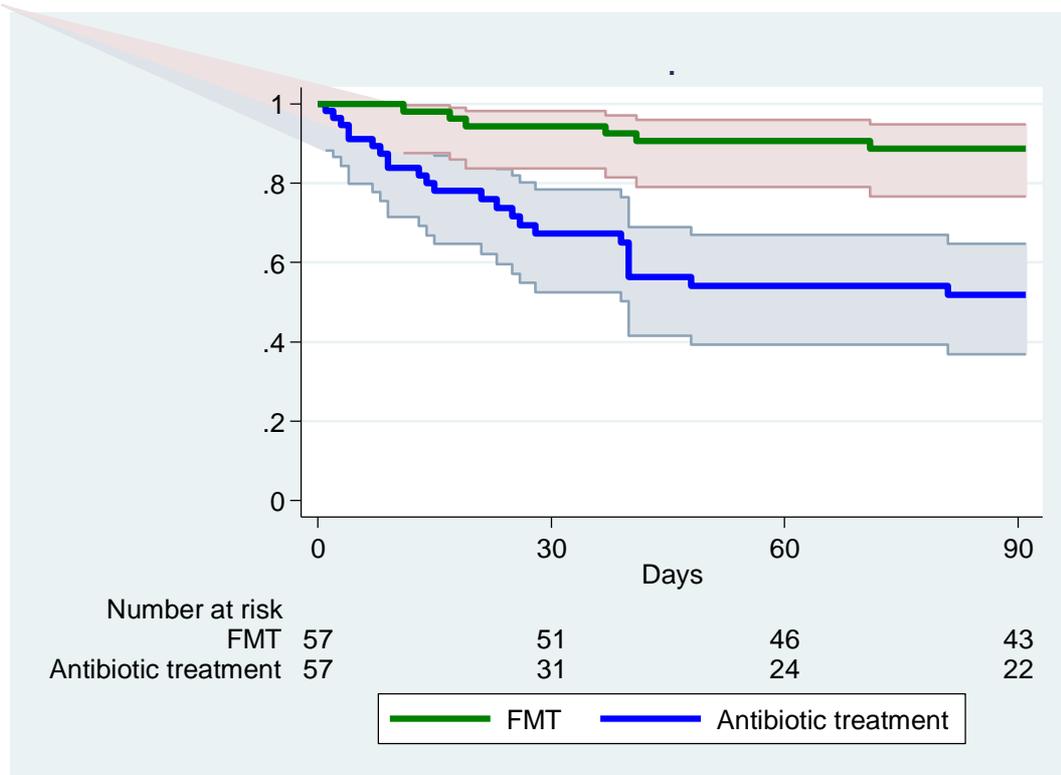
554 **with antibiotics.**
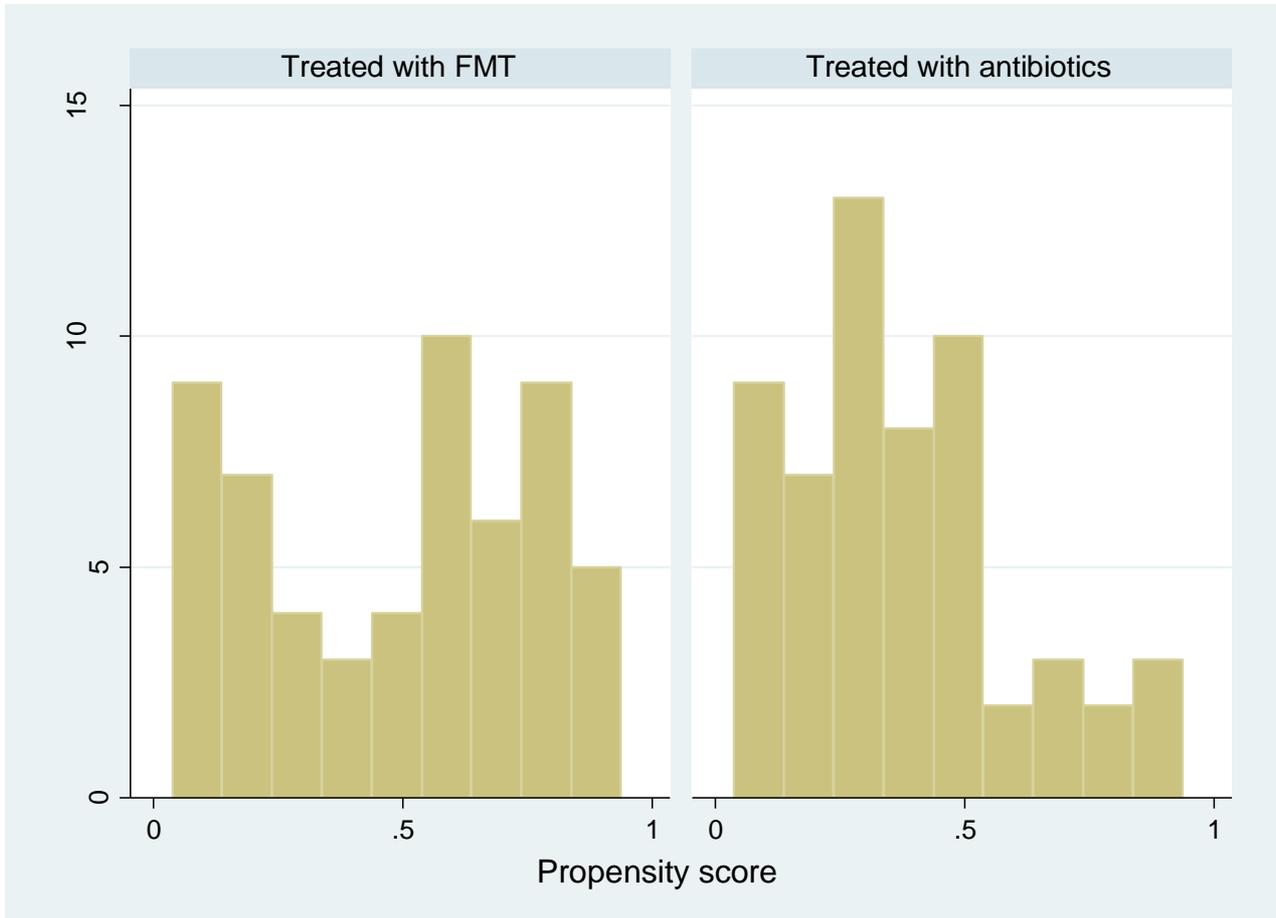
555

556

557

558

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.**

561

562

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

564

565 **APPENDIX**

566

567 **Definitions**

568   RCDI was defined as diarrhea and positivity for the *C. difficile* toxin stool test within 8

569 weeks from the end of the previous antibiotic treatment (15). Patients were classified, based on their

570 clinical picture, according to the latest recommendations from the Infectious Disease Society of

571 America for the management of CDI (44). Criteria for defining non-severe CDI were leukocytosis

572 with a white blood cell count of ≤15 000 cells/mL and a serum creatinine level <1.5 mg/dL. Severe

573 CDI was defined by leukocytosis with a white blood cell count of ≥15 000 cells/mL or a serum

574 creatinine level >1.5 mg/dL. Finally, fulminant CDI was diagnosed in the presence of hypotension

575 or shock, ileus, or megacolon (44). Nosocomial CDI was defined as CDI diagnosed more than 48

576 hours after hospital admission.

577   An episode of BSI was defined, according to recommendations from the Centers for Disease

578 Control and Prevention, as the presence of one or more recognized pathogens in at least one blood

579 culture or, for potential skin contaminants, the positivity of two or more separate blood cultures

580 within 48 hours, in the presence of at least two signs or symptoms of a systemic inflammatory

581 response (45). Candidaemia was defined as one or more blood cultures positive for *Candida* genus,

582 together with clinical signs and symptoms of infection (46).

583   A primary BSI was defined as one occurring without a known source of infection. A

584 secondary BSI was defined as one arising from a recognized source. CVCs were accounted to be

585 the likely source of infection if blood cultures from the catheter lumen, but not from a peripheral

586 vein, were positive within 2 hours (47).

587

588 **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

615   diseases caused by prions; recent parasitosis or infection from rotavirus, Giardia lamblia, or other

616   microbes with gastrointestinal involvement; recent (less than 6 months) travel in tropical countries,

617   countries at high risk of communicable diseases, or traveller's diarrhea; recent (less than 6 months)

618   history of vaccination with a live attenuated virus; healthcare or animal workers.

619   B) Gastrointestinal, metabolic and neurological disordes, including: irritable bowel

620   syndrome, inflammatory bowel disease, functional chronic constipation, celiac disease, or other

621   chronic gastrointestinal diseases; chronic, systemic autoimmune disorders with gastrointestinal

622   involvement; gastrointestinal cancer or polyposis (or high risk for these disorders); recent diarrhea

623   or hematochezia; neurological disorders and/or psychiatric conditions; overweight or obesity (body

624   mass index >25)

625   C) The use of drugs that could impair the composition of the intestinal microbiota,

626   including: recent (<3 months) exposure to antibiotics, immunosuppressants, chemotherapeuthics, or

627   chronic therapy with proton pump inhibitors.

628   All potential donors who passed the questionnaire underwent blood exams (including:

629   serology for cytomegalovirus, Epstein-Barr virus, hepatitis A, hepatitis B, hepatitis C, and hepatitis

630   E viruses, syphilis, HIV-1 and HIV-2 viruses, *Entamoeba histolytica*; complete blood count, C-

631   reactive protein, erythrocyte sedimentation rate, albumin, creatinine, electrolytes,

632   aminotransferases, bilirubin, gamma-glutamyltransferase, alkaline phosphatase) and stool exams

633   (including: culture and toxin for *C. difficile*; detection of enteric pathogens, including Salmonella,

634   Shigella, Campylobacter, Escherichia coli O157 H7, Yersinia, vancomycin-resistant enterococci,

635   methicillin-resistant Staphylococcus aureus, or Gram-negative multidrug-resistant bacteria;

636   Norovirus; antigens and/or acid fast staining for *Giardia lamblia* and *Criptosporidium parvum*;

637   Protozoa (including *Blastocystis hominis*) and helminths; faecal occult blood testing) to exclude

638   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