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## **Bacterial diversity and specific taxa are associated with decolonization of carbapenemase-producing enterobacterales after fecal microbiota transplantation**

Benjamin Davido, Andrea R Watson, Pierre de Truchis, Gianluca Galazzo, Aurelien Dinh, Rui Batista, Elisabeth M Terveer, Christine Lawrence, Hugues Michelon, Marion Jobard, et al.

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# Journal of Infection

## Bacterial Diversity and Specific Taxa are Associated with Decolonization of Carbapenemase-Producing Enterobacterales after Fecal Microbiota Transplantation --Manuscript Draft--

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<b>Abstract:</b>	<p><b>Objectives:</b> We evaluated the effect of fecal microbiota transplantation (FMT) on the clearance of carbapenemase-producing Enterobacterales (CPE) carriage.</p> <p><b>Methods:</b> We performed a prospective, multi-center study, conducted among patients who received a single dose of FMT from one of four healthy donors. The primary endpoint was complete clearance of CPE carriage two weeks after FMT with a secondary endpoint at three months. Shotgun metagenomic sequencing was performed to assess gut microbiota composition of donors and recipients before and after FMT.</p> <p><b>Results:</b> Twenty CPE-colonized patients were included in the study, where post-FMT 20% (n=4/20) of patients met the primary endpoint and 40% (n=8/20) of patients met the secondary endpoint. Kaplan-Meier curves between patients with FMT intervention and the control group (n=82) revealed a similar rate of decolonization between groups. Microbiota composition analyses revealed that response to FMT was not donor-dependent. Responders had a significantly lower relative abundance of CPE species pre-FMT than non-responders, and 14 days post-FMT responders had significantly higher bacterial species richness and alpha diversity compared to non-responders (p&lt;0.05). Responder fecal samples were also enriched in specific species, with significantly higher relative abundances of <i>Faecalibacterium prausnitzii</i>, <i>Parabacteroides distasonis</i>, <i>Collinsella aerofaciens</i>, <i>Alistipes finegoldii</i> and <i>Blautia_A sp900066335</i> (q&lt;0.01) compared to non-responders.</p> <p><b>Conclusion:</b> FMT administration did not achieve statistical significance for CPE decolonization, but was correlated with the relative abundance of specific bacterial taxa, including CPE species.</p>

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1th January 2024  
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Dear Sir,

Please find enclosed our manuscript entitled “**Specific bacterial signatures in the microbiome are associated with decolonization of Carbapenemase-Producing Enterobacterales (CPE) during Fecal Microbiota Transplantation**” which I would like to publish in your renowned journal.

Historically, we published data in your journal data regarding potential impact of donors during FMT, pioneering this strategy since 2015 after the ECCMID congress to promote decolonization of multidrug-resistant organism (MDRO).

Besides, we published in 2017 in your journal (J of Inf) about the decolonization of VRE using FMT “Impact of faecal microbiota transplantation to eradicate vancomycin-resistant enterococci (VRE) colonization in humans”.

Today, we present the second part of our work, revealing that FMT in routine cannot resolve the issue of antimicrobial resistance. However, our findings show that responders to FMT (decolonized patients) exhibit particular microbiome signatures associated with the eradication of CPE carriage. Moreover, our data in the interventional group (n=20) support that FMT may serve more as a proxy to reduce the risk of infection rather than decolonization. We observed in 2/2 patients with a past medical history of recurrent UTI that both were free of symptoms during a one-year follow-up.

To the best of our knowledge, only 2 clinical trials have published on FMT to eradicate MDRO colonization. One, by Huttner et al. in CID among 10 patients who previously underwent selective digestive decontamination, and showed negative results. The other, very recently by Woodworth et al., reported in Science Trans Research about ESBL-producing Enterobacterales carriage and demonstrated its interest among 5 immunocompromised patients (kidney transplanted).

Our present work adds precious data to clinical physician and confirm that using FMT in routine among all colonized patients is not a relevant strategy. Still, specific patterns of patients could warrant its efficacy.

All authors are responsible for reported research and have participated in the concept and design, analysis and interpretation of data, drafting or revising, and have approved this manuscript.

I confirm that all the authors have seen and approved the manuscript, and that the manuscript has neither been previously published nor is being considered for publication elsewhere.

I hope you will consider this work for publication in your journal.

Sincerely yours,



B. Davido, MD, PhD

**Reviewers' comments:**

**Reviewer #1: It should be clearly stated that the route of administration, the protocol of FMT administration, single dose, etc. could influence real decolonization process. Decolonization occurred in patients who had lower CPE abundance before FMT. This means the FMT could be successful but the regimen the Authors used was inappropriate. So if you state that FMT doesn't work it is not true - FMT in this regimen doesn't work.**

A: We understand the reviewer's comment even if we did not write anywhere in the manuscript that "FMT does not work". We tried to mitigate such affirmation and integrated reviewer's comments.

Line 244 we clarified that we used a specific regimen for FMT in our study, right inside the paragraph that was already discussing different methods between authors. as follows ". Indeed, it should be considered that the route of administration used in the present study, as well as the protocol of FMT involving a single dose, may have negatively influenced the decolonization process."

**I think also the seq data should be published and accession numbers provided etc.**

A: As asked the seq data will be shared by Vedanta on request in order to meet both reviewers' demand and expectations. For that we uploaded the requested data on NCBI SRA under study ID PRJNA1121295 and for that we added a paragraph in the methods section.

Nevertheless, we decided to still restrict access to the data and we will embargo these sequences until after the manuscript is accepted for publication. However, we will make sequences available upon the execution of a Data Transfer Agreement with Vedanta if requested. Requests can be directed to [legal@vedantabio.com](mailto:legal@vedantabio.com)

**The conclusions from this study must be fairly discussed.**

A: Line 373 we clarified that the present work was about "complete" eradication of carriage.

Line 375 of the discussion we added the following sentence "suggesting that another FMT regimen could potentially yield more successful outcomes in this indication."

Likewise, in the conclusion of the abstract it is specified that we had a particular regimen as follows "FMT administration using the proposed regimen did not achieve statistical significance for complete CPE decolonization"

**Reviewer #2: The authors have made several revisions to the manuscript in response to prior comments and it appears substantially improved. While it is appreciated that this journal may not require data upload and sharing, for clarity, the authors can upload the data at this stage and include the accession number in this publication but still restrict access to be available upon request / embargo access until the Vedanta discovery manuscript is published. Otherwise, there is no guarantee that these data will eventually be uploaded, which should absolutely be expected of these projects to support reproducibility and fuller use of the data for the field.**

A: As asked the seq data will be shared by Vedanta on request in order to meet both reviewers' demand and expectations. For that we uploaded the requested data on NCBI SRA under study ID PRJNA1121295 and for that we added a paragraph in the methods section.

Nevertheless, we decided to still restrict access to the data and we will embargo these sequences until after the manuscript is accepted for publication. However, we will make sequences available upon the execution of a Data Transfer Agreement with Vedanta if requested. Requests can be directed to [legal@vedantabio.com](mailto:legal@vedantabio.com)

**The authors have not included a CONSORT style diagram, which was requested in the comments after the initial review and are recommended by consensus reporting guidelines (<https://www.equator-network.org/reporting-guidelines/consort/>). One should be created and included as a supplemental figure.**

A: Actually, we did not respond positively to this proposal in the first revision because CONSORT is meant for randomized clinical trials, not observational studies. As it has been re-asked in this R2, we propose to create a STROBE flowchart that reflects our study and is introduced as Figure 1.

**Line 238-239: The authors have not qualified their broad conclusion that 'FMT has limited ability to decolonize CPE' as suggested in initial submission. Data in this study demonstrate that a single FMT dose administered via enteric feeding tube has limited decolonization efficacy, not that FMT has limited ability. Why else would the authors suggest further standardization and study of FMT for this indication in lines 242-243? This seems important to clarify as a main conclusion.**

A: On this comment, you join reviewer #1 and we decided to clarify this statement and rephrase the whole paragraph as follows: "Consistent with findings previously reported by our group 22,23 and others 17, our results showed that a single dose of FMT administered via enteric feeding tube has limited efficacy to decolonize CPE. Nevertheless, the efficacy of FMT against MDROs is variable and successful decolonization of CPE or ESBL producing Enterobacteriales following FMT administration has been reported 29,30. Factors contributing to the variability in FMT efficacy in such indication include number of FMT doses, donor selection, FMT preparation, route of FMT administration and pre-treatment regimen 16,17,29,31. Indeed, it should be considered that the route of administration used in the present study, as well as the protocol of FMT involving a single dose, may have negatively influenced the decolonization process."

Also, in the conclusion it is mitigated as follows "Our study shows the importance of considering different factors that influence FMT efficacy in donors and recipients to achieve optimal therapeutic results, suggesting that another FMT regimen could potentially yield more successful outcomes in this indication."

**Line 277-279: It looks like the infection analysis in the Woodworth paper included 16 contemporaneous controls, not 5.**

A: Correct, we confounded the number of patients who had FMT at the first stage of the procedure. It is now corrected with the accurate value.

**Line 156, t-test is usually considered parametric, not non-parametric**

A: We completely agree, thank you for pointing out that typo.

**Bacterial Diversity and Specific Taxa are Associated with Decolonization of Carbapenemase-Producing Enterobacterales after Fecal Microbiota Transplantation**

Benjamin Davido<sup>1,2</sup>, Andrea R. Watson<sup>3</sup>, Pierre de Truchis<sup>1</sup>, Gianluca Galazzo<sup>3</sup>, Aurelien Dinh<sup>1,2</sup>, Rui Batista<sup>4</sup>, Elisabeth M Terveer<sup>5</sup>, Christine Lawrence<sup>6</sup>, Hugues Michelon<sup>7</sup>, Marion Jobard<sup>4</sup>, Azzam Saleh-Mghir<sup>1,8</sup>, Ed J Kuijper<sup>5</sup>, Silvia Caballero<sup>3</sup>

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I confirm that all listed authors have contributed to this work and approved the paper. Manuscript has not been submitted elsewhere.

Running title: Fecal Microbiota Transplantation for decolonization

Word count: 3995 words

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This work was presented in part at the IDWeek Conference held on 11-15 October 2023 in Boston, Massachusetts.

Highlights:

- Post-FMT data revealed microbiota changes in multidrug-resistant colonized patients
- FMT unexpectedly promoted the reduction of superinfections (n=2/2)
- Using FMT solely for decolonization might not be the most relevant purpose



## 1 **Abstract**

2 **Objectives:** We evaluated the effect of fecal microbiota transplantation (FMT) on the clearance of  
3 carbapenemase-producing Enterobacterales (CPE) carriage.

4 **Methods:** We performed a prospective, multi-center study, conducted among patients who received a  
5 single dose of FMT from one of four healthy donors. The primary endpoint was complete clearance of CPE  
6 carriage two weeks after FMT with a secondary endpoint at three months. Shotgun metagenomic  
7 sequencing was performed to assess gut microbiota composition of donors and recipients before and  
8 after FMT.

9 **Results:** Twenty CPE-colonized patients were included in the study, where post-FMT 20% (n=4/20) of  
10 patients met the primary endpoint and 40% (n=8/20) of patients met the secondary endpoint. Kaplan-  
11 Meier curves between patients with FMT intervention and the control group (n=82) revealed a similar  
12 rate of decolonization between groups.

13 Microbiota composition analyses revealed that response to FMT was not donor-dependent. Responders  
14 had a significantly lower relative abundance of CPE species pre-FMT than non-responders, and 14 days  
15 post-FMT responders had significantly higher bacterial species richness and alpha diversity compared to  
16 non-responders ( $p < 0.05$ ). Responder fecal samples were also enriched in specific species, with  
17 significantly higher relative abundances of *Faecalibacterium prausnitzii*, *Parabacteroides distasonis*,  
18 *Collinsella aerofaciens*, *Alistipes finegoldii* and *Blautia\_A sp900066335* ( $q < 0.01$ ) compared to non-  
19 responders.

20 **Conclusion:** FMT administration using the proposed regimen did not achieve statistical significance for  
21 complete CPE decolonization but was correlated with the relative abundance of specific bacterial taxa,  
22 including CPE species.

23

24 Key words: FMT, carbapenemase-producing Enterobacterales, decolonization, carriage

25

26 **Introduction**

27 Gastrointestinal (GI) colonization with multidrug-resistant organisms (MDROs) is a major risk factor for  
28 infection and mortality in high-risk patient populations <sup>1-5</sup>. In 2015, The European Centre for Disease  
29 Prevention and Control (ECDC) reported that infections with MDROs accounted for approximately 33,000  
30 hospital deaths in Europe <sup>6</sup>. Additionally, in 2019, up to 1.27 million deaths were attributable to  
31 antimicrobial resistance worldwide <sup>7</sup>. Of particular concern are carbapenemase-producing  
32 Enterobacterales (CPE), which have been classified by the World Health Organization as one of the most  
33 critical antibiotic-resistant pathogen groups for which new therapies are urgently needed <sup>8</sup>. The  
34 prevalence of CPE is increasing worldwide and in France alone, for instance, the number of CPE cases  
35 increased from 248 to approximately 1704 (6-fold) from 2012 – 2018 <sup>9</sup>. Likewise, a recent study conducted  
36 by the European Antimicrobial Resistance Surveillance Network (EARS-Net) revealed that the number of  
37 deaths attributable to carbapenem-resistant Enterobacterales in Europe increased by 4 to 6-fold between  
38 2007 and 2015, especially in countries such as Italy, Greece and Romania where CPE are highly prevalent  
39 <sup>6</sup>.

40 MDRO colonization has been shown to increase the risk of infection by 14% in a median follow-up period  
41 of 30 days <sup>10</sup>. Spontaneous decolonization of CPE is slow, ranging from 12% decolonization within 30 days  
42 in our hospital's dedicated ward for CPE-colonized patients under reinforced isolation precautions <sup>11</sup>, and  
43 up to 25% decolonization after 30 days among healthcare residents <sup>12</sup>. As a result, decolonization  
44 strategies including selective digestive decontamination (SDD) and fecal microbiota transplantation (FMT)  
45 have been implemented to accelerate MDRO decolonization and reduce infection risk <sup>13-16</sup>. While SDD

46 against CPE significantly reduced infection risk following successful decolonization, it was associated with  
47 the development of antibiotic resistance and colonization recurrence<sup>13,14,17</sup>. These risks have limited the  
48 use of SDD for infection prevention and guidelines from the European Society for Clinical Microbiology  
49 and Infectious Diseases strongly recommend against the use of oral antibiotics for MDRO decolonization  
50<sup>18</sup>. As an alternative, FMT has been shown to be a safer and more effective strategy for decolonization of  
51 CPE and other MDROs, with efficacy rates ranging from 20-90%<sup>16</sup> and a significant reduction in the  
52 number of MDRO infections following FMT treatment<sup>19-21</sup>.

53 Although several case reports have been published supporting the role of FMT in MDRO decolonization,  
54 our group reported the first case series (n=8) where we showed that 2/6 CPE carriers decolonized 1 month  
55 after FMT<sup>22</sup>. Due to its small size, our study lacked sufficient power to draw definitive conclusions, which  
56 led us to carry out a larger prospective multicenter FMT trial (FEDEX) with preliminary findings showing a  
57 CPE decolonization rate of 50%<sup>23</sup>. Importantly, this result is concordant with another randomized  
58 controlled clinical trial published by Huttner *et al.* (n=22)<sup>17</sup>. Here we report the final results of the FEDEX  
59 study in CPE-colonized immunocompromised patients and describe the impact of FMT on patient  
60 microbiota composition.

61

## 62 **Materials and methods**

### 63 *Setting*

64 FEDEX was a prospective multi-center study designed to evaluate the use of FMT for decolonization of  
65 CPE from the GI tract. The trial was conducted in three French university hospitals in Paris between  
66 January 2015 and December 2019. FMT administration was performed at Hôpital Raymond Poincaré,  
67 Garches, France.

68

69 *Participants*

70 Patients  $\geq 18$  years with confirmed CPE colonization and able to provide informed consent were included  
71 in the study. CPE colonization was determined by at least three consecutive positive rectal swabs at  
72 weekly intervals, including the week prior to FMT. Exclusion criteria were immunosuppression (human  
73 immunodeficiency virus with CD4  $< 200/\text{mm}^3$ ), immunosuppressive therapy (including corticosteroids  $> 60$   
74 mg/day for more than five days, chemotherapy, stem cell transplantation under immunosuppressant  
75 drugs and other inherited immunosuppressive disorder), antibiotic treatment at the time of FMT, previous  
76 infection with *Clostridioides difficile* treated with FMT, as well as individuals who were pregnant or  
77 breastfeeding, unable to provide informed consent, under tutorship or guardianship, homeless or without  
78 health insurance. Patients non-eligible for FMT intervention were assigned to the non-FMT cohort.

79

80 *Data collection*

81 The following data were collected from all study participants:

- 82 - Patient characteristics: age, sex, comorbidities, Charlson Comorbidity Index at admission,  
83 hospitalization 6 months prior to enrollment, duration of carriage, and travel abroad.
- 84 - Episodes of infection caused by CPE.
- 85 - Antibiotic administration and duration of treatment a month prior to CPE screening.
- 86 - CPE species ID and mechanism of resistance.
- 87 - Biological abnormalities including white blood cell count, CRP and albumin at the time of FMT.
- 88 - Functional autonomy scale based on the iso-resource group (IRG), a need-of-care scale ranging  
89 from 1 (i.e., fully dependent/bedridden) to 6 (i.e., fully independent) as previously described in

90 nursing homes<sup>24</sup>.

91

## 92 *Donor screening and FMT preparation*

93 Following French Health authorities' recommendations described by Sokol *et al.*<sup>25</sup>, potential donors were  
94 pre-screened using a standardized questionnaire regarding their age, body mass index, comorbidities,  
95 drug treatments and previous travels. Blood and feces were tested for transmissible agents (e.g.,  
96 *Clostridioides difficile*; *Salmonella spp.*; MDROs; *Giardia lamblia*; human immunodeficiency virus; hepatitis  
97 A, B and C virus; norovirus; cytomegalovirus; Protozoa and helminths). Donors were rescreened on the  
98 day of donation for any at risk events and behaviors since the first screening visit. Four eligible male  
99 donors (Donors 1-4) were selected for the first part of study and a female donor (Donor 5) was used  
100 thereafter (starting from April 2019). All donors were <40 years old. Fecal material was prepared  
101 aerobically by mixing 70-100g of freshly discharged stool with 250 mL of 0.9% NaCl and 10% glycerol  
102 solution, aliquoted after sieving and centrifugation and preserved at -80°C for 12 months.

103

## 104 *Study procedure*

105 Two days prior to FMT, patients were administered the lowest dose of a proton pump inhibitor (i.e.,  
106 esomeprazole 20 mg) to neutralize stomach acid. No antibiotic pre-treatment was administered. The day  
107 before transplantation, patients received an oral laxative containing 2.4g of senna extract (X-prep  
108 preparation). Immediately afterwards, patient benefited from the insertion of a nasoduodenal tube to  
109 ensure the administration of FMT the following day. Approximately 12 hours before the FMT procedure,  
110 a frozen aliquot of fecal material preparation was thawed at +2/+8°C and divided into five 50-mL opaque  
111 syringes for administration via the nasoduodenal route. Prior to discharge, patients were closely  
112 monitored for 24 hours following FMT and evaluated for potential adverse events, in particular aspiration

113 (pneumonia) and GI symptoms such as abdominal discomfort or vomiting.

114

#### 115 *Microbiological methods*

116 To assess CPE colonization, rectal swabs collected from each patient were cultured on selective media  
117 (CHROMID® CARBA SMART by bioMérieux, Marcy l’Etoile, France) and on Drigalski agar plates, a  
118 selective and differential medium used for the isolation of Enterobacterales and other non-fermenters  
119 from clinical specimens. When bacterial growth was observed, species identification was performed by  
120 MALDI-TOF mass spectrometry (MALDI Biotyper, Bruker Daltonique, Wissenbourg, France). CPE  
121 colonization was confirmed by taking another rectal swab prior to FMT and using rapid PCR testing for  
122 OXA-48, KPC, VIM, IMP-1 and NDM (PCR Xpert® CarbaR by Cepheid®, 81470 Maurens-Scopont, France).  
123 The presence of enzymes associated with antibiotic resistance was confirmed using a beta-lactam test for  
124 ESBL production (Laboratoire Biorad, 92430, Marnes-La-Coquette, France).

125

#### 126 *Outcomes measures*

127 The main outcome measure was time to successful decolonization following FMT, as determined by at  
128 least two consecutive negative rectal swabs (PCR and culture) with a 24-h interval on days 7(±1), 14(±1),  
129 21(±2), 28(±3) and 90(±7) following the procedure. Patients that remained negative for CPE over at least  
130 two consecutive time points were considered decolonized. The primary endpoint for decolonization was  
131 set at day 14 post-FMT based on findings from animal decolonization studies <sup>26,27</sup>. A secondary  
132 decolonization endpoint was set at 3 months following transplant.

133

#### 134 *Metagenomic analysis*

135 Fecal samples were collected at baseline (pre-FMT) and at all timepoints post-FMT from 12 patients (out  
136 of 20) and their corresponding donors (3 out of 5), for a total of 68 samples. Stools were stored at -80°C  
137 (to preserve DNA) in 2 different tubes: 1 aliquoting tube containing roughly 1 ml of fixative for shotgun  
138 sequencing purposes (OMNigene GUT OMR-200, DNA Genotek Inc, Ottawa, ON, Canada) and a 15-ml  
139 conical tube containing 6 ml of 30% glycerol for bacterial preservation and pathogen quantification  
140 purposes. DNA was extracted from fecal samples for donor 1 and 2, and samples from 4 recipients, using  
141 the MoBio MagAttract DNA/RNA isolation kit with ClearMag particles on a KingFisher instrument. Library  
142 preparation was performed with Illumina's Nextera XT protocol. For fecal samples of donor 5 and the 8  
143 remaining recipients, DNA was extracted using the PowerSoil Pro DNA extraction kit automated for high  
144 throughput on the QiaCube HT using Powerbead Pro Plates with 0.5mm and 0.1mm ceramic beads. Library  
145 preparation for these samples was performed with a procedure adapted from the Illumina DNA Prep kit.  
146 All prepared libraries were sequenced using an Illumina NextSeq instrument, with a median sequencing  
147 depth of approximately 27.9 million reads (IQR 22,268,980 - 35,096,830 reads). K-mer based taxonomic  
148 profiling of all metagenomes was performed using the One Codex web platform, and short-read  
149 taxonomic assignments made by One Codex were used for host-read removal<sup>28</sup>. Normalized abundance  
150 of functional pathways was measured using HUMAnN 3.0. Of note, controls were not analyzed through  
151 metagenomics, as they were part of another study in routine care and received no intervention.

152

### 153 *Statistical analysis*

154 Results are described as n (%) or median [interquartile range (IQR)]. Analysis of decolonization following  
155 FMT was performed using GraphPad Prism v.8.0.2 (GraphPad Software Inc., La Jolla, CA). A Kaplan-Meier  
156 analysis using the Log-rank (Mantel-Cox) test was used to plot rate of decolonization, and statistical tests  
157 were performed using the parametric Student's *t*-test considering sample size (n<30) or log-rank (Mantel-

158 Cox test) for survival curves. Microbiota analyses were performed using R v4.2.2 and visualized using  
159 ggplot2. The R package vegan v2.6.4 “diversity” and “vegdist” functions were used to calculate Shannon  
160 diversity and Bray-Curtis dissimilarity indices, respectively, and the “specnumber” function was used to  
161 calculate species richness. The functional pathways selected for tests of association with recipient CPE  
162 clearance at day 14 were identified through principal component analysis (PCA) of all recipient samples  
163 and subsequent selection of the 5 pathways corresponding to the strongest PCA loadings. Statistical tests  
164 were performed using the Wilcoxon Rank-Sum test with false discovery rate (FDR) adjustment. Taxon  
165 associations with response were calculated using MaAslin2 v1.12.0 with default parameters, which ran a  
166 log-transformed linear model and performed FDR adjustment.

167

#### 168 *Study approval*

169 The FEDEX study was approved by The French National Agency for Medicines and Health Products Safety  
170 (ANSM; authorization N°140990A-41). The trial was registered under EudraCT No. 2014-003048-11, and  
171 available on <https://clinicaltrials.gov/> (NCT03029078). This study was conducted in accordance with the  
172 declaration of Helsinki and good clinical practice. Written informed consent for participation in this study  
173 was obtained from all study participants.

174

#### 175 *Data availability*

176 In accordance with Vedanta Biosciences, raw stool metagenomics datasets and disaggregated clinical  
177 metadata from the FEDEX cohort have been have been deposited at the NCBI Sequence Read Archive  
178 (SRA) (<https://www.ncbi.nlm.nih.gov/sra>) under study ID PRJNA1121295 for controlled access. Data will  
179 be made available upon publication.



## 180 **Results**

### 181 *Population characteristics*

182 After initial screening a total of 29 patients participated in the FEDEX study, 9 of whom were colonized by  
183 vancomycin-resistant enterococci and were excluded from the present analysis (see Flowchart in **Figure**  
184 **1**). Thus, 20 participants were colonized with CPE at D0. For this sub-cohort of patients, the median age  
185 was 72.5 years (IQR 62.25-81.75), median Charlson Comorbidity index was 5 (IQR 3.75-7), median albumin  
186 level was 29.0 g/L (IQR 25.25-31.25) and median GIR score was 4.5 (IQR 2-5.25). The colonizing CPE  
187 organisms included *Klebsiella pneumoniae* (n=15), *Escherichia coli* (n=9), *Enterobacter cloacae* (n=1) and  
188 *Citrobacter koseri* (n=1). A subset of these patients (n=10; 50%) were co-colonized with multiple CPE  
189 organisms as well as ESBL-producing Enterobacterales. The median duration of carriage before FMT was  
190 62.5 days (IQR 48.75-122.5). A summary of the patients' baseline characteristics is shown in Table 1.

191

### 192 *Decolonization following FMT*

193 Fourteen days after FMT, 4 out of 20 patients (20%) tested negative for CPE and met the primary endpoint  
194 for the study. Thirty days post-FMT, 2 additional patients (30%) became negative for CPE followed by two  
195 others by day ninety, resulting in an overall CPE decolonization rate of 40% (8/20) three months post-  
196 transplant (secondary endpoint). The decolonization rate over time is shown as a percentage on a Kaplan-  
197 Meier curve between CPE cases in the FMT group versus spontaneous CPE decolonization in our historical  
198 non-FMT cohort<sup>11</sup> (**Figure 2**). Patients in the comparator arm (n=82) had clinical profiles similar to patients  
199 in the FMT group (see supplementary Table) but were not considered for FMT either because they decided  
200 not to participate in the study or because they failed to meet the inclusion criteria, including medical  
201 transfer in our hospital for FMT intervention. Although the percentage of patients with CPE carriage  
202 decreased within the first 14 days after intervention, this difference was not statistically significant (p=0.1)

203 and was ultimately similar between the groups ( $p=0.9$ , **Figure 2**). Furthermore, there was no statistically  
204 significant difference in decolonization efficacy between the four FMT donors used in the first half of the  
205 study ( $n=2/12$ ) and the fifth FMT donor used thereafter ( $n=2/8$ ) ( $p=0.99$ ). It is worth noting that the 2  
206 responders in the second half of the study were also colonized with ESBL-producing Enterobacterales and  
207 had a history of recurrent urinary tract infection (UTI) caused by these organisms. They did not have any  
208 further UTI episodes for up to 12 months following transplant. To the best of our knowledge no patient in  
209 the control group had a previous medical history of recurrent UTI. Finally, there were no complications  
210 during the FMT procedure and no serious adverse events reported at follow-up, except for mild transient  
211 GI complaints ( $n=4$ ).

212

### 213 *Microbiota analyses*

214 Metagenomic sequencing was performed on fecal samples collected before and after FMT for 12 patients  
215 and their 3 corresponding donors. Fecal material derived from Donor 1, Donor 2 and Donor 5 was used to  
216 treat one, three and eight patients in this sub-cohort, respectively. Four of 12 patients (33%) met the  
217 primary endpoint for decolonization. We analyzed the gut microbiota composition and taxonomic  
218 diversity in responders and non-responders at baseline and 1 week, 2 weeks, 1 month and 2-3 months  
219 after fecal transplantation. We found that the number of detected bacterial species, as measured by  
220 species richness, was significantly higher in responders than in non-responders 2 weeks and 1 month after  
221 FMT and was comparable to the bacterial richness observed in the donors (**Figure 3A**). Consistent with  
222 these findings, the gut microbiota alpha diversity, measured by the Shannon index, was significantly  
223 higher in responders compared to non-responders at 2 weeks, 1 month and 2-3 months post-transplant  
224 (**Figure 3B**). These observations suggested that engraftment of likely donor-derived taxa was more  
225 prevalent in responders than in non-responders. To evaluate this further, we measured the Euclidean

226 distance between recipient samples and the centroid of their corresponding donor samples, calculated  
227 using the first 2 principal co-ordinates of a Bray-Curtis dissimilarity matrix, and found the median distance  
228 to be shorter among responders than non-responders at all timepoints following transplant with the  
229 greatest distance at 1-month post-FMT ( $p=0.152$  after FDR adjustment) (**Figure 4**). This finding  
230 demonstrated a trend where the microbiota of patients that decolonized CPE was more similar to the  
231 microbiota of their donors compared to patients that failed to respond.

232 Next, we examined whether specific donor-derived bacterial taxa were associated with response. We  
233 identified *Faecalibacterium prausnitzii*, *Parabacteroides distasonis*, *Collinsella aerofaciens*, *Alistipes*  
234 *fingoldii* and *Blautia\_A sp900066335* to be significantly increased in responders post-FMT, whereas  
235 *Klebsiella pneumoniae* was significantly more abundant in non-responders (**Figure 5**). We did not find any  
236 such associations between functional pathway abundance and recipient response ( $p>0.05$ )  
237 (Supplementary Figure 1). We hypothesized that CPE burden at baseline could be a predictor of effective  
238 decolonization, or lack thereof, following fecal transplantation. To evaluate this hypothesis, we compared  
239 the relative abundance of CPE species in responders and non-responders before and after transplant. We  
240 found the relative abundance of CPE species was significantly higher in non-responders compared to  
241 responders at baseline ( $p=0.036$ ) and 1-week post-FMT ( $p=0.036$ ) (**Figure 6**), suggesting that complete  
242 elimination of CPE carriage may be challenging in patients with high CPE burden.

## 243 **Discussion**

244 Consistent with findings previously reported by our group<sup>22,23</sup> and others<sup>17</sup>, our results showed that a  
245 single dose of FMT administered via enteric feeding tube has limited efficacy to decolonize CPE.  
246 Nevertheless, the efficacy of FMT against MDROs is variable and successful decolonization of CPE or ESBL  
247 producing Enterobacterales following FMT administration has been reported<sup>29,30</sup>. Factors contributing to  
248 the variability in FMT efficacy in such indication include number of FMT doses, donor selection, FMT

249 preparation, route of FMT administration and pre-treatment regimen <sup>16,17,29,31</sup>. Indeed, it should be  
250 considered that the route of administration used in the present study, as well as the protocol of FMT  
251 involving a single dose, may have negatively influenced the decolonization process. As a result, there is an  
252 urgent need to standardize the FMT process in this population for more consistent results. Despite the  
253 variability in efficacy, FMT is still a safer option than decolonization strategies involving targeted  
254 antibiotics, where the emergence of antibiotic-resistant isolates and colonization recurrence have been  
255 observed <sup>13,14</sup>. Importantly, there was no colonization recurrence after 1 month among responders in the  
256 FEDEX study.

257 Although we did not achieve a statistically significant difference in CPE decolonization following FMT, it  
258 should be noted that in the non-responder group, one patient with previously documented CPE  
259 bacteremia did not experience any further CPE infection episodes post-FMT despite remaining colonized.  
260 This observation aligns with findings from Ghani *et al.*, who reported a reduction in the number of MDRO  
261 infections after FMT administration in the absence of successful decolonization <sup>21</sup>, suggesting that  
262 pathogen eradication from the intestine is not required to achieve clinical benefit. The authors speculated  
263 that FMT could lead to partial decolonization, an event that could be clinically meaningful. In line with this  
264 hypothesis, we found that non-responders exhibited a lower relative abundance of CPE species 1 week  
265 post-FMT compared to baseline. This suggests a likely reduction in CPE burden following transplant.  
266 However, absolute quantification of CPE load at baseline and post-FMT without any media enrichment is  
267 needed to determine the full impact of FMT on CPE colonization levels.

268 Furthermore, 2/2 non-immunocompromised patients with recurrent UTI due to ESBL-producing  
269 Enterobacterales did not experience any UTI episodes for up to 12 months after FMT administration. One  
270 33-year-old individual had previously been admitted twice for prostatitis within the 12-months prior to  
271 intervention and had required two courses of carbapenems (with the last injection occurring 3 months  
272 before the FMT). The other individual was a 57-year-old female with recurrent UTI due to neurogenic

273 bladder, and had historically presented with pyelonephritis every 1-2 months treated by cefoxitin using  
274 outpatient parenteral antimicrobial therapy (with the last injection occurring 2 months before the FMT).

275 The intestine can serve as a reservoir for UTI-causing organisms<sup>4,32</sup> and FMT interventional studies against  
276 *C. difficile* infection (CDI) have shown that FMT can significantly reduce the frequency of UTI episodes in  
277 CDI patients with a history of recurrent UTI even in the absence of decolonization<sup>21,33</sup>. Conversely,  
278 transmission events of antibiotic-susceptible uropathogenic strains and subsequent infections post-FMT  
279 have been described, advocating for meticulous FMT donor screening<sup>34</sup>. Despite the small sample size,  
280 our observations are consistent with these findings and add to the growing body of evidence supporting  
281 the role of FMT in infection prevention, especially those caused by MDROs. This hypothesis is supported  
282 by a recent study by Woodworth *et al.*<sup>35</sup>, designed to monitor adverse events during FMT delivered  
283 through retention enema. In the study, UTI was classified as a severe adverse event in a study of 11 kidney  
284 transplant individuals under immunosuppressive therapies thus harboring a potent disrupted microbiota,  
285 responsible for further modification of gut bacterial colonization as previously shown in the literature<sup>36-</sup>  
286<sup>38</sup>. Indeed, in the present work Woodworth *et al.* reported that after a 6-month observation period, two  
287 individuals experienced MDRO UTI with a delayed onset of symptoms compared to contemporaneous  
288 controls (n=16)<sup>35</sup>.

289 Microbiome analyses of stool samples collected from our patients pre- and post-FMT demonstrated that  
290 post-FMT, responders had more diverse microbiotas than non-responders, and responders' microbiotas  
291 resembled those of their corresponding donors more closely. Likewise, Bilinski *et al.*<sup>30</sup> previously showed  
292 that richness and diversity of the transplanted material was significantly different between responders  
293 and non-responders.

294 In our present work we showed that among responders, *Faecalibacterium prausnitzii*, *Parabacteroides*  
295 *distasonis*, *Collinsella aerofaciens*, *Alistipes finegoldii* and *Blautia\_A sp900066335* had significantly higher

296 relative abundances and were therefore associated with successful CPE decolonization post-FMT.  
297 *Faecalibacterium* species have been previously reported to be correlated with Enterobacterales  
298 suppression likely due to their ability to produce metabolites, specifically short chain fatty acids (SCFA),  
299 that can inhibit the growth of Gram-negative pathogens<sup>4,39</sup>. It is noteworthy that Woodworth *et al.*<sup>35</sup>  
300 also described specific taxa, including *Alistipes* and *Faecalibacterium* species, associated with the  
301 decolonization of ESBL-producing Enterobacterales in a small sample size trial (n=11). Likewise, Bilinski *et*  
302 *al.*<sup>30</sup> described microbial signatures associated with decolonization using FMT that pertained to  
303 *Barnesiella*, *Bacteroides*, and *Butyricimonas* species. Additionally, Leo *et al.* reported that decolonization  
304 was associated with the presence of *Bifidobacterium* strains that were enriched in individuals who had  
305 cleared multidrug-resistant Enterobacterales one month after the return from travel to tropical regions  
306<sup>40</sup>.

307 Although specific bacterial taxa have been associated with MDRO eradication, these differ across studies  
308<sup>30,35</sup>, suggesting that a taxonomically-independent common function or set of functions is more important  
309 than species identity. We found no significant associations between functional pathway abundance and  
310 response; however, functional analysis capabilities were limited to genes and functions present in a  
311 reference database and the use of non-assembled metagenomic short-reads. Future work applying  
312 assembly-based approaches that can leverage more sensitive functional annotation methods may reveal  
313 further insights.

314 The microbiota of non-responders exhibited a higher relative abundance of *Klebsiella pneumoniae*, which  
315 represented  $\leq 12\%$  of the patients' microbiota, compared to responders where *Klebsiella pneumoniae*  
316 levels were nearly undetectable. Importantly, we found that the relative abundance of CPE organisms  
317 before FMT was much lower in responders than in non-responders, suggesting that CPE colonization levels  
318 may be correlated with FMT response, defined by complete elimination of carriage. This finding, however,  
319 does not suggest that patients with high levels of CPE colonization will not respond to FMT or other

320 microbiome-based therapies. In fact, these individuals may benefit from multiple rounds of FMT  
321 treatment as demonstrated by studies where administration of more than 2 FMT doses led to successful  
322 MDRO decolonization after a single dose failed to do so <sup>29,31,35</sup>. However, while multiple FMT doses may  
323 be a potential approach to lower CPE levels enough to reduce infection risk and limit transmission, in the  
324 absence of encapsulated FMT undergoing multiple rounds of FMT via nasoduodenal tube may increase  
325 the risk for serious adverse events, including bacteremia and perforations <sup>41</sup>. Moreover, an important  
326 medical consideration for MDRO-colonized patients is that CPE carriers are often placed under contact  
327 isolation in a dedicated ward resulting in higher healthcare costs and treatment delays <sup>42,43</sup>. Therefore,  
328 FMT administration for MDRO decolonization could be a viable intervention for CPE carriers undergoing  
329 surgery as well as serve as a measure for outbreak control <sup>44</sup>. In line with this, Saidaini *et al.* showed that  
330 in a case-control study in MDRO-colonized patients, FMT administration accelerated discharge of patients  
331 that experienced decolonization (n=8/10) compared to those in the control group <sup>45</sup>. In contrast, our  
332 findings showed no difference in terms of decolonization (up to 90 days) between the patients who  
333 underwent FMT and our control group consisting of 82 individuals.

334 In 2019, a multicenter, randomized controlled study was conducted to evaluate the efficacy of a single  
335 dose of FMT against decolonization of multidrug-resistant Enterobacterales in 39 patients <sup>17</sup>. Prior to FMT,  
336 patients in the intervention group received a 5-day antibiotic course which markedly reduced MDRO  
337 levels. At 1-month, the percentage of patients that remained positive for MDRO colonization was 61.9%  
338 and 79% in the FMT and control groups, respectively. The low efficacy rate reported could be attributed  
339 to poor bacterial viability in the fecal transplants following antibiotic pre-treatment for gram-negative  
340 bacteria. Therefore, further study of microbiota conditioning regimens, including pre-treatment with  
341 specific antibiotic classes and length of antibiotic course is needed in other indications beyond CDI. Bowel  
342 lavage may be a more effective pre-treatment option based on the high decolonization rates reported <sup>31</sup>.  
343 Similarly, patients in the FEDEX study received a bowel prep without antibiotics. Furthermore, studies

344 have shown that administration of antibiotics within 7 days post-FMT is the most common cause of  
345 decolonization failure<sup>23,31,44</sup>. Although our results are not statistically significant due to the limited number  
346 of patients studied, they support this finding (17% of antibiotic use within 30 days post-FMT in the  
347 responder group versus 36% in the non-responder group).

348 Our study has several limitations. First, despite our efforts to match patients with a control group, the  
349 study was not randomized with a placebo group. Second, although the sample size is small (n=20) and  
350 statistical analyses to evaluate potential risk factors of failure were not performed, it is one of the larger  
351 studies reported to date. Third, FMT efficacy was based on complete CPE eradication, an outcome that  
352 may be too high a bar to measure FMT success. Fourth, it could be argued that we did not use pooled  
353 stools for our trial, a has received interest for treatment of microbiota-associated chronic diseases<sup>46</sup>, but  
354 such a strategy was not considered relevant at the time of the FEDEX study (prior to 2017) and was  
355 contrary to the intended research goal (evaluating the impact of a unique donor). Furthermore, our FMTs  
356 were prepared aerobically rather than anaerobically. However, a recent meta-analysis (2021) reported  
357 two studies where FMT material was prepared under anaerobic and aerobic conditions for the treatment  
358 of recurrent CDI, and efficacy was shown to be comparable (80.3% and 84.4%, respectively)<sup>47</sup>. To our  
359 knowledge, no study to date has directly compared the effect of aerobic vs. anaerobic fecal material  
360 processing conditions on the effectiveness of FMT.

361 Finally, the main limitation of testing for decolonization in the clinic is that the current methods fail to  
362 take into account pathogen load and decolonization is defined by the absence of MDRO detection.  
363 Although testing over weeks/months may not be sufficient to show persistent eradication, MDRO  
364 elimination within the first 1-3 months after FMT may have long-term benefits, as demonstrated by the  
365 FMT recipient who decolonized and remained UTI-free for over a year. Furthermore, previous reports  
366 have shown that pathogen eradication is not needed to provide clinical benefit suggesting that keeping  
367 MDRO levels below a certain threshold is key for limiting extraintestinal infections with the colonizing



368 organism <sup>21</sup>. Finally, other underlying factors involved in decolonization, including antibiotic  
369 administration, and diet may impact the gut microbiome composition and create a temporary state of  
370 dysbiosis where colonization recurrence with either the same or a different MDRO strain may occur.

371 Despite these limitations, our results might encourage future FMT trials to switch to quantitative  
372 approaches to accurately assess MDRO colonization levels and shift the study goal towards infection  
373 prevention, rather than focusing solely on carriage elimination. Indeed, FMT might relate to other  
374 important outcomes, such as ongoing transmission or symptomatic infection, as suggested in our finding.  
375 Therefore, all of these questions raised deserved further investigation to better tackle antimicrobial  
376 resistance and infection prevention control.

377 In conclusion, our findings revealed that the engraftment of specific donor-derived taxa and CPE levels  
378 pre-FMT were significantly correlated with FMT efficacy in a non-immunocompromised population.  
379 However, we showed that instead of achieving significant complete CPE decolonization, it promoted  
380 significant modification of microbiota richness and diversity, which may be associated with subsequent  
381 less infections (UTI). Our study shows the importance of considering different factors that influence FMT  
382 efficacy in donors and recipients to achieve optimal therapeutic results, suggesting that another FMT  
383 regimen could potentially yield more successful outcomes in this indication. Such a strategy might  
384 contribute to the development of unique live biotherapeutics for specific clinical profiles, and ultimately  
385 provide an antibiotic-free antibacterial strategy to fight against antimicrobial-resistance, as seen for  
386 treatment of *C. difficile* infections <sup>48</sup> .

387

### 388 **Legends of the Figures**

389 Figure 1: STROBE Flowchart of the FEDEX study. Flowchart in accordance with the STROBE (Strengthening  
390 the Reporting of Observational Studies in Epidemiology) statement <http://www.strobestatement.org>

391 Figure 2: Kaplan-Meier representation for decolonization between spontaneous decolonization (control  
392 group, n=82) and FMT intervention (n=20). Dashed lines indicate primary and secondary endpoints.

393

394 Figure 3: Gut microbiota analysis between responders and non-responders

395 *Box and whisker plots indicating the (A) species richness and (B) Shannon diversity index of donor samples*  
396 *(grey), samples from patients with CPE clearance on day 14 (teal), and patients without CPE clearance on*  
397 *day 14 (orange). The median ( $\pm$ interquartile range) is plotted for each group at the indicated timepoints.*  
398 *Whiskers extend to the lowest and highest values no greater than 1.5x the interquartile range from the*  
399 *closest hinge. Patients with CPE clearance on day 14 have significantly higher species richness and/or*  
400 *Shannon diversity index than patients without CPE clearance on day 14 at 2 weeks, 1 month, and 2-3*  
401 *months post-FMT (Wilcoxon Rank-Sum test with FDR adjustment).*

402

403 Figure 4: Microbiota distance between FMT recipients and donors

404 *Box and whisker plots indicating the Euclidean distance of samples from patients with CPE clearance on*  
405 *day 14 (teal), and patients without CPE clearance on day 14 (orange) to the centroid of their corresponding*  
406 *donor samples, calculated using the first 2 principal co-ordinates of a Bray-Curtis dissimilarity matrix*  
407 *between samples. The median ( $\pm$ interquartile range) is plotted for each group at the indicated timepoints.*  
408 *Whiskers extend to the lowest and highest values no greater than 1.5x the interquartile range from the*  
409 *closest hinge. While not statistically significant, patients with CPE clearance on day 14 have lower mean*  
410 *distance from their donors than patients without CPE clearance at all post-FMT timepoints (Wilcoxon*  
411 *Rank-Sum test with FDR adjustment).*

412

413 Figure 5: Bacterial taxa associated with response

414 Box and whisker plots of the percent relative abundance for the indicated species in all samples from  
415 patients who ultimately had-CPE clearance on day 14 (teal), and all samples from patients who did not  
416 ultimately have CPE clearance on day 14 (orange). *The median ( $\pm$ interquartile range) is plotted for each  
417 species in each group. Whiskers extend to the lowest and highest values no greater than 1.5x the  
418 interquartile range from the closest hinge. The displayed taxa were significantly altered between patients  
419 with and without CPE clearance at day 14 across all samples (MaAsLin2 log-transformed linear model with  
420 FDR adjustment).*

421

422 Figure 6: Abundance of CPE species pre- and post-FMT

423 *Box and whisker plots indicating the cumulative percent relative abundance of CPE species in samples from*  
424 *patients with CPE clearance on day 14 (teal) and patients without CPE clearance on day 14 (orange). The*  
425 *median ( $\pm$ interquartile range) is plotted for each group at the indicated timepoints. Whiskers extend to the*  
426 *lowest and highest values no greater than 1.5x the interquartile range from the closest hinge. Patients*  
427 *with CPE clearance on day 14 have significantly lower CPE relative abundance than patients without CPE*  
428 *clearance on day 14 at baseline and 1 week post-FMT (Wilcoxon Rank-Sum test with FDR adjustment).*

429

430 Supplementary Figure 1: Functional pathway analyses between responders and non-responders

431 *A) Principal component analysis (PCA) plot of the normalized abundance of functional pathways in patients*  
432 *with CPE clearance on day 14 (blue) and patients without CPE clearance on day 14 (red). Arrows represent*  
433 *the 5 strongest PCA loadings. B) Box and whisker plots indicating the normalized pathway abundance of*  
434 *the functional pathways corresponding to the 5 strongest PCA loadings in samples from patients with CPE*

435 *clearance on day 14 (teal) and patients without CPE clearance on day 14 (orange). The median*  
436 *( $\pm$ interquartile range) is plotted for each group at the indicated timepoints. Whiskers extend to the lowest*  
437 *and highest values no greater than 1.5x the interquartile range from the closest hinge. There is no*  
438 *significant difference in normalized pathway abundance between patients with CPE clearance on day 14*  
439 *and patients without CPE clearance on day 14 (Wilcoxon Rank-Sum test with FDR adjustment).*

440

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443

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447

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452

453 **Declaration of interests**

454 A.R.W, G.G and S.C are employees of Vedanta Biosciences and have an equity interest in the company.  
455 Vedanta Biosciences holds patents related to this work. The authors vouch for the accuracy and  
456 completeness of the data and data analyses. This content is solely the responsibility of the authors.

457

#### 458 **Author Contributions**

459 BD, PDT and SC designed research (project conception, development of overall research plan, and study  
460 oversight). BD, ASM and AD conducted research and were responsible for the management and  
461 coordination of the trial, including recruitment, conduct of the study, data collection, management of  
462 investigational products, etc. ARW was in charge of monitoring the study progress. GG, SC and ARW  
463 performed metagenomics analyses. RB, EMT, EJK, HM and MJ were responsible of stools conservation and  
464 selection. CL and ASM performed microbiological cultures and PCR. BD, SC, ARW and PDT interpreted the  
465 results, wrote the manuscript and had primary responsibility for the final content. All authors have read  
466 and approved the final manuscript.

#### 467 **Trial Registration**

468 The trial was registered in 2014 under NCT03029078 and is available on <https://clinicaltrials.gov/>.

469

#### 470 **References**

471 1 Vehreschild M. J. G. T., Hamprecht A., Peterson L., Schubert S., Hantschel M., Peter S., et al. A  
472 multicentre cohort study on colonization and infection with ESBL-producing Enterobacteriaceae in high-  
473 risk patients with haematological malignancies. *Journal of Antimicrobial Chemotherapy*  
474 2014;**69**(12):3387–92. Doi: 10.1093/jac/dku305.

475 2 Taur Ying, Xavier Joao B, Lipuma Lauren, Ubeda Carles, Goldberg Jenna, Gobourne Asia, et al.  
476 Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem  
477 cell transplantation. *Clin Infect Dis* 2012;**55**(7):905–14. Doi: 10.1093/cid/cis580.

- 478 3 McConville Thomas Howe, Sullivan Sean Berger, Gomez-Simmonds Angela, Whittier Susan,  
479 Uhlemann Anne-Catrin. Carbapenem-resistant Enterobacteriaceae colonization (CRE) and subsequent  
480 risk of infection and 90-day mortality in critically ill patients, an observational study. *PLoS One*  
481 2017;**12**(10):e0186195. Doi: 10.1371/journal.pone.0186195.
- 482 4 Magruder Matthew, Sholi Adam N, Gong Catherine, Zhang Lisa, Edusei Emmanuel, Huang  
483 Jennifer, et al. Gut uropathogen abundance is a risk factor for development of bacteriuria and urinary  
484 tract infection. *Nat Commun* 2019;**10**(1):5521. Doi: 10.1038/s41467-019-13467-w.
- 485 5 Ferstl Philip G, Filmann Natalie, Heilgenthal Eva-Maria, Schnitzbauer Andreas A, Bechstein Wolf  
486 O, Kempf Volkhard A J, et al. Colonization with multidrug-resistant organisms is associated with in  
487 increased mortality in liver transplant candidates. *PLoS One* 2021;**16**(1):e0245091. Doi:  
488 10.1371/journal.pone.0245091.
- 489 6 Cassini Alessandro, Högberg Liselotte Diaz, Plachouras Diamantis, Quattrocchi Annalisa, Hoxha  
490 Ana, Simonsen Gunnar Skov, et al. Attributable deaths and disability-adjusted life-years caused by  
491 infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a  
492 population-level modelling analysis. *Lancet Infect Dis* 2019;**19**(1):56–66. Doi: 10.1016/S1473-  
493 3099(18)30605-4.
- 494 7 Murray Christopher JL, Ikuta Kevin Shunji, Sharara Fablina, Swetschinski Lucien, Robles Aguilar  
495 Gisela, Gray Authia, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic  
496 analysis. *The Lancet* 2022;**399**(10325):629–55. Doi: 10.1016/S0140-6736(21)02724-0.
- 497 8 Tacconelli Evelina, Carrara Elena, Savoldi Alessia, Harbarth Stephan, Mendelson Marc, Monnet  
498 Dominique L, et al. Discovery, research, and development of new antibiotics: the WHO priority list of  
499 antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**(3):318–27. Doi: 10.1016/S1473-  
500 3099(17)30753-3.
- 501 9 Colomb-Cotinat M, Soing-Altrach S, Leon A, Savitch Y, Poujol I, Naas T, et al. Emerging  
502 extensively drug-resistant bacteria (eXDR) in France in 2018. *Med Mal Infect* 2020;**50**(8):715–22. Doi:  
503 10.1016/j.medmal.2020.01.011.
- 504 10 Willems Roel P.J., van Dijk Karin, Vehreschild Maria J.G.T., Biehl Lena M., Ket Johannes C.F.,  
505 Remmelzwaal Sharon, et al. Incidence of infection with multidrug-resistant Gram-negative bacteria and  
506 vancomycin-resistant enterococci in carriers: a systematic review and meta-regression analysis. *Lancet*  
507 *Infect Dis* 2023;**23**(6):719–31. Doi: 10.1016/S1473-3099(22)00811-8.
- 508 11 Davido Benjamin, Moussiegt Aurore, Dinh Aurélien, Bouchand Frédérique, Matt Morgan, Senard  
509 Olivia, et al. Germs of thrones - spontaneous decolonization of Carbapenem-Resistant  
510 Enterobacteriaceae (CRE) and Vancomycin-Resistant Enterococci (VRE) in Western Europe: is this myth  
511 or reality? *Antimicrob Resist Infect Control* 2018;**7**(1):100. Doi: 10.1186/s13756-018-0390-5.
- 512 12 Bar-Yoseph Haggai, Hussein Khetam, Braun Eyal, Paul Mical. Natural history and decolonization  
513 strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-  
514 analysis. *Journal of Antimicrobial Chemotherapy* 2016;**71**(10):2729–39. Doi: 10.1093/jac/dkw221.
- 515 13 Machuca Isabel, Gutiérrez-Gutiérrez Belén, Pérez Cortés Salvador, Gracia-Ahufinger Irene,  
516 Serrano Josefina, Madrigal María Dolores, et al. Oral decontamination with aminoglycosides is

517 associated with lower risk of mortality and infections in high-risk patients colonized with colistin-  
518 resistant, KPC-producing *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2016;**71**(11):3242–9. Doi:  
519 10.1093/jac/dkw272.

520 14 Oren I, Sprecher H, Finkelstein R, Hadad S, Neuberger A, Hussein K, et al. Eradication of  
521 carbapenem-resistant Enterobacteriaceae gastrointestinal colonization with nonabsorbable oral  
522 antibiotic treatment: A prospective controlled trial. *Am J Infect Control* 2013;**41**(12):1167–72. Doi:  
523 10.1016/J.AJIC.2013.04.018.

524 15 Tascini Carlo, Sbrana Francesco, Flammini Sarah, Tagliaferri Enrico, Arena Fabio, Leonildi  
525 Alessandro, et al. Oral gentamicin gut decontamination for prevention of KPC-producing *Klebsiella*  
526 *pneumoniae* infections: relevance of concomitant systemic antibiotic therapy. *Antimicrob Agents*  
527 *Chemother* 2014;**58**(4):1972–6. Doi: 10.1128/AAC.02283-13.

528 16 Bilsen Manu P, Lambregts Merel M C, van Prehn Joffrey, Kuijper Ed J. Faecal microbiota  
529 replacement to eradicate antimicrobial resistant bacteria in the intestinal tract - a systematic review.  
530 *Curr Opin Gastroenterol* 2022;**38**(1):15–25. Doi: 10.1097/MOG.0000000000000792.

531 17 Huttner B D, de Lastours V, Wassenberg M, Maharshak N, Mauris A, Galperine T, et al. A 5-day  
532 course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant  
533 Enterobacteriaceae: a randomized clinical trial. *Clin Microbiol Infect* 2019;**25**(7):830–8. Doi:  
534 10.1016/j.cmi.2018.12.009.

535 18 Tacconelli E., Mazzaferri F., de Smet A. M., Bragantini D., Eggimann P., Huttner B. D., et al.  
536 ESCMID-EUCIC clinical guidelines on decolonization of multidrug-resistant Gram-negative bacteria  
537 carriers. *Clin Microbiol Infect* 2019;**25**(7):807–17. Doi: 10.1016/J.CMI.2019.01.005.

538 19 Crum-Cianflone Nancy F, Sullivan Eva, Ballon-Landa Gonzalo. Fecal microbiota transplantation  
539 and successful resolution of multidrug-resistant-organism colonization. *J Clin Microbiol*  
540 2015;**53**(6):1986–9. Doi: 10.1128/JCM.00820-15.

541 20 Stripling Joshua, Kumar Ranjit, Baddley John W, Nellore Anoma, Dixon Paula, Howard Donna, et  
542 al. Loss of Vancomycin-Resistant Enterococcus Fecal Dominance in an Organ Transplant Patient With  
543 *Clostridium difficile* Colitis After Fecal Microbiota Transplant. *Open Forum Infect Dis* 2015;**2**(2):ofv078.  
544 Doi: 10.1093/ofid/ofv078.

545 21 Ghani Rohma, Mullish Benjamin H, McDonald Julie A K, Ghazy Anan, Williams Horace R T,  
546 Brannigan Eimear T, et al. Disease Prevention Not Decolonization: A Model for Fecal Microbiota  
547 Transplantation in Patients Colonized With Multidrug-resistant Organisms. *Clin Infect Dis*  
548 2021;**72**(8):1444–7. Doi: 10.1093/cid/ciaa948.

549 22 Davido B., Batista R., Michelon H., Lepointeur M., Bouchand F., Lepeule R., et al. Is faecal  
550 microbiota transplantation an option to eradicate highly drug-resistant enteric bacteria carriage? *Journal*  
551 *of Hospital Infection* 2017;**95**(4):433–7. Doi: 10.1016/j.jhin.2017.02.001.

552 23 Dinh A., Fessi H., Duran C., Batista R., Michelon H., Bouchand F., et al. Clearance of carbapenem-  
553 resistant Enterobacteriaceae vs vancomycin-resistant enterococci carriage after faecal microbiota  
554 transplant: a prospective comparative study. *Journal of Hospital Infection* 2018. Doi:  
555 10.1016/j.jhin.2018.02.018.

- 556 24 Fries Brant E., Schneider Don P., Foley William J., Gavazzi Marie, Burke Robert, Cornelius  
557 Elizabeth. Refining a case-mix measure for nursing homes: Resource utilization groups (rug-iii). *Med Care*  
558 1994;**32**(7):668–85. Doi: 10.1097/00005650-199407000-00002.
- 559 25 Sokol Harry, Galperine Tatiana, Kapel Nathalie, Bourlioux Pierre, Seksik Philippe, Barbut Frederic,  
560 et al. Faecal microbiota transplantation in recurrent *Clostridium difficile* infection: Recommendations  
561 from the French Group of Faecal microbiota Transplantation. *Dig Liver Dis* 2016;**48**(3):242–7. Doi:  
562 10.1016/j.dld.2015.08.017.
- 563 26 Caballero Silvia, Kim Sohn, Carter Rebecca A, Leiner Ingrid M, Sušac Bože, Miller Liza, et al.  
564 Cooperating Commensals Restore Colonization Resistance to Vancomycin-Resistant *Enterococcus*  
565 *faecium*. *Cell Host Microbe* 2017;**21**(5):592-602.e4. Doi: 10.1016/j.chom.2017.04.002.
- 566 27 Caballero Silvia, Carter Rebecca, Ke Xu, Sušac Bože, Leiner Ingrid M., Kim Grace J., et al. Distinct  
567 but Spatially Overlapping Intestinal Niches for Vancomycin-Resistant *Enterococcus faecium* and  
568 Carbapenem-Resistant *Klebsiella pneumoniae*. *PLoS Pathog* 2015;**11**(9):1–20. Doi:  
569 10.1371/journal.ppat.1005132.
- 570 28 Minot S S, Krumm N, Greenfield N B. One Codex: a sensitive and accurate data platform for  
571 genomic microbial identification. *Preprint at BioRxiv* 2015. Doi: <https://doi.org/10.1101/027607>.
- 572 29 Singh Ramandeep, de Groot Pieter F., Geerlings Suzanne E., Hodiament Caspar J., Belzer Clara,  
573 Berge Ineke J. M. ten, et al. Fecal microbiota transplantation against intestinal colonization by extended  
574 spectrum beta-lactamase producing *Enterobacteriaceae*: a proof of principle study. *BMC Res Notes*  
575 2018;**11**(1):190. Doi: 10.1186/s13104-018-3293-x.
- 576 30 Bilinski Jaroslaw, Grzesiowski Pawel, Sorensen Nikolaj, Madry Krzysztof, Muszynski Jacek, Robak  
577 Katarzyna, et al. Fecal Microbiota Transplantation in Patients with Blood Disorders Inhibits Gut  
578 Colonization with Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. *Clinical*  
579 *Infectious Diseases* 2017. Doi: 10.1093/cid/cix252.
- 580 31 Biliński Jarosław, Grzesiowski Paweł, Muszyński Jacek, Wróblewska Marta, Mądry Krzysztof,  
581 Robak Katarzyna, et al. Fecal Microbiota Transplantation Inhibits Multidrug-Resistant Gut Pathogens:  
582 Preliminary Report Performed in an Immunocompromised Host. *Arch Immunol Ther Exp (Warsz)*  
583 2016;**64**(3):255–8. Doi: 10.1007/s00005-016-0387-9.
- 584 32 Niki M., Hirai I., Yoshinaga A., Ulzii-Orshikh L., Nakata A., Yamamoto A., et al. Extended-spectrum  
585  $\beta$ -lactamase-producing *Escherichia coli* strains in the feces of carriers contribute substantially to urinary  
586 tract infections in these patients. *Infection* 2011;**39**(5):467–71. Doi: 10.1007/s15010-011-0128-2.
- 587 33 Tariq Raseen, Pardi Darrell S., Tosh Pritish K., Walker Randall C., Razonable Raymund R., Khanna  
588 Sahil. Fecal Microbiota Transplantation for Recurrent *Clostridium difficile* Infection Reduces Recurrent  
589 Urinary Tract Infection Frequency. *Clinical Infectious Diseases* 2017. Doi: 10.1093/cid/cix618.
- 590 34 Vendrik Karuna E.W., de Meij Tim G.J., Bökenkamp Arend, Ooijevaar Rogier E., Groenewegen  
591 Bas, Hendrickx Antoni P.A., et al. Transmission of Antibiotic-Susceptible *Escherichia coli* Causing Urinary  
592 Tract Infections in a Fecal Microbiota Transplantation Recipient: Consequences for Donor Screening?  
593 *Open Forum Infect Dis* 2022;**9**(7). Doi: 10.1093/ofid/ofac324.



- 594 35 Woodworth Michael H., Conrad Roth E., Haldopoulos Marina, Pouch Stephanie M., Babiker  
595 Ahmed, Mehta Aneesh K., et al. Fecal microbiota transplantation promotes reduction of antimicrobial  
596 resistance by strain replacement. *Sci Transl Med* 2023;**15**(720):eabo2750. Doi:  
597 10.1126/SCITRANSLMED.ABO2750.
- 598 36 Tourret Jérôme, Willing Benjamin P., Dion Sara, MacPherson Jayden, Denamur Erick, Finlay Brett  
599 B. Immunosuppressive Treatment Alters Secretion of Ileal Antimicrobial Peptides and Gut Microbiota,  
600 and Favors Subsequent Colonization by Uropathogenic Escherichia coli. *Transplantation* 2017;**101**(1):74–  
601 82. Doi: 10.1097/TP.0000000000001492.
- 602 37 Gibson Caitlin M., Childs-Kean Lindsey M., Naziruddin Zahra, Howell Crystal K. The alteration of  
603 the gut microbiome by immunosuppressive agents used in solid organ transplantation. *Transpl Infect Dis*  
604 2021;**23**(1). Doi: 10.1111/TID.13397.
- 605 38 Gabarre Paul, Loens Christopher, Tamzali Yanis, Barrou Benoit, Jaisser Frédéric, Tourret Jérôme.  
606 Immunosuppressive therapy after solid organ transplantation and the gut microbiota: Bidirectional  
607 interactions with clinical consequences. *Am J Transplant* 2022;**22**(4):1014–30. Doi: 10.1111/AJT.16836.
- 608 39 Sorbara Matthew T, Dubin Krista, Littmann Eric R, Moody Thomas U, Fontana Emily, Seok Ruth,  
609 et al. Inhibiting antibiotic-resistant Enterobacteriaceae by microbiota-mediated intracellular  
610 acidification. *J Exp Med* 2019;**216**(1):84–98. Doi: 10.1084/jem.20181639.
- 611 40 Leo Stefano, Lazarevic Vladimir, Gaïa Nadia, Estellat Candice, Girard Myriam, Matheron Sophie,  
612 et al. The intestinal microbiota predisposes to traveler’s diarrhea and to the carriage of multidrug-  
613 resistant Enterobacteriaceae after traveling to tropical regions. *Gut Microbes* 2019;**10**(5). Doi:  
614 10.1080/19490976.2018.1564431.
- 615 41 Baxter M., Colville A. Adverse events in faecal microbiota transplant: a review of the literature. *J*  
616 *Hosp Infect* 2016;**92**(2):117–27. Doi: 10.1016/J.JHIN.2015.10.024.
- 617 42 Tackin A, Lawrence C, Godin E, Verheye J C, Davido B. [Management in a dedicated ward of  
618 patients colonized with highly resistant bacteria: Is it worth it?]. *Med Mal Infect* 2020;**50**(5):454–5. Doi:  
619 10.1016/j.medmal.2020.01.007.
- 620 43 van Dijk M D, Voor In ’t Holt A F, Polinder S, Severin J A, Vos M C. The daily direct costs of  
621 isolating patients identified with highly resistant micro-organisms in a non-outbreak setting. *J Hosp*  
622 *Infect* 2021;**109**:88–95. Doi: 10.1016/j.jhin.2020.12.013.
- 623 44 Davido B., Batista R., Fessi H., Salomon J., Dinh A. Impact of faecal microbiota transplantation to  
624 eradicate vancomycin-resistant enterococci (VRE) colonization in humans. *Journal of Infection*  
625 2017;**75**(4). Doi: 10.1016/j.jinf.2017.06.001.
- 626 45 Saïdani Nadia, Lagier Jean-Christophe, Cassir Nadim, Million Matthieu, Baron Sophie, Dubourg  
627 Grégory, et al. Faecal microbiota transplantation shortens the colonisation period and allows re-entry of  
628 patients carrying carbapenamase-producing bacteria into medical care facilities. *Int J Antimicrob Agents*  
629 2019;**53**(4):355–61. Doi: 10.1016/j.ijantimicag.2018.11.014.

630 46 Kazerouni Abbas, Wein Lawrence M. Exploring the Efficacy of Pooled Stools in Fecal Microbiota  
631 Transplantation for Microbiota-Associated Chronic Diseases. *PLoS One* 2017;**12**(1):e0163956. Doi:  
632 10.1371/journal.pone.0163956.

633 47 Cold Frederik, Baunwall Simon Mark Dahl, Dahlerup Jens Frederik, Petersen Andreas Munk, Hvas  
634 Christian Lodberg, Hansen Lars Hestbjerg. Systematic review with meta-analysis: encapsulated faecal  
635 microbiota transplantation – evidence for clinical efficacy. *Therap Adv Gastroenterol* 2021;**14**. Doi:  
636 10.1177/17562848211041004/SUPPL\_FILE/SJ-DOCX-2-TAG-10.1177\_17562848211041004.DOCX.

637 48 Orenstein Robert, Hecht Gail, Harvey Adam, Tillotson Glenn, Khanna Sahil. Two-year durability  
638 of REBYOTA™ (RBL), a live biotherapeutic for the prevention of recurrent *Clostridioides difficile*  
639 infections . *Open Forum Infect Dis* 2023;**10**(9). Doi: 10.1093/ofid/ofad456.

640

641

1 **Abstract**

2 **Objectives:** We evaluated the effect of fecal microbiota transplantation (FMT) on the clearance of  
3 carbapenemase-producing Enterobacterales (CPE) carriage.

4 **Methods:** We performed a prospective, multi-center study, conducted among patients who received a  
5 single dose of FMT from one of four healthy donors. The primary endpoint was complete clearance of CPE  
6 carriage two weeks after FMT with a secondary endpoint at three months. Shotgun metagenomic  
7 sequencing was performed to assess gut microbiota composition of donors and recipients before and  
8 after FMT.

9 **Results:** Twenty CPE-colonized patients were included in the study, where post-FMT 20% (n=4/20) of  
10 patients met the primary endpoint and 40% (n=8/20) of patients met the secondary endpoint. Kaplan-  
11 Meier curves between patients with FMT intervention and the control group (n=82) revealed a similar  
12 rate of decolonization between groups.

13 Microbiota composition analyses revealed that response to FMT was not donor-dependent. Responders  
14 had a significantly lower relative abundance of CPE species pre-FMT than non-responders, and 14 days  
15 post-FMT responders had significantly higher bacterial species richness and alpha diversity compared to  
16 non-responders ( $p < 0.05$ ). Responder fecal samples were also enriched in specific species, with  
17 significantly higher relative abundances of *Faecalibacterium prausnitzii*, *Parabacteroides distasonis*,  
18 *Collinsella aerofaciens*, *Alistipes finegoldii* and *Blautia\_A sp900066335* ( $q < 0.01$ ) compared to non-  
19 responders.

20 **Conclusion:** FMT administration [using the proposed regimen](#) did not achieve statistical significance for  
21 [complete](#) CPE decolonization, but was correlated with the relative abundance of specific bacterial taxa,  
22 including CPE species.

23

24 Key words: FMT, carbapenemase-producing Enterobacterales, decolonization, carriage

25

26 **Introduction**

27 Gastrointestinal (GI) colonization with multidrug-resistant organisms (MDROs) is a major risk factor for  
28 infection and mortality in high-risk patient populations <sup>1-5</sup>. In 2015, The European Centre for Disease  
29 Prevention and Control (ECDC) reported that infections with MDROs accounted for approximately 33,000  
30 hospital deaths in Europe <sup>6</sup>. Additionally, in 2019, up to 1.27 million deaths were attributable to  
31 antimicrobial resistance worldwide <sup>7</sup>. Of particular concern are carbapenemase-producing  
32 Enterobacterales (CPE), which have been classified by the World Health Organization as one of the most  
33 critical antibiotic-resistant pathogen groups for which new therapies are urgently needed <sup>8</sup>. The  
34 prevalence of CPE is increasing worldwide and in France alone, for instance, the number of CPE cases  
35 increased from 248 to approximately 1704 (6-fold) from 2012 – 2018 <sup>9</sup>. Likewise, a recent study conducted  
36 by the European Antimicrobial Resistance Surveillance Network (EARS-Net) revealed that the number of  
37 deaths attributable to carbapenem-resistant Enterobacterales in Europe increased by 4 to 6-fold between  
38 2007 and 2015, especially in countries such as Italy, Greece and Romania where CPE are highly prevalent  
39 <sup>6</sup>.

40 MDRO colonization has been shown to increase the risk of infection by 14% in a median follow-up period  
41 of 30 days <sup>10</sup>. Spontaneous decolonization of CPE is slow, ranging from 12% decolonization within 30 days  
42 in our hospital's dedicated ward for CPE-colonized patients under reinforced isolation precautions <sup>11</sup>, and  
43 up to 25% decolonization after 30 days among healthcare residents <sup>12</sup>. As a result, decolonization  
44 strategies including selective digestive decontamination (SDD) and fecal microbiota transplantation (FMT)  
45 have been implemented to accelerate MDRO decolonization and reduce infection risk <sup>13-16</sup>. While SDD

46 against CPE significantly reduced infection risk following successful decolonization, it was associated with  
47 the development of antibiotic resistance and colonization recurrence<sup>13,14,17</sup>. These risks have limited the  
48 use of SDD for infection prevention and guidelines from the European Society for Clinical Microbiology  
49 and Infectious Diseases strongly recommend against the use of oral antibiotics for MDRO decolonization  
50<sup>18</sup>. As an alternative, FMT has been shown to be a safer and more effective strategy for decolonization of  
51 CPE and other MDROs, with efficacy rates ranging from 20-90%<sup>16</sup> and a significant reduction in the  
52 number of MDRO infections following FMT treatment<sup>19-21</sup>.

53 Although several case reports have been published supporting the role of FMT in MDRO decolonization,  
54 our group reported the first case series (n=8) where we showed that 2/6 CPE carriers decolonized 1 month  
55 after FMT<sup>22</sup>. Due to its small size, our study lacked sufficient power to draw definitive conclusions, which  
56 led us to carry out a larger prospective multicenter FMT trial (FEDEX) with preliminary findings showing a  
57 CPE decolonization rate of 50%<sup>23</sup>. Importantly, this result is concordant with another randomized  
58 controlled clinical trial published by Huttner *et al.* (n=22)<sup>17</sup>. Here we report the final results of the FEDEX  
59 study in CPE-colonized immunocompromised patients and describe the impact of FMT on patient  
60 microbiota composition.

61

## 62 **Materials and methods**

### 63 *Setting*

64 FEDEX was a prospective multi-center study designed to evaluate the use of FMT for decolonization of  
65 CPE from the GI tract. The trial was conducted in three French university hospitals in Paris between  
66 January 2015 and December 2019. FMT administration was performed at Hôpital Raymond Poincaré,  
67 Garches, France.

68

69 *Participants*

70 Patients  $\geq 18$  years with confirmed CPE colonization and able to provide informed consent were included  
71 in the study. CPE colonization was determined by at least three consecutive positive rectal swabs at  
72 weekly intervals, including the week prior to FMT. Exclusion criteria were immunosuppression (human  
73 immunodeficiency virus with  $CD4 < 200/mm^3$ ), immunosuppressive therapy (including corticosteroids  $> 60$   
74 mg/day for more than five days, chemotherapy, stem cell transplantation under immunosuppressant  
75 drugs and other inherited immunosuppressive disorder), antibiotic treatment at the time of FMT, previous  
76 infection with *Clostridioides difficile* treated with FMT, as well as individuals who were pregnant or  
77 breastfeeding, unable to provide informed consent, under tutorship or guardianship, homeless or without  
78 health insurance. Patients non-eligible for FMT intervention were assigned to the non-FMT cohort.

79

80 *Data collection*

81 The following data were collected from all study participants:

- 82 - Patient characteristics: age, sex, comorbidities, Charlson Comorbidity Index at admission,  
83 hospitalization 6 months prior to enrollment, duration of carriage, and travel abroad.
- 84 - Episodes of infection caused by CPE.
- 85 - Antibiotic administration and duration of treatment a month prior to CPE screening.
- 86 - CPE species ID and mechanism of resistance.
- 87 - Biological abnormalities including white blood cell count, CRP and albumin at the time of FMT.
- 88 - Functional autonomy scale based on the iso-resource group (IRG), a need-of-care scale ranging  
89 from 1 (i.e., fully dependent/bedridden) to 6 (i.e., fully independent) as previously described in

90 nursing homes<sup>24</sup>.

91

92 *Donor screening and FMT preparation*

93 Following French Health authorities' recommendations described by Sokol *et al.*<sup>25</sup>, potential donors were  
94 pre-screened using a standardized questionnaire regarding their age, body mass index, comorbidities,  
95 drug treatments and previous travels. Blood and feces were tested for transmissible agents (e.g.,  
96 *Clostridioides difficile*; *Salmonella spp.*; MDROs; *Giardia lamblia*; human immunodeficiency virus; hepatitis  
97 A, B and C virus; norovirus; cytomegalovirus; Protozoa and helminths). Donors were rescreened on the  
98 day of donation for any at risk events and behaviors since the first screening visit. Four eligible male  
99 donors (Donors 1-4) were selected for the first part of study and a female donor (Donor 5) was used  
100 thereafter (starting from April 2019). All donors were <40 years old. Fecal material was prepared  
101 aerobically by mixing 70-100g of freshly discharged stool with 250 mL of 0.9% NaCl and 10% glycerol  
102 solution, aliquoted after sieving and centrifugation and preserved at -80°C for 12 months.

103

104 *Study procedure*

105 Two days prior to FMT, patients were administered the lowest dose of a proton pump inhibitor (i.e.,  
106 esomeprazole 20 mg) to neutralize stomach acid. No antibiotic pre-treatment was administered. The day  
107 before transplantation, patients received an oral laxative containing 2.4g of senna extract (X-prep  
108 preparation). Immediately afterwards, patient benefited from the insertion of a nasoduodenal tube to  
109 ensure the administration of FMT the following day. Approximately 12 hours before the FMT procedure,  
110 a frozen aliquot of fecal material preparation was thawed at +2/+8°C and divided into five 50-mL opaque  
111 syringes for administration via the nasoduodenal route. Prior to discharge, patients were closely  
112 monitored for 24 hours following FMT and evaluated for potential adverse events, in particular aspiration

113 (pneumonia) and GI symptoms such as abdominal discomfort or vomiting.

114

#### 115 *Microbiological methods*

116 To assess CPE colonization, rectal swabs collected from each patient were cultured on selective media  
117 (CHROMID<sup>®</sup> CARBA SMART by bioMérieux, Marcy l'Etoile, France) and on Drigalski agar plates, a  
118 selective and differential medium used for the isolation of Enterobacterales and other non-fermenters  
119 from clinical specimens. . When bacterial growth was observed, species identification was performed by  
120 MALDI-TOF mass spectrometry (MALDI Biotyper, Bruker Daltonique, Wissenbourg, France). CPE  
121 colonization was confirmed by taking another rectal swab prior to FMT and using rapid PCR testing for  
122 OXA-48, KPC, VIM, IMP-1 and NDM (PCR Xpert<sup>®</sup> CarbaR by Cepheid<sup>®</sup>, 81470 Maurens-Scopont, France).  
123 The presence of enzymes associated with antibiotic resistance was confirmed using a beta-lactam test for  
124 ESBL production (Laboratoire Biorad, 92430, Marnes-La-Coquette, France).

125

#### 126 *Outcomes measures*

127 The main outcome measure was time to successful decolonization following FMT, as determined by at  
128 least two consecutive negative rectal swabs (PCR and culture) with a 24-h interval on days 7( $\pm$ 1), 14( $\pm$ 1),  
129 21( $\pm$ 2), 28( $\pm$ 3) and 90( $\pm$ 7) following the procedure. Patients that remained negative for CPE over at least  
130 two consecutive time points were considered decolonized. The primary endpoint for decolonization was  
131 set at day 14 post-FMT based on findings from animal decolonization studies <sup>26,27</sup>. A secondary  
132 decolonization endpoint was set at 3 months following transplant.

133

#### 134 *Metagenomic analysis*



135 Fecal samples were collected at baseline (pre-FMT) and at all timepoints post-FMT from 12 patients (out  
136 of 20) and their corresponding donors (3 out of 5), for a total of 68 samples. Stools were stored at -80°C  
137 (to preserve DNA) in 2 different tubes: 1 aliquoting tube containing roughly 1 ml of fixative for shotgun  
138 sequencing purposes (OMNIgene GUT OMR-200, DNA Genotek Inc, Ottawa, ON, Canada) and a 15-ml  
139 conical tube containing 6 ml of 30% glycerol for bacterial preservation and pathogen quantification  
140 purposes. DNA was extracted from fecal samples for donor 1 and 2, and samples from 4 recipients, using  
141 the MoBio MagAttract DNA/RNA isolation kit with ClearMag particles on a KingFisher instrument. Library  
142 preparation was performed with Illumina's Nextera XT protocol. For fecal samples of donor 5 and the 8  
143 remaining recipients, DNA was extracted using the PowerSoil Pro DNA extraction kit automated for high  
144 throughput on the QiaCube HT using Powerbead Pro Plates with 0.5mm and 0.1mm ceramic beads. Library  
145 preparation for these samples was performed with a procedure adapted from the Illumina DNA Prep kit.  
146 All prepared libraries were sequenced using an Illumina NextSeq instrument, with a median sequencing  
147 depth of approximately 27.9 million reads (IQR 22,268,980 - 35,096,830 reads). K-mer based taxonomic  
148 profiling of all metagenomes was performed using the One Codex web platform, and short-read  
149 taxonomic assignments made by One Codex were used for host-read removal<sup>28</sup>. Normalized abundance  
150 of functional pathways was measured using HUMAnN 3.0. Of note, controls were not analyzed through  
151 metagenomics, as they were part of another study in routine care and received no intervention.

152

### 153 *Statistical analysis*

154 Results are described as n (%) or median [interquartile range (IQR)]. Analysis of decolonization following  
155 FMT was performed using GraphPad Prism v.8.0.2 (GraphPad Software Inc., La Jolla, CA). A Kaplan-Meier  
156 analysis using the Log-rank (Mantel-Cox) test was used to plot rate of decolonization, and statistical tests  
157 were performed using the ~~non~~-parametric Student's *t*-test considering sample size (n<30) or log-rank

158 (Mantel-Cox test) for survival curves. Microbiota analyses were performed using R v4.2.2 and visualized  
159 using ggplot2. The R package vegan v2.6.4 “diversity” and “vegdist” functions were used to calculate  
160 Shannon diversity and Bray-Curtis dissimilarity indices, respectively, and the “specnumber” function was  
161 used to calculate species richness. The functional pathways selected for tests of association with recipient  
162 CPE clearance at day 14 were identified through principal component analysis (PCA) of all recipient  
163 samples and subsequent selection of the 5 pathways corresponding to the strongest PCA loadings.  
164 Statistical tests were performed using the Wilcoxon Rank-Sum test with false discovery rate (FDR)  
165 adjustment. Taxon associations with response were calculated using MaAslin2 v1.12.0 with default  
166 parameters, which ran a log-transformed linear model and performed FDR adjustment.

167

#### 168 *Study approval*

169 The FEDEX study was approved by The French National Agency for Medicines and Health Products Safety  
170 (ANSM; authorization N°140990A-41). The trial was registered under EudraCT No. 2014-003048-11, and  
171 available on <https://clinicaltrials.gov/> (NCT03029078). This study was conducted in accordance with the  
172 declaration of Helsinki and good clinical practice. Written informed consent for participation in this study  
173 was obtained from all study participants.

174

#### 175 *Data availability*

176 *In accordance with Vedanta Biosciences, raw stool metagenomics datasets and disaggregated clinical*  
177 *metadata from the FEDEX cohort have been deposited at the NCBI Sequence Read Archive*  
178 *(SRA) (<https://www.ncbi.nlm.nih.gov/sra>) under study ID PRJNA1121295 for controlled access. Data will*  
179 *be made available upon publication.*

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180 **Results**

181 *Population characteristics*

182 [After initial screening](#) a total of 29 patients participated in the FEDEX study, 9 of whom were colonized  
183 by vancomycin-resistant enterococci and were excluded from the present analysis [\(see Flowchart in](#)  
184 [Figure 1\)](#). Thus, 20 participants were colonized with CPE at D0. For this sub-cohort of patients, the median  
185 age was 72.5 years (IQR 62.25-81.75), median Charlson Comorbidity index was 5 (IQR 3.75-7), median  
186 albumin level was 29.0 g/L (IQR 25.25-31.25) and median GIR score was 4.5 (IQR 2-5.25). The colonizing  
187 CPE organisms included *Klebsiella pneumoniae* (n=15), *Escherichia coli* (n=9), *Enterobacter cloacae* (n=1)  
188 and *Citrobacter koseri* (n=1). A subset of these patients (n=10; 50%) were co-colonized with multiple CPE  
189 organisms as well as ESBL-producing Enterobacterales. The median duration of carriage before FMT was  
190 62.5 days (IQR 48.75-122.5). A summary of the patients' baseline characteristics is shown in Table 1.

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192 *Decolonization following FMT*

193 Fourteen days after FMT, 4 out of 20 patients (20%) tested negative for CPE and met the primary endpoint  
194 for the study. Thirty days post-FMT, 2 additional patients (30%) became negative for CPE followed by two  
195 others by day ninety, resulting in an overall CPE decolonization rate of 40% (8/20) three months post-  
196 transplant (secondary endpoint). The decolonization rate over time is shown as a percentage on a Kaplan-  
197 Meier curve between CPE cases in the FMT group versus spontaneous CPE decolonization in our historical  
198 non-FMT cohort <sup>11</sup> ([Figure 12](#)). Patients in the comparator arm (n=82) had clinical profiles similar to  
199 patients in the FMT group (see supplementary Table) but were not considered for FMT either because  
200 they decided not to participate in the study or because they failed to meet the inclusion criteria, including  
201 medical transfer in our hospital for FMT intervention. Although the percentage of patients with CPE  
202 carriage decreased within the first 14 days after intervention, this difference was not statistically

203 significant ( $p=0.1$ ) and was ultimately similar between the groups ( $p=0.9$ , **Figure 42**). Furthermore, there  
204 was no statistically significant difference in decolonization efficacy between the four FMT donors used in  
205 the first half of the study ( $n=2/12$ ) and the fifth FMT donor used thereafter ( $n=2/8$ ) ( $p=0.99$ ). It is worth  
206 noting that the 2 responders in the second half of the study were also colonized with ESBL-producing  
207 Enterobacterales and had a history of recurrent urinary tract infection (UTI) caused by these organisms.  
208 They did not have any further UTI episodes for up to 12 months following transplant. To the best of our  
209 knowledge no patient in the control group had a previous medical history of recurrent UTI. Finally, there  
210 were no complications during the FMT procedure and no serious adverse events reported at follow-up,  
211 except for mild transient GI complaints ( $n=4$ ).

212

#### 213 *Microbiota analyses*

214 Metagenomic sequencing was performed on fecal samples collected before and after FMT for 12 patients  
215 and their 3 corresponding donors. Fecal material derived from Donor 1, Donor 2 and Donor 5 was used to  
216 treat one, three and eight patients in this sub-cohort, respectively. Four of 12 patients (33%) met the  
217 primary endpoint for decolonization. We analyzed the gut microbiota composition and taxonomic  
218 diversity in responders and non-responders at baseline and 1 week, 2 weeks, 1 month and 2-3 months  
219 after fecal transplantation. We found that the number of detected bacterial species, as measured by  
220 species richness, was significantly higher in responders than in non-responders 2 weeks and 1 month after  
221 FMT and was comparable to the bacterial richness observed in the donors (**Figure 2A3A**). Consistent with  
222 these findings, the gut microbiota alpha diversity, measured by the Shannon index, was significantly  
223 higher in responders compared to non-responders at 2 weeks, 1 month and 2-3 months post-transplant  
224 (**Figure 32B**). These observations suggested that engraftment of likely donor-derived taxa was more  
225 prevalent in responders than in non-responders. To evaluate this further, we measured the Euclidean

226 distance between recipient samples and the centroid of their corresponding donor samples, calculated  
227 using the first 2 principal co-ordinates of a Bray-Curtis dissimilarity matrix, and found the median distance  
228 to be shorter among responders than non-responders at all timepoints following transplant with the  
229 greatest distance at 1-month post-FMT ( $p=0.152$  after FDR adjustment) (Figure 34). This finding  
230 demonstrated a trend where the microbiota of patients that decolonized CPE was more similar to the  
231 microbiota of their donors compared to patients that failed to respond.

232 Next, we examined whether specific donor-derived bacterial taxa were associated with response. We  
233 identified *Faecalibacterium prausnitzii*, *Parabacteroides distasonis*, *Collinsella aerofaciens*, *Alistipes*  
234 *finegoldii* and *Blautia\_A sp900066335* to be significantly increased in responders post-FMT, whereas  
235 *Klebsiella pneumoniae* was significantly more abundant in non-responders (Figure 54). We did not find  
236 any such associations between functional pathway abundance and recipient response ( $p>0.05$ )  
237 (Supplementary Figure 1). We hypothesized that CPE burden at baseline could be a predictor of effective  
238 decolonization, or lack thereof, following fecal transplantation. To evaluate this hypothesis, we compared  
239 the relative abundance of CPE species in responders and non-responders before and after transplant. We  
240 found the relative abundance of CPE species was significantly higher in non-responders compared to  
241 responders at baseline ( $p=0.036$ ) and 1-week post-FMT ( $p=0.036$ ) (Figure 65), suggesting that complete  
242 elimination of CPE carriage may be challenging in patients with high CPE burden.

## 243 Discussion

244 Consistent with findings previously reported by our group<sup>22,23</sup> and others<sup>17</sup>, our results showed that a  
245 single dose of FMT administered via enteric feeding tube has limited ability/efficacy to decolonize CPE.  
246 Nevertheless, the efficacy of FMT against CPE-MDROs is variable and successful decolonization of CPE or  
247 ESBL producing Enterobacterales following FMT administration has been reported<sup>29,30</sup>. Factors  
248 contributing to the variability in FMT efficacy against MDROs in such indication include number of FMT

249 doses, donor selection, FMT preparation, route of FMT administration and pre-treatment regimen  
250 <sup>16,17,29,31</sup>. Indeed, it should be considered that the route of administration used in the present study, as  
251 well as the protocol of FMT involving a single dose, may have negatively influenced the decolonization  
252 process. As a result, there is an urgent need to standardize the FMT process in this population for more  
253 consistent results. Despite the variability in efficacy, FMT is still a safer option than decolonization  
254 strategies involving targeted antibiotics, where the emergence of antibiotic-resistant isolates and  
255 colonization recurrence have been observed <sup>13,14</sup>. Importantly, there was no colonization recurrence after  
256 1 month among responders in the FEDEX study.

257 Although we did not achieve a statistically significant difference in CPE decolonization following FMT, it  
258 should be noted that in the non-responder group, one patient with previously documented CPE  
259 bacteremia did not experience any further CPE infection episodes post-FMT despite remaining colonized.  
260 This observation aligns with findings from Ghani *et al.*, who reported a reduction in the number of MDRO  
261 infections after FMT administration in the absence of successful decolonization <sup>21</sup>, suggesting that  
262 pathogen eradication from the intestine is not required to achieve clinical benefit. The authors speculated  
263 that FMT could lead to partial decolonization, an event that could be clinically meaningful. In line with this  
264 hypothesis, we found that non-responders exhibited a lower relative abundance of CPE species 1 week  
265 post-FMT compared to baseline. This suggests a likely reduction in CPE burden following transplant.  
266 However, absolute quantification of CPE load at baseline and post-FMT without any media enrichment is  
267 needed to determine the full impact of FMT on CPE colonization levels.

268 Furthermore, 2/2 non-immunocompromised patients with recurrent UTI due to ESBL-producing  
269 Enterobacterales did not experience any UTI episodes for up to 12 months after FMT administration. One  
270 33-year-old individual had previously been admitted twice for prostatitis within the 12-months prior to  
271 intervention and had required two courses of carbapenems (with the last injection occurring 3 months  
272 before the FMT). The other individual was a 57-year-old female with recurrent UTI due to neurogenic

273 bladder, and had historically presented with pyelonephritis every 1-2 months treated by cefoxitin using  
274 outpatient parenteral antimicrobial therapy (with the last injection occurring 2 months before the FMT).

275 The intestine can serve as a reservoir for UTI-causing organisms<sup>4,32</sup> and FMT interventional studies against  
276 *C. difficile* infection (CDI) have shown that FMT can significantly reduce the frequency of UTI episodes in  
277 CDI patients with a history of recurrent UTI even in the absence of decolonization<sup>21,33</sup>. Conversely,  
278 transmission events of antibiotic-susceptible uropathogenic strains and subsequent infections post-FMT  
279 have been described, advocating for meticulous FMT donor screening<sup>34</sup>. Despite the small sample size,  
280 our observations are consistent with these findings and add to the growing body of evidence supporting  
281 the role of FMT in infection prevention, especially those caused by MDROs. This hypothesis is supported  
282 by a recent study by Woodworth *et al.*<sup>35</sup>, designed to monitor adverse events during FMT delivered  
283 through retention enema. In the study, UTI was classified as a severe adverse event in a study of 11 kidney  
284 transplant individuals under immunosuppressive therapies thus harboring a potent disrupted microbiota,  
285 responsible for further modification of gut bacterial colonization as previously shown in the literature<sup>36-</sup>  
286<sup>38</sup>. Indeed, in the present work Woodworth *et al.* reported that after a 6-month observation period, two  
287 individuals experienced MDRO UTI with a delayed onset of symptoms compared to contemporaneous  
288 controls (n=165)<sup>35</sup>.

289 Microbiome analyses of stool samples collected from our patients pre- and post-FMT demonstrated that  
290 post-FMT, responders had more diverse microbiotas than non-responders, and responders' microbiotas  
291 resembled those of their corresponding donors more closely. Likewise, Bilinski *et al.*<sup>30</sup> previously showed  
292 that richness and diversity of the transplanted material was significantly different between responders  
293 and non-responders.

294 In our present work we showed that among responders, *Faecalibacterium prausnitzii*, *Parabacteroides*  
295 *distasonis*, *Collinsella aerofaciens*, *Alistipes finegoldii* and *Blautia\_A sp900066335* had significantly higher

296 relative abundances and were therefore associated with successful CPE decolonization post-FMT.  
297 *Faecalibacterium* species have been previously reported to be correlated with Enterobacterales  
298 suppression likely due to their ability to produce metabolites, specifically short chain fatty acids (SCFA),  
299 that can inhibit the growth of Gram-negative pathogens<sup>4,39</sup>. It is noteworthy that Woodworth *et al.*<sup>35</sup>  
300 also described specific taxa, including *Alistipes* and *Faecalibacterium* species, associated with the  
301 decolonization of ESBL-producing Enterobacterales in a small sample size trial (n=11). Likewise, Bilinski *et*  
302 *al.*<sup>30</sup> described microbial signatures associated with decolonization using FMT that pertained to  
303 *Barnesiella*, *Bacteroides*, and *Butyrivimonas* species. Additionally, Leo *et al.* reported that decolonization  
304 was associated with the presence of *Bifidobacterium* strains that were enriched in individuals who had  
305 cleared multidrug-resistant Enterobacterales one month after the return from travel to tropical regions  
306<sup>40</sup>.

307 Although specific bacterial taxa have been associated with MDRO eradication, these differ across studies  
308<sup>30,35</sup>, suggesting that a taxonomically-independent common function or set of functions is more important  
309 than species identity. We found no significant associations between functional pathway abundance and  
310 response; however, functional analysis capabilities were limited to genes and functions present in a  
311 reference database and the use of non-assembled metagenomic short-reads. Future work applying  
312 assembly-based approaches that can leverage more sensitive functional annotation methods may reveal  
313 further insights.

314 The microbiota of non-responders exhibited a higher relative abundance of *Klebsiella pneumoniae*, which  
315 represented  $\leq 12\%$  of the patients' microbiota, compared to responders where *Klebsiella pneumoniae*  
316 levels were nearly undetectable. Importantly, we found that the relative abundance of CPE organisms  
317 before FMT was much lower in responders than in non-responders, suggesting that CPE colonization levels  
318 may be correlated with FMT response, defined by complete elimination of carriage. This finding, however,  
319 does not suggest that patients with high levels of CPE colonization will not respond to FMT or other



320 microbiome-based therapies. In fact, these individuals may benefit from multiple rounds of FMT  
321 treatment as demonstrated by studies where administration of more than 2 FMT doses led to successful  
322 MDRO decolonization after a single dose failed to do so <sup>29,31,35</sup>. However, while multiple FMT doses may  
323 be a potential approach to lower CPE levels enough to reduce infection risk and limit transmission, in the  
324 absence of encapsulated FMT undergoing multiple rounds of FMT via nasoduodenal tube may increase  
325 the risk for serious adverse events, including bacteremia and perforations <sup>41</sup>. Moreover, an important  
326 medical consideration for MDRO-colonized patients is that CPE carriers are often placed under contact  
327 isolation in a dedicated ward resulting in higher healthcare costs and treatment delays <sup>42,43</sup>. Therefore,  
328 FMT administration for MDRO decolonization could be a viable intervention for CPE carriers undergoing  
329 surgery as well as serve as a measure for outbreak control <sup>44</sup>. In line with this, Saidaini *et al.* showed that  
330 in a case-control study in MDRO-colonized patients, FMT administration accelerated discharge of patients  
331 that experienced decolonization (n=8/10) compared to those in the control group <sup>45</sup>. In contrast, our  
332 findings showed no difference in terms of decolonization (up to 90 days) between the patients who  
333 underwent FMT and our control group consisting of 82 individuals.

334 In 2019, a multicenter, randomized controlled study was conducted to evaluate the efficacy of a single  
335 dose of FMT against decolonization of multidrug-resistant Enterobacterales in 39 patients <sup>17</sup>. Prior to FMT,  
336 patients in the intervention group received a 5-day antibiotic course which markedly reduced MDRO  
337 levels. At 1-month, the percentage of patients that remained positive for MDRO colonization was 61.9%  
338 and 79% in the FMT and control groups, respectively. The low efficacy rate reported could be attributed  
339 to poor bacterial viability in the fecal transplants following antibiotic pre-treatment for gram-negative  
340 bacteria. Therefore, further study of microbiota conditioning regimens, including pre-treatment with  
341 specific antibiotic classes and length of antibiotic course is needed in other indications beyond CDI. Bowel  
342 lavage may be a more effective pre-treatment option based on the high decolonization rates reported <sup>31</sup>.  
343 Similarly, patients in the FEDEX study received a bowel prep without antibiotics. Furthermore, studies

344 have shown that administration of antibiotics within 7 days post-FMT is the most common cause of  
345 decolonization failure<sup>23,31,44</sup>. Although our results are not statistically significant due to the limited number  
346 of patients studied, they support this finding (17% of antibiotic use within 30 days post-FMT in the  
347 responder group versus 36% in the non-responder group).

348 Our study has several limitations. First, despite our efforts to match patients with a control group, the  
349 study was not randomized with a placebo group. Second, although the sample size is small (n=20) and  
350 statistical analyses to evaluate potential risk factors of failure were not performed, it is one of the larger  
351 studies reported to date. Third, FMT efficacy was based on complete CPE eradication, an outcome that  
352 may be too high a bar to measure FMT success. Fourth, it could be argued that we did not use pooled  
353 stools for our trial, a has received interest for treatment of microbiota-associated chronic diseases<sup>46</sup>, but  
354 such a strategy was not considered relevant at the time of the FEDEX study (prior to 2017) and was  
355 contrary to the intended research goal (evaluating the impact of a unique donor). Furthermore, our FMTs  
356 were prepared aerobically rather than anaerobically. However, a recent meta-analysis (2021) reported  
357 two studies where FMT material was prepared under anaerobic and aerobic conditions for the treatment  
358 of recurrent CDI, and efficacy was shown to be comparable (80.3% and 84.4%, respectively)<sup>47</sup>. To our  
359 knowledge, no study to date has directly compared the effect of aerobic vs. anaerobic fecal material  
360 processing conditions on the effectiveness of FMT.

361 Finally, the main limitation of testing for decolonization in the clinic is that the current methods fail to  
362 take into account pathogen load and decolonization is defined by the absence of MDRO detection.  
363 Although testing over weeks/months may not be sufficient to show persistent eradication, MDRO  
364 elimination within the first 1-3 months after FMT may have long-term benefits, as demonstrated by the  
365 FMT recipient who decolonized and remained UTI-free for over a year. Furthermore, previous reports  
366 have shown that pathogen eradication is not needed to provide clinical benefit suggesting that keeping  
367 MDRO levels below a certain threshold is key for limiting extraintestinal infections with the colonizing

368 organism <sup>21</sup>. Finally, other underlying factors involved in decolonization, including antibiotic  
369 administration, and diet may impact the gut microbiome composition and create a temporary state of  
370 dysbiosis where colonization recurrence with either the same or a different MDRO strain may occur.

371 Despite these limitations, our results might encourage future FMT trials to switch to quantitative  
372 approaches to accurately assess MDRO colonization levels and shift the study goal towards infection  
373 prevention, rather than focusing solely on carriage elimination. Indeed, FMT might relate to other  
374 important outcomes, such as ongoing transmission or symptomatic infection, as suggested in our finding.  
375 Therefore, all of these questions raised deserved further investigation to better tackle antimicrobial  
376 resistance and infection prevention control.

377 In conclusion, our findings revealed that the engraftment of specific donor-derived taxa and CPE levels  
378 pre-FMT were significantly correlated with FMT efficacy in a non-immunocompromised population.

379 However, we showed that instead of achieving significant complete CPE decolonization, it promoted  
380 significant modification of microbiota richness and diversity, which may be associated with subsequent  
381 less infections (UTI). Our study shows the importance of considering different factors that influence FMT  
382 efficacy in donors and recipients to achieve optimal therapeutic results, suggesting that another FMT  
383 regimen could potentially yield more successful outcomes in this indication. Such a strategy might  
384 contribute to the development of unique live biotherapeutics for specific clinical profiles, and ultimately  
385 provide an antibiotic-free antibacterial strategy to fight against antimicrobial-resistance, as seen for  
386 treatment of *C. difficile* infections <sup>48</sup> .

387

### 388 **Legends of the Figures**

389 [Figure 1: STROBE Flowchart of the FEDEX study. Flowchart in accordance with the STROBE \(Strengthening](#)  
390 [the Reporting of Observational Studies in Epidemiology\) statement <http://www.strobstatement.org>](http://www.strobstatement.org)

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391  
392 [Figure 42](#): Kaplan-Meier representation for decolonization between spontaneous decolonization (control  
393 group, n=82) and FMT intervention (n=20). Dashed lines indicate primary and secondary endpoints.

394

395 [Figure 32](#): Gut microbiota analysis between responders and non-responders  
396 *Box and whisker plots indicating the (A) species richness and (B) Shannon diversity index of donor samples*  
397 *(grey), samples from patients with CPE clearance on day 14 (teal), and patients without CPE clearance on*  
398 *day 14 (orange). The median ( $\pm$ interquartile range) is plotted for each group at the indicated timepoints.*  
399 *Whiskers extend to the lowest and highest values no greater than 1.5x the interquartile range from the*  
400 *closest hinge. Patients with CPE clearance on day 14 have significantly higher species richness and/or*  
401 *Shannon diversity index than patients without CPE clearance on day 14 at 2 weeks, 1 month, and 2-3*  
402 *months post-FMT (Wilcoxon Rank-Sum test with FDR adjustment).*

403

404 [Figure 34](#): Microbiota distance between FMT recipients and donors  
405 *Box and whisker plots indicating the Euclidean distance of samples from patients with CPE clearance on*  
406 *day 14 (teal), and patients without CPE clearance on day 14 (orange) to the centroid of their corresponding*  
407 *donor samples, calculated using the first 2 principal co-ordinates of a Bray-Curtis dissimilarity matrix*  
408 *between samples. The median ( $\pm$ interquartile range) is plotted for each group at the indicated timepoints.*  
409 *Whiskers extend to the lowest and highest values no greater than 1.5x the interquartile range from the*  
410 *closest hinge. While not statistically significant, patients with CPE clearance on day 14 have lower mean*  
411 *distance from their donors than patients without CPE clearance at all post-FMT timepoints (Wilcoxon*  
412 *Rank-Sum test with FDR adjustment).*

413

414 [Figure 45](#): Bacterial taxa associated with response

415 Box and whisker plots of the percent relative abundance for the indicated species in all samples from  
416 patients who ultimately had-CPE clearance on day 14 (teal), and all samples from patients who did not  
417 ultimately have CPE clearance on day 14 (orange). *The median ( $\pm$ interquartile range) is plotted for each  
418 species in each group. Whiskers extend to the lowest and highest values no greater than 1.5x the  
419 interquartile range from the closest hinge. The displayed taxa were significantly altered between patients  
420 with and without CPE clearance at day 14 across all samples (MaAsLin2 log-transformed linear model with  
421 FDR adjustment).*

422

423 [Figure 65](#): Abundance of CPE species pre- and post-FMT

424 Box and whisker plots indicating the cumulative percent relative abundance of CPE species in samples from  
425 patients with CPE clearance on day 14 (teal) and patients without CPE clearance on day 14 (orange). *The  
426 median ( $\pm$ interquartile range) is plotted for each group at the indicated timepoints. Whiskers extend to the  
427 lowest and highest values no greater than 1.5x the interquartile range from the closest hinge. Patients  
428 with CPE clearance on day 14 have significantly lower CPE relative abundance than patients without CPE  
429 clearance on day 14 at baseline and 1 week post-FMT (Wilcoxon Rank-Sum test with FDR adjustment).*

430

431 [Supplementary Figure 1](#): Functional pathway analyses between responders and non-responders

432 A) *Principal component analysis (PCA) plot of the normalized abundance of functional pathways in patients  
433 with CPE clearance on day 14 (blue) and patients without CPE clearance on day 14 (red). Arrows represent  
434 the 5 strongest PCA loadings. B) Box and whisker plots indicating the normalized pathway abundance of*

435 *the functional pathways corresponding to the 5 strongest PCA loadings in samples from patients with CPE*  
436 *clearance on day 14 (teal) and patients without CPE clearance on day 14 (orange). The median*  
437 *( $\pm$ interquartile range) is plotted for each group at the indicated timepoints. Whiskers extend to the lowest*  
438 *and highest values no greater than 1.5x the interquartile range from the closest hinge. There is no*  
439 *significant difference in normalized pathway abundance between patients with CPE clearance on day 14*  
440 *and patients without CPE clearance on day 14 (Wilcoxon Rank-Sum test with FDR adjustment).*

441

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444

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448

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453

454 **Declaration of interests**

455 A.R.W, G.G and S.C are employees of Vedanta Biosciences and have an equity interest in the company.  
456 Vedanta Biosciences holds patents related to this work. The authors vouch for the accuracy and  
457 completeness of the data and data analyses. This content is solely the responsibility of the authors.

458

#### 459 **Author Contributions**

460 BD, PDT and SC designed research (project conception, development of overall research plan, and study  
461 oversight). BD, ASM and AD conducted research and were responsible for the management and  
462 coordination of the trial, including recruitment, conduct of the study, data collection, management of  
463 investigational products, etc. ARW was in charge of monitoring the study progress. GG, SC and ARW  
464 performed metagenomics analyses. RB, EMT, EJK, HM and MJ were responsible of stools conservation and  
465 selection. CL and ASM performed microbiological cultures and PCR. BD, SC, ARW and PDT interpreted the  
466 results, wrote the manuscript and had primary responsibility for the final content. All authors have read  
467 and approved the final manuscript.

#### 468 **Trial Registration**

469 The trial was registered in 2014 under NCT03029078 and is available on <https://clinicaltrials.gov/>.

470

#### 471 **References**

- 472 1 Vehreschild M. J. G. T., Hamprecht A., Peterson L., Schubert S., Hantschel M., Peter S., et al. A  
473 multicentre cohort study on colonization and infection with ESBL-producing Enterobacteriaceae in high-  
474 risk patients with haematological malignancies. *Journal of Antimicrobial Chemotherapy*  
475 2014;**69**(12):3387–92. Doi: 10.1093/jac/dku305.
- 476 2 Taur Ying, Xavier Joao B, Lipuma Lauren, Ubeda Carles, Goldberg Jenna, Gobourne Asia, et al.  
477 Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem  
478 cell transplantation. *Clin Infect Dis* 2012;**55**(7):905–14. Doi: 10.1093/cid/cis580.

- 479 3 McConville Thomas Howe, Sullivan Sean Berger, Gomez-Simmonds Angela, Whittier Susan,  
 480 Uhlemann Anne-Catrin. Carbapenem-resistant Enterobacteriaceae colonization (CRE) and subsequent  
 481 risk of infection and 90-day mortality in critically ill patients, an observational study. *PLoS One*  
 482 2017;**12**(10):e0186195. Doi: 10.1371/journal.pone.0186195.
- 483 4 Magruder Matthew, Sholi Adam N, Gong Catherine, Zhang Lisa, Edusei Emmanuel, Huang  
 484 Jennifer, et al. Gut uropathogen abundance is a risk factor for development of bacteriuria and urinary  
 485 tract infection. *Nat Commun* 2019;**10**(1):5521. Doi: 10.1038/s41467-019-13467-w.
- 486 5 Ferstl Philip G, Filmann Natalie, Heilgental Eva-Maria, Schnitzbauer Andreas A, Bechstein Wolf  
 487 O, Kempf Volkhard A J, et al. Colonization with multidrug-resistant organisms is associated with in  
 488 increased mortality in liver transplant candidates. *PLoS One* 2021;**16**(1):e0245091. Doi:  
 489 10.1371/journal.pone.0245091.
- 490 6 Cassini Alessandro, Högberg Liselotte Diaz, Plachouras Diamantis, Quattrocchi Annalisa, Hoxha  
 491 Ana, Simonsen Gunnar Skov, et al. Attributable deaths and disability-adjusted life-years caused by  
 492 infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a  
 493 population-level modelling analysis. *Lancet Infect Dis* 2019;**19**(1):56–66. Doi: 10.1016/S1473-  
 494 3099(18)30605-4.
- 495 7 Murray Christopher JL, Ikuta Kevin Shunji, Sharara Fablina, Swetschinski Lucien, Robles Aguilar  
 496 Gisela, Gray Authia, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic  
 497 analysis. *The Lancet* 2022;**399**(10325):629–55. Doi: 10.1016/S0140-6736(21)02724-0.
- 498 8 Tacconelli Evelina, Carrara Elena, Savoldi Alessia, Harbarth Stephan, Mendelson Marc, Monnet  
 499 Dominique L, et al. Discovery, research, and development of new antibiotics: the WHO priority list of  
 500 antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**(3):318–27. Doi: 10.1016/S1473-  
 501 3099(17)30753-3.
- 502 9 Colomb-Cotinat M, Soing-Altrach S, Leon A, Savitch Y, Poujol I, Naas T, et al. Emerging  
 503 extensively drug-resistant bacteria (eXDR) in France in 2018. *Med Mal Infect* 2020;**50**(8):715–22. Doi:  
 504 10.1016/j.medmal.2020.01.011.
- 505 10 Willems Roel P.J., van Dijk Karin, Vehreschild Maria J.G.T., Biehl Lena M., Ket Johannes C.F.,  
 506 Rimmelzwaal Sharon, et al. Incidence of infection with multidrug-resistant Gram-negative bacteria and  
 507 vancomycin-resistant enterococci in carriers: a systematic review and meta-regression analysis. *Lancet*  
 508 *Infect Dis* 2023;**23**(6):719–31. Doi: 10.1016/S1473-3099(22)00811-8.
- 509 11 Davido Benjamin, Moussiégt Aurore, Dinh Aurélien, Bouchand Frédérique, Matt Morgan, Senard  
 510 Olivia, et al. Germs of thrones - spontaneous decolonization of Carbapenem-Resistant  
 511 Enterobacteriaceae (CRE) and Vancomycin-Resistant Enterococci (VRE) in Western Europe: is this myth  
 512 or reality? *Antimicrob Resist Infect Control* 2018;**7**(1):100. Doi: 10.1186/s13756-018-0390-5.
- 513 12 Bar-Yoseph Haggai, Hussein Khetam, Braun Eyal, Paul Mical. Natural history and decolonization  
 514 strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-  
 515 analysis. *Journal of Antimicrobial Chemotherapy* 2016;**71**(10):2729–39. Doi: 10.1093/jac/dkw221.
- 516 13 Machuca Isabel, Gutiérrez-Gutiérrez Belén, Pérez Cortés Salvador, Gracia-Ahufinger Irene,  
 517 Serrano Josefina, Madrigal María Dolores, et al. Oral decontamination with aminoglycosides is

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518 associated with lower risk of mortality and infections in high-risk patients colonized with colistin-  
519 resistant, KPC-producing *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2016;**71**(11):3242–9. Doi:  
520 10.1093/jac/dkw272.

521 14 Oren I, Sprecher H, Finkelstein R, Hadad S, Neuberger A, Hussein K, et al. Eradication of  
522 carbapenem-resistant Enterobacteriaceae gastrointestinal colonization with nonabsorbable oral  
523 antibiotic treatment: A prospective controlled trial. *Am J Infect Control* 2013;**41**(12):1167–72. Doi:  
524 10.1016/J.AJIC.2013.04.018.

525 15 Tascini Carlo, Sbrana Francesco, Flammini Sarah, Tagliaferri Enrico, Arena Fabio, Leonildi  
526 Alessandro, et al. Oral gentamicin gut decontamination for prevention of KPC-producing *Klebsiella*  
527 *pneumoniae* infections: relevance of concomitant systemic antibiotic therapy. *Antimicrob Agents*  
528 *Chemother* 2014;**58**(4):1972–6. Doi: 10.1128/AAC.02283-13.

529 16 Bilsen Manu P, Lambregts Merel M C, van Prehn Joffrey, Kuijper Ed J. Faecal microbiota  
530 replacement to eradicate antimicrobial resistant bacteria in the intestinal tract - a systematic review.  
531 *Curr Opin Gastroenterol* 2022;**38**(1):15–25. Doi: 10.1097/MOG.0000000000000792.

532 17 Huttner B D, de Lastours V, Wassenberg M, Maharshak N, Mauris A, Galperine T, et al. A 5-day  
533 course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant  
534 Enterobacteriaceae: a randomized clinical trial. *Clin Microbiol Infect* 2019;**25**(7):830–8. Doi:  
535 10.1016/j.cmi.2018.12.009.

536 18 Tacconelli E., Mazzaferri F., de Smet A. M., Bragantini D., Eggimann P., Huttner B. D., et al.  
537 ESCMID-EUCIC clinical guidelines on decolonization of multidrug-resistant Gram-negative bacteria  
538 carriers. *Clin Microbiol Infect* 2019;**25**(7):807–17. Doi: 10.1016/J.CMI.2019.01.005.

539 19 Crum-Cianflone Nancy F, Sullivan Eva, Ballon-Landa Gonzalo. Fecal microbiota transplantation  
540 and successful resolution of multidrug-resistant-organism colonization. *J Clin Microbiol*  
541 2015;**53**(6):1986–9. Doi: 10.1128/JCM.00820-15.

542 20 Stripling Joshua, Kumar Ranjit, Baddley John W, Nellore Anoma, Dixon Paula, Howard Donna, et  
543 al. Loss of Vancomycin-Resistant Enterococcus Fecal Dominance in an Organ Transplant Patient With  
544 *Clostridium difficile* Colitis After Fecal Microbiota Transplant. *Open Forum Infect Dis* 2015;**2**(2):ofv078.  
545 Doi: 10.1093/ofid/ofv078.

546 21 Ghani Rohma, Mullish Benjamin H, McDonald Julie A K, Ghazy Anan, Williams Horace R T,  
547 Brannigan Eimear T, et al. Disease Prevention Not Decolonization: A Model for Fecal Microbiota  
548 Transplantation in Patients Colonized With Multidrug-resistant Organisms. *Clin Infect Dis*  
549 2021;**72**(8):1444–7. Doi: 10.1093/cid/ciaa948.

550 22 Davido B., Batista R., Michelon H., Lepointeur M., Bouchand F., Lepeule R., et al. Is faecal  
551 microbiota transplantation an option to eradicate highly drug-resistant enteric bacteria carriage? *Journal*  
552 *of Hospital Infection* 2017;**95**(4):433–7. Doi: 10.1016/j.jhin.2017.02.001.

553 23 Dinh A., Fessi H., Duran C., Batista R., Michelon H., Bouchand F., et al. Clearance of carbapenem-  
554 resistant Enterobacteriaceae vs vancomycin-resistant enterococci carriage after faecal microbiota  
555 transplant: a prospective comparative study. *Journal of Hospital Infection* 2018. Doi:  
556 10.1016/j.jhin.2018.02.018.

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- 557 24 Fries Brant E., Schneider Don P., Foley William J., Gavazzi Marie, Burke Robert, Cornelius  
558 Elizabeth. Refining a case-mix measure for nursing homes: Resource utilization groups (rug-iii). *Med Care*  
559 1994;**32**(7):668–85. Doi: 10.1097/00005650-199407000-00002.
- 560 25 Sokol Harry, Galperine Tatiana, Kapel Nathalie, Bourlioux Pierre, Seksik Philippe, Barbut Frederic,  
561 et al. Faecal microbiota transplantation in recurrent *Clostridium difficile* infection: Recommendations  
562 from the French Group of Faecal microbiota Transplantation. *Dig Liver Dis* 2016;**48**(3):242–7. Doi:  
563 10.1016/j.dld.2015.08.017.
- 564 26 Caballero Silvia, Kim Sohn, Carter Rebecca A, Leiner Ingrid M, Sušac Bože, Miller Liza, et al.  
565 Cooperating Commensals Restore Colonization Resistance to Vancomycin-Resistant *Enterococcus*  
566 *faecium*. *Cell Host Microbe* 2017;**21**(5):592–602.e4. Doi: 10.1016/j.chom.2017.04.002.
- 567 27 Caballero Silvia, Carter Rebecca, Ke Xu, Sušac Bože, Leiner Ingrid M., Kim Grace J., et al. Distinct  
568 but Spatially Overlapping Intestinal Niches for Vancomycin-Resistant *Enterococcus faecium* and  
569 Carbapenem-Resistant *Klebsiella pneumoniae*. *PLoS Pathog* 2015;**11**(9):1–20. Doi:  
570 10.1371/journal.ppat.1005132.
- 571 28 Minot S S, Krumm N, Greenfield N B. One Codex: a sensitive and accurate data platform for  
572 genomic microbial identification. *Preprint at BioRxiv* 2015. Doi: <https://doi.org/10.1101/027607>.
- 573 29 Singh Ramandeep, de Groot Pieter F., Geerlings Suzanne E., Hodiamont Caspar J., Belzer Clara,  
574 Berge Ineke J. M. ten, et al. Fecal microbiota transplantation against intestinal colonization by extended  
575 spectrum beta-lactamase producing Enterobacteriaceae: a proof of principle study. *BMC Res Notes*  
576 2018;**11**(1):190. Doi: 10.1186/s13104-018-3293-x.
- 577 30 Bilinski Jaroslaw, Grzesiowski Pawel, Sorensen Nikolaj, Madry Krzysztof, Muszynski Jacek, Robak  
578 Katarzyna, et al. Fecal Microbiota Transplantation in Patients with Blood Disorders Inhibits Gut  
579 Colonization with Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. *Clinical*  
580 *Infectious Diseases* 2017. Doi: 10.1093/cid/cix252.
- 581 31 Biliński Jarosław, Grzesiowski Paweł, Muszyński Jacek, Wróblewska Marta, Mądry Krzysztof,  
582 Robak Katarzyna, et al. Fecal Microbiota Transplantation Inhibits Multidrug-Resistant Gut Pathogens:  
583 Preliminary Report Performed in an Immunocompromised Host. *Arch Immunol Ther Exp (Warsz)*  
584 2016;**64**(3):255–8. Doi: 10.1007/s00005-016-0387-9.
- 585 32 Niki M., Hirai I., Yoshinaga A., Ulzii-Orshikh L., Nakata A., Yamamoto A., et al. Extended-spectrum  
586  $\beta$ -lactamase-producing *Escherichia coli* strains in the feces of carriers contribute substantially to urinary  
587 tract infections in these patients. *Infection* 2011;**39**(5):467–71. Doi: 10.1007/s15010-011-0128-2.
- 588 33 Tariq Raseen, Pardi Darrell S., Tosh Pritish K., Walker Randall C., Razonable Raymond R., Khanna  
589 Sahil. Fecal Microbiota Transplantation for Recurrent *Clostridium difficile* Infection Reduces Recurrent  
590 Urinary Tract Infection Frequency. *Clinical Infectious Diseases* 2017. Doi: 10.1093/cid/cix618.
- 591 34 Vendrik Karuna E.W., de Meij Tim G.J., Bökenkamp Arend, Ooijevaar Rogier E., Groenewegen  
592 Bas, Hendrickx Antoni P.A., et al. Transmission of Antibiotic-Susceptible *Escherichia coli* Causing Urinary  
593 Tract Infections in a Fecal Microbiota Transplantation Recipient: Consequences for Donor Screening?  
594 *Open Forum Infect Dis* 2022;**9**(7). Doi: 10.1093/ofid/ofac324.

595 35 Woodworth Michael H., Conrad Roth E., Haldopoulos Marina, Pouch Stephanie M., Babiker  
596 Ahmed, Mehta Aneesh K., et al. Fecal microbiota transplantation promotes reduction of antimicrobial  
597 resistance by strain replacement. *Sci Transl Med* 2023;**15**(720):eabo2750. Doi:  
598 10.1126/SCITRANSLMED.ABO2750.

599 36 Tourret Jérôme, Willing Benjamin P., Dion Sara, MacPherson Jayden, Denamur Erick, Finlay Brett  
600 B. Immunosuppressive Treatment Alters Secretion of Ileal Antimicrobial Peptides and Gut Microbiota,  
601 and Favors Subsequent Colonization by Uropathogenic *Escherichia coli*. *Transplantation* 2017;**101**(1):74–  
602 82. Doi: 10.1097/TP.0000000000001492.

603 37 Gibson Caitlin M., Childs-Kean Lindsey M., Naziruddin Zahra, Howell Crystal K. The alteration of  
604 the gut microbiome by immunosuppressive agents used in solid organ transplantation. *Transpl Infect Dis*  
605 2021;**23**(1). Doi: 10.1111/TID.13397.

606 38 Gabarre Paul, Loens Christopher, Tamzali Yanis, Barrou Benoit, Jaisser Frédéric, Tourret Jérôme.  
607 Immunosuppressive therapy after solid organ transplantation and the gut microbiota: Bidirectional  
608 interactions with clinical consequences. *Am J Transplant* 2022;**22**(4):1014–30. Doi: 10.1111/AJT.16836.

609 39 Sorbara Matthew T, Dubin Krista, Littmann Eric R, Moody Thomas U, Fontana Emily, Seok Ruth,  
610 et al. Inhibiting antibiotic-resistant Enterobacteriaceae by microbiota-mediated intracellular  
611 acidification. *J Exp Med* 2019;**216**(1):84–98. Doi: 10.1084/jem.20181639.

612 40 Leo Stefano, Lazarevic Vladimir, Gaïa Nadia, Estellat Candice, Girard Myriam, Matheron Sophie,  
613 et al. The intestinal microbiota predisposes to traveler’s diarrhea and to the carriage of multidrug-  
614 resistant Enterobacteriaceae after traveling to tropical regions. *Gut Microbes* 2019;**10**(5). Doi:  
615 10.1080/19490976.2018.1564431.

616 41 Baxter M., Colville A. Adverse events in faecal microbiota transplant: a review of the literature. *J*  
617 *Hosp Infect* 2016;**92**(2):117–27. Doi: 10.1016/J.JHIN.2015.10.024.

618 42 Tackin A, Lawrence C, Godin E, Verheye J C, Davido B. [Management in a dedicated ward of  
619 patients colonized with highly resistant bacteria: Is it worth it?]. *Med Mal Infect* 2020;**50**(5):454–5. Doi:  
620 10.1016/j.medmal.2020.01.007.

621 43 van Dijk M D, Voor In ’t Holt A F, Polinder S, Severin J A, Vos M C. The daily direct costs of  
622 isolating patients identified with highly resistant micro-organisms in a non-outbreak setting. *J Hosp*  
623 *Infect* 2021;**109**:88–95. Doi: 10.1016/j.jhin.2020.12.013.

624 44 Davido B., Batista R., Fessi H., Salomon J., Dinh A. Impact of faecal microbiota transplantation to  
625 eradicate vancomycin-resistant enterococci (VRE) colonization in humans. *Journal of Infection*  
626 2017;**75**(4). Doi: 10.1016/j.jinf.2017.06.001.

627 45 Saïdani Nadia, Lagier Jean-Christophe, Cassir Nadim, Million Matthieu, Baron Sophie, Dubourg  
628 Grégory, et al. Faecal microbiota transplantation shortens the colonisation period and allows re-entry of  
629 patients carrying carbapenamase-producing bacteria into medical care facilities. *Int J Antimicrob Agents*  
630 2019;**53**(4):355–61. Doi: 10.1016/j.ijantimicag.2018.11.014.

631 46 Kazerouni Abbas, Wein Lawrence M. Exploring the Efficacy of Pooled Stools in Fecal Microbiota  
632 Transplantation for Microbiota-Associated Chronic Diseases. *PLoS One* 2017;**12**(1):e0163956. Doi:  
633 10.1371/journal.pone.0163956.

634 47 Cold Frederik, Baunwall Simon Mark Dahl, Dahlerup Jens Frederik, Petersen Andreas Munk, Hvas  
635 Christian Lodberg, Hansen Lars Hestbjerg. Systematic review with meta-analysis: encapsulated faecal  
636 microbiota transplantation – evidence for clinical efficacy. *Therap Adv Gastroenterol* 2021;**14**. Doi:  
637 10.1177/17562848211041004/SUPPL\_FILE/SJ-DOCX-2-TAG-10.1177\_17562848211041004.DOCX.

638 48 Orenstein Robert, Hecht Gail, Harvey Adam, Tillotson Glenn, Khanna Sahil. Two-year durability  
639 of REBYOTA™ (RBL), a live biotherapeutic for the prevention of recurrent *Clostridioides difficile*  
640 infections. *Open Forum Infect Dis* 2023;**10**(9). Doi: 10.1093/ofid/ofad456.

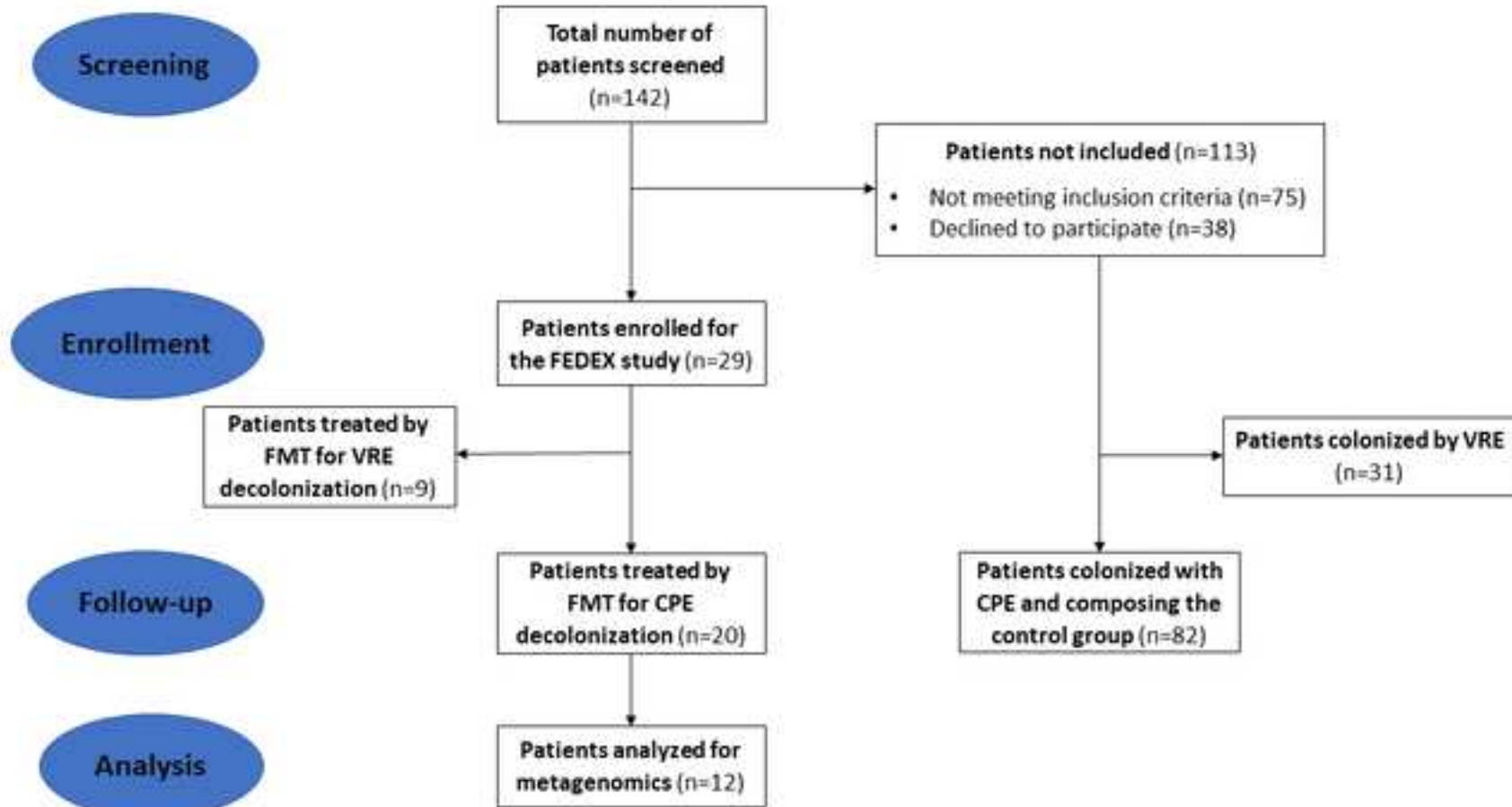
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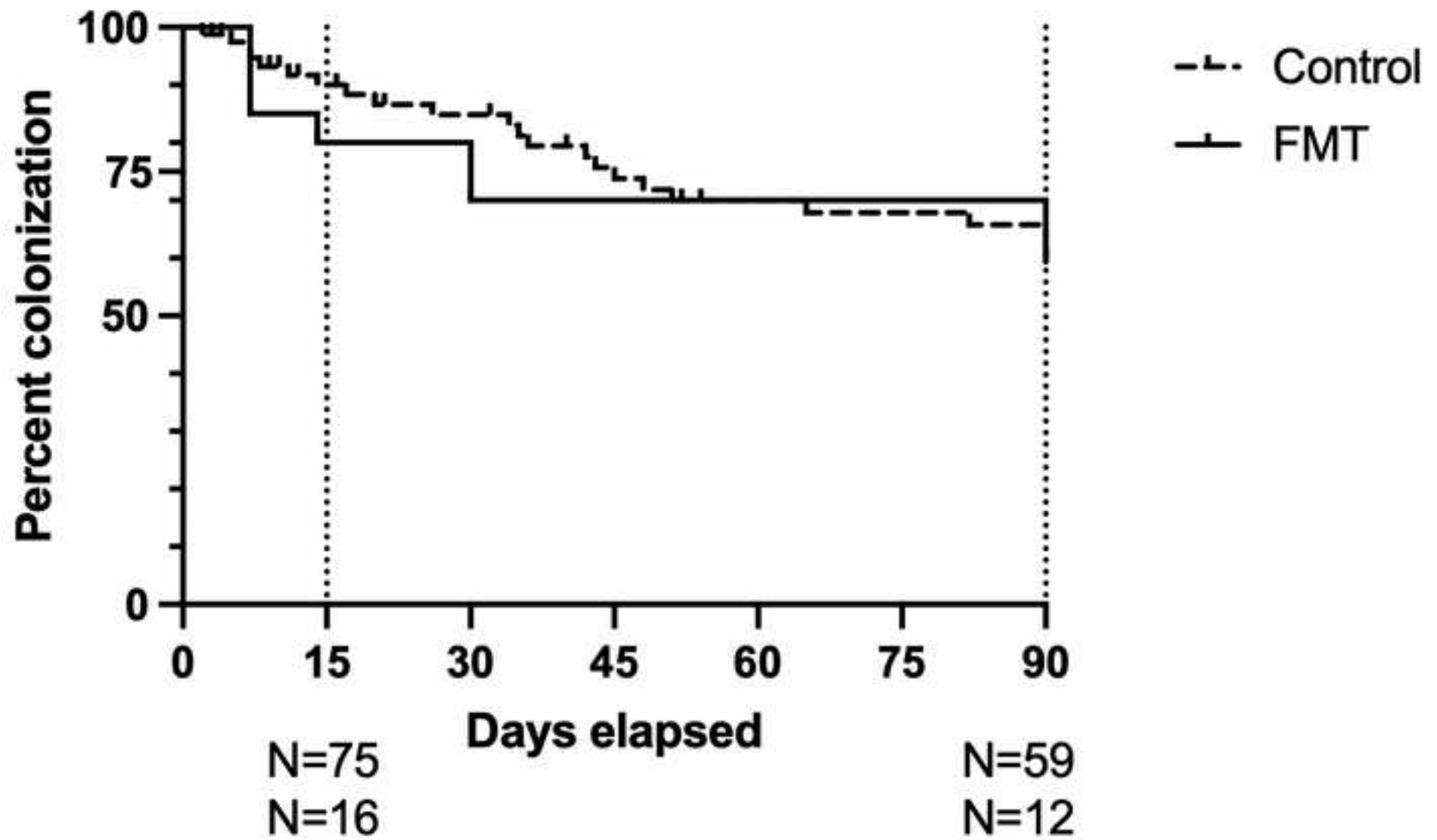
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Table 1: Descriptive factors which may have contributed to decolonization using FMT at 1 month

Variables	Success (n=6)	Failure (n=14)	p-value
<b>Characteristics at baseline</b>			
Age in years, median (IQR)	64.5 (59-71)	73.5 (70-83)	0.2
Hospitalization within previous 6 months, n (%)	5 (83)	12 (86)	0.9
Stay abroad, n (%)	3 (50)	10 (71)	0.6
Duration of carriage in days, median (IQR)	82.5 (61-115)	60 (46-119)	0.6
Previous antibiotic intake within the month, n (%)	4 (67)	10 (71)	0.9
- Duration of treatment in days ,median (IQR)	2 (2-7)	7 (7-14)	0.5
Charlson Score, median (IQR)	5 (4-6)	5 (4-7)	0.5
GIR score, median (IQR)	3.5 (1.5-5.5)	5 (2.25-5)	0.6
Previously infected by a MDRO, n (%)	2 (33)	1 (7)	0.2
<b>Biological abnormalities</b>			
WBC count per mm <sup>3</sup> , median (IQR)	10600 (8775-11750)	8450 (6825-9175)	0.2
PMN count per mm <sup>3</sup> , median (IQR)	7170 (6645-8190)	5235 (3750-6152)	0.1
Lymphocytes count per mm <sup>3</sup> , median (IQR)	1670 (1147.5-1900)	1795 (1425-2387)	0.4
Monocytes count per mm <sup>3</sup> , median (IQR)	815 (597-1115)	660 (487.5-812)	0.4
CRP level in mg/L, median (IQR)	25.5 (8-56)	16.5 (9.5-41)	0.9
Albumin level in g/L, median (IQR)	30 (29-31)	26.5 (24-32)	0.7
<b>Type of microorganisms*</b>			
<i>E. coli</i> , n (%)	4 (67)	5 (36)	0.3
<i>K. pneumoniae</i> , n (%)	4 (67)	11 (78)	0.6
<i>E. cloacae</i> , n (%)	1 (17)	0	0.3
Others, n (%)	0	1 (7)	0.9
Co-colonization, n (%)	4 (67)	6 (43)	0.6
<b>Mechanism of resistance</b>			
OXA48-type, n (%)	6 (100)	11 (78)	0.5
NDM1-type, n (%)	0	3 (21)	0.5
ESBL-producing co-colonization, n (%)	4 (67)	6 (43)	0.6
<b>Antimicrobial therapy after FMT</b>			
Antibiotic intake after FMT during follow-up, n (%)	1 (17)	5 (36)	0.6
- Duration of treatment in days, median (IQR)	7 (7-7)	7 (7-45)	0.7

\* Total percentage was >100% considering some patients carried 2 types of microorganisms





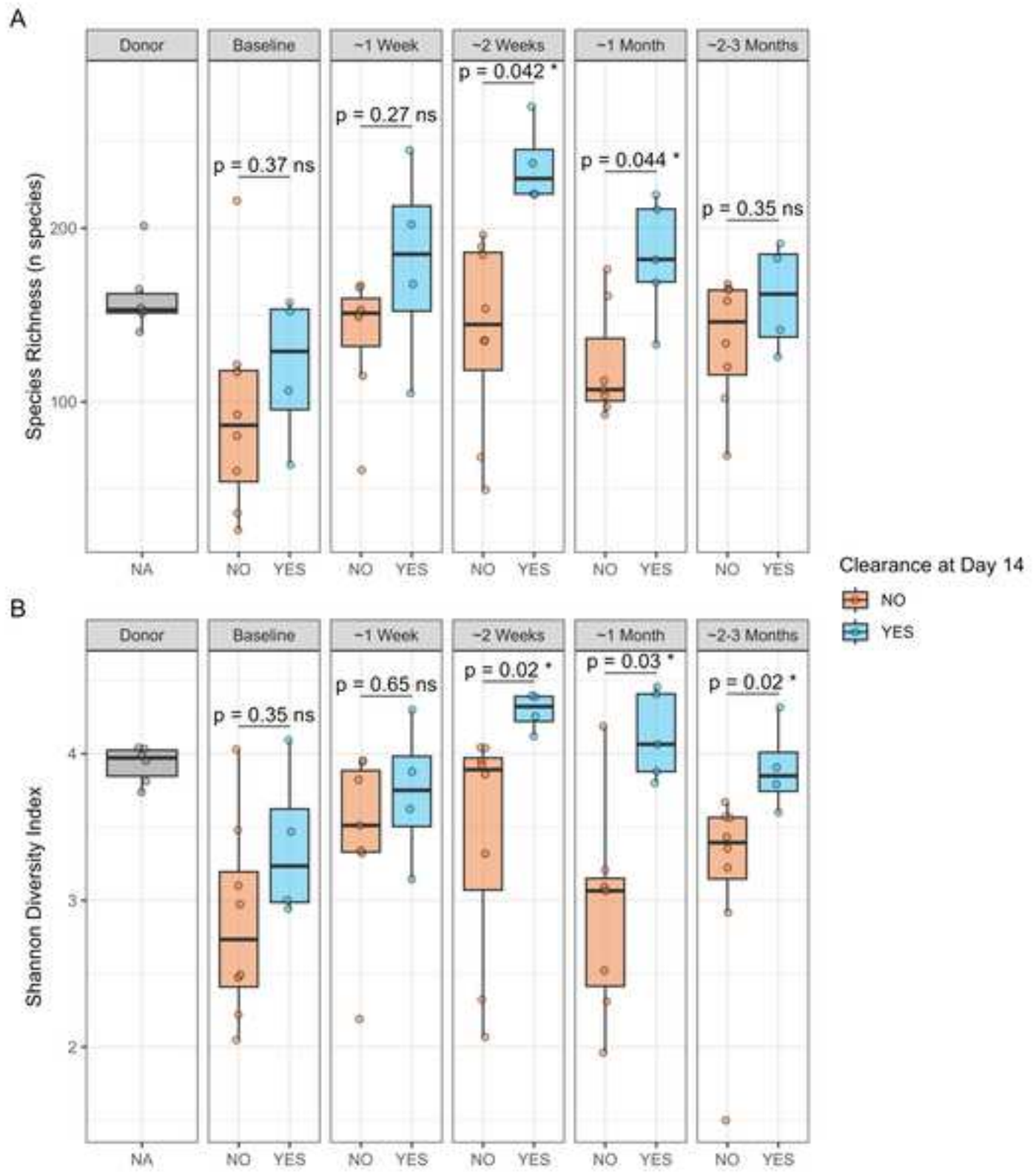
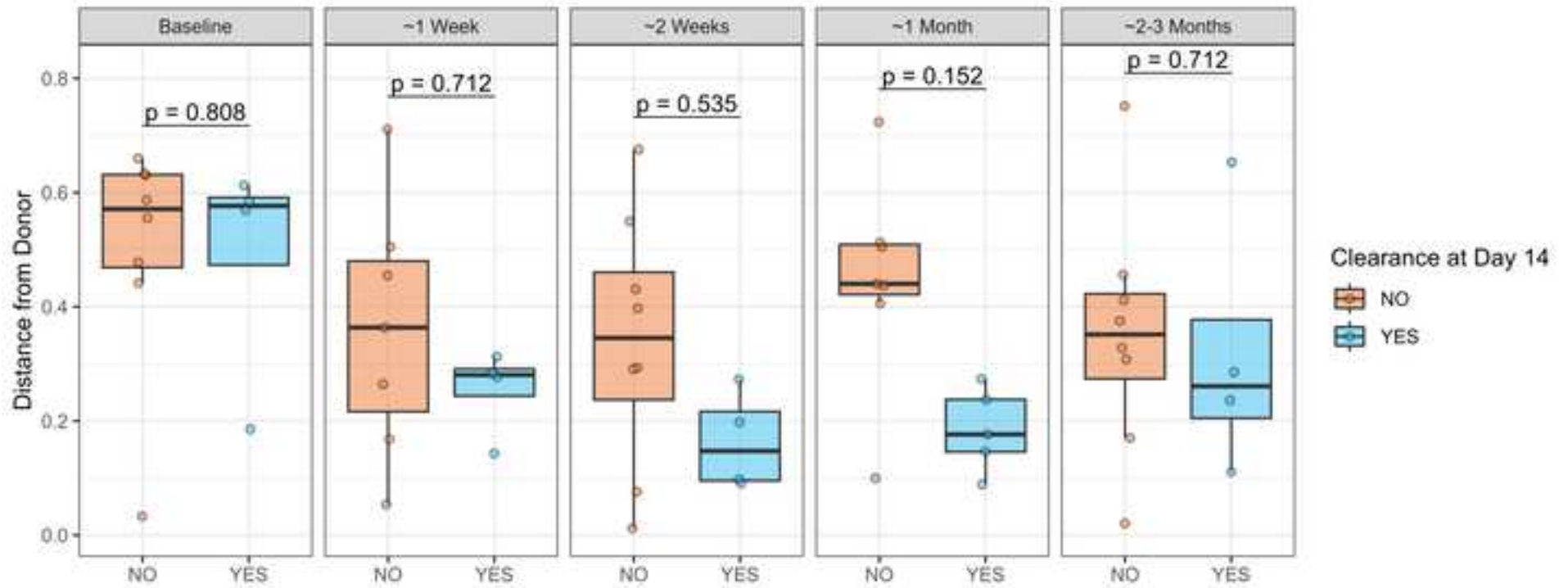
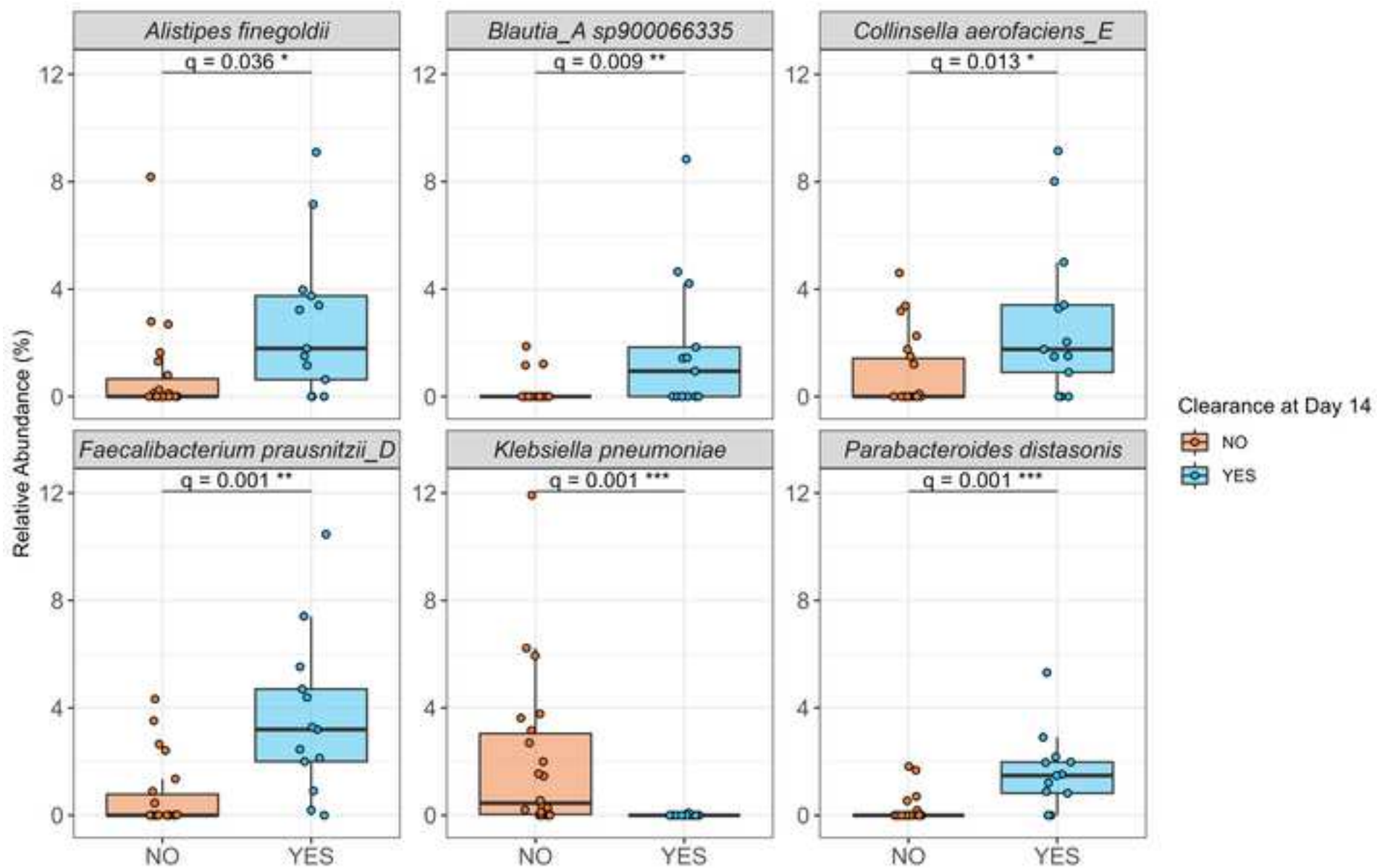




Figure4

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**Supplementary file**  
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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for Journal of Infection and was not involved in the editorial review or the decision to publish this article.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



Dr Benjamin DAVIDO

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