

1 **Alterations in intestinal Proteobacteria and antimicrobial resistance gene burden in**
2 **individuals administered microbial ecosystem therapeutic (MET-2) for recurrent**
3 ***Clostridioides difficile* infection**

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24 **Abstract**

25 Intestinal colonisation with pathogens and antimicrobial resistant organisms (AROs) is
26 associated with increased risk of infection. Fecal microbiota transplant (FMT) has successfully
27 been used to cure recurrent *Clostridioides difficile* infection (rCDI) and to decolonise intestinal
28 AROs. However, FMT has significant practical barriers to implementation. A microbial
29 consortium, microbial ecosystem therapeutic (MET)-2, is an alternative to FMT for the treatment
30 of rCDI. It is unknown whether MET-2 is associated with decreases in pathogens and
31 antimicrobial resistance genes (ARGs). We conducted a post-hoc metagenomic analysis of stool
32 collected from two interventional studies of MET-2 (published) and FMT (unpublished) for rCDI
33 treatment to understand if MET-2 had similar effects to FMT for decreasing pathogens and
34 ARGs as well as increasing anaerobes. Patients were included in the current study if baseline
35 stool had Proteobacteria relative abundance $\geq 10\%$ by metagenomic sequencing. We assessed
36 pre- and post-treatment Proteobacteria, obligate anaerobe and butyrate-producer relative
37 abundances and total ARGs. MET-2 and FMT were associated with decreases in Proteobacteria
38 relative abundance as well as increases in obligate anaerobe and butyrate-producer relative
39 abundances. The microbiota response remained stable over 4 or 6 months for MET-2 and FMT,
40 respectively. MET-2, but not FMT, was associated with a decrease in the total number of ARGs.
41 MET-2 is a potential therapeutic strategy for ARO/ARG decolonisation and anaerobe repletion.

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48 **Introduction**

49 The human gut microbiome has been implicated as a potential source of infection among
50 hospitalized individuals [1,2]. Gastrointestinal carriage of pathogenic antimicrobial resistant
51 organisms (AROs) with increased abundance of Proteobacteria and decreased abundance of
52 obligate anaerobes and butyrate-producers are associated with elevated risks of infection in these
53 individuals [3–5]. Gut commensal anaerobes provide colonisation resistance against
54 opportunistic pathogens and are important mediators of immune system function and regulation
55 [6]. Butyrate, an anaerobic by-product of dietary fibre fermentation, limits the overgrowth and
56 translocation of opportunistic pathogens by promoting epithelial barrier function, intestinal
57 hypoxia, macrophage antimicrobial function, and immune system homeostasis [6–9].

58 Antibiotic use, including prophylaxis and selective decontamination of the digestive tract,
59 are effective infection prevention strategies in some populations [5,10]. However, antibiotic use
60 is associated with toxicity, disruption of the gut microbiota, and the growth promotion of AROs
61 [11–13]. Thus, alternatives to antibiotics for infection prevention and control are needed.

62 Fecal microbiota transplant (FMT) is effective for the treatment of recurrent
63 *Clostridioides difficile* infection (rCDI) and is associated with decreased incidence of
64 bloodstream infection in this population [14,15]. FMT may be a potential strategy for
65 decolonising intestinal AROs where eradication rates have ranged from 37.5 – 87.5% in mostly
66 small observational studies lacking a placebo control [16]. However, FMT is limited by safety
67 and scalability challenges [17,18]. Microbial ecosystem therapeutic (MET)-2, is a bacterial
68 consortium of 39 isolated organisms and has been demonstrated in a recent clinical trial to cure
69 rCDI in 79% of trial participants [19]. Since patients with rCDI are often colonized with AROs
70 and have increased abundances of Proteobacteria including clinically-relevant members of

71 Enterobacteriaceae in the gut microbiome [16,20], we postulated that MET-2 administration may
72 have similar effects to FMT for decreasing Proteobacteria and antimicrobial resistant gene
73 (ARG) abundance in stool.

74 In this study, we performed a post-hoc metagenomic analysis of stool microbiome
75 composition in recipients of MET-2 or FMT for rCDI from two cohorts to assess their effects on
76 the abundances of Proteobacteria, ARGs, obligate anaerobes, and butyrate-producers in pre- and
77 post-therapy stool samples.

78 **Methods**

79 **Study design and participants**

80 This is a study of patients 18 years or older with rCDI, defined as one or more
81 recurrences of CDI, who participated in separate prospective cohorts evaluating the effects of
82 either a microbial consortium (MET-2), which has been previously described [19], or FMT
83 (unpublished) on rCDI recurrence. As part of the Microbiota Therapeutics Outcomes Program,
84 the FMT for rCDI study is currently ongoing in Toronto, Ontario, Canada. Donor screening
85 protocol, the preparation of the FMT, as well as participant inclusion and exclusion criteria are
86 outlined in Supplementary Methods 1.

87 Briefly, both cohorts were on antibiotic therapies prior to the therapeutic intervention.

88 Patients who received FMT underwent bowel preparation prior to FMT. FMT was administered
89 via enema 3 times over the course of 7 days. Patients receiving MET-2 did not receive bowel
90 preparation prior to the intervention. Initially, 10 MET-2 capsules were taken orally for 2 days,
91 and then 3 capsules were taken orally for 8 days. In the current study, we selected individuals
92 from either cohort with $\geq 10\%$ Proteobacteria relative abundance in the baseline stool sample

93 based on metagenomic analyses and did not receive additional MET-2 or FMT. This study had
94 research ethics approval.

95 **Sample collection and processing**

96 Stool sample collection from the MET-2 study was described previously [19]. Briefly,
97 stool samples were collected at baseline prior to MET-2, as well as approximately 2 weeks, 1
98 month, and 4 months post-MET-2. For the FMT study, stool samples were collected at baseline
99 prior to FMT, as well as 1, 3, and 6 months post-FMT. All stool samples were stored at -80°C
100 until further use. Stool samples (0.25 g) were subject to DNA extraction using the DNeasy
101 PowerSoil Pro Kit (Qiagen) and stored at -20°C. DNA concentration was measured using the
102 Qubit Fluorometer (Thermo Fisher) following the manufacturer's instructions. Prior to library
103 preparation, DNA was diluted to approximately 100 ng in DNase/RNase free water. Sequencing
104 libraries were generated using the DNA Prep kit (Illumina) and the IDT for Illumina UD indexes
105 (Illumina). Libraries were stored at -20°C. Libraries were manually pooled and sequenced at 2
106 x150 bp using the SP flowcell on the NovaSeq 6000 at the Princess Margaret Genomics Centre.

107 **Outcomes**

108 The primary outcome was change in Proteobacteria relative abundance between baseline
109 and approximately 1-month (30 days \pm 10 days) post-intervention (MET-2 and FMT). The
110 secondary outcomes included total ARGs as well as obligate anaerobe and butyrate-producer
111 relative abundance between baseline and 1-month post-intervention (MET-2 and FMT). Lastly,
112 exploratory longitudinal analyses of Proteobacteria, obligate anaerobe, and butyrate-producer
113 relative abundances up to 4 months in the MET-2 interventional group and 6 months in the FMT
114 interventional group were performed.

115 **Sequence data processing**

116 Sequence quality was assessed with FastQC v.0.11.9 [21]. As the quality was high, no
117 sequence trimming was performed. Nextera adapters were trimmed with Trimmomatic v0.39
118 [22]. Human and phiX reads were removed with KneadData v.0.7.2 [23]. Taxa were identified
119 from quality-processed reads using Metaphlan3 v.3.0.13 [24]. To ensure an even sampling depth
120 for ARG detection, quality processed-reads were subsampled to 12,328,297 reads, which
121 represents the lowest sequencing depth that retained all baseline and 1-month samples from the
122 MET-2 and FMT interventional groups. Based on the performance characteristics of sequencing
123 metagenomic samples for the detection of ARGs [25], 12,328,297 reads per sample provides a
124 detection frequency $\geq 90\%$ for all ARGs to relative abundances of $\geq 3\%$. The subsampled reads
125 were assembled into contigs using metaSPades v.3.15.3 [26] with the recommended kmer
126 lengths of 21, 33, 55, and 77. To predict the ARGs from metagenome-assembled contigs, RGI
127 *main* v.5.1.0 of the CARD [27] was used on default settings (perfect and strict hits identified
128 only), specifying DIAMOND v.0.8.36 [28] as the local aligner and the *-low_quality* flag. RGI's
129 *heatmap* v.5.1.0 function was used to categorize ARGs based on drug class-associated resistance.
130 Published 16S rRNA sequences of stool samples from vancomycin-treated patients with CDI
131 pre-treatment and approximately 1-month post-treatment were analyzed to measure
132 Proteobacteria relative abundance, as a reference [29,30], using QIMME2 v.2022.8 [31].

133 **Proteobacteria quantitative polymerase chain reaction (qPCR) for absolute abundance
134 quantification**

135 Density of γ -Proteobacteria in each fecal sample and DNA extraction negative controls
136 were measured using qPCR, with the forward primer (5'-TCGTCAGCTCGTGTGTA-3'), the
137 reverse primer (5'-CGTAAGGGCCATGATG-3')[32] and probe (HEX-5'-AACGAGCGC-
138 ZEN-AACCCTTWTCCY-3'-FQ-IABk) (Integrated DNA Technologies).

139 **Microbiota analyses and anaerobe classification**

140 Proteobacteria content in each sample was summarized at the species level and overall
141 relative abundance was quantified at the phylum level. Enterobacteriaceae, γ -Proteobacteria
142 relative abundances were also quantified. Obligate anaerobe and butyrate-producer diversity in
143 each sample was summarized and relative abundance determined at the species level.

144 We used Bergey's Manual of Systematic Bacteriology volumes 2 – 5 to manually classify
145 species-level taxa as obligate anaerobes and butyrate-producers based on descriptors in the
146 manuals such as "strictly anaerobic", "anaerobic" or "obligate anaerobe" as well as "produces
147 butyrate", "forms butyrate", or "butyric acid is an end product" [33–37]. If the manual did not
148 have descriptive terms for butyrate production, taxa were not considered butyrate-producers.

149 Microbiome measurements were assessed at baseline, 30 days (\pm 10 days) and up to 4 or 6
150 months post-intervention for MET-2 and FMT recipients, respectively.

151 **Statistical analyses**

152 Samples were grouped by intervention received (MET-2 or FMT) and stratified by
153 timepoint. To compare relative abundances between baseline and 1-month post-intervention
154 samples, 0.000001 was added to relative abundances for all taxa (to account for zeros), then log
155 transformed. Pairwise analyses were performed using the non-parametric Wilcoxon matched-
156 pairs signed-rank test on log-transformed relative abundances and total number of ARGs within
157 interventional groups between the baseline and 1-month post-intervention timepoints. The non-
158 parametric Mann-Whitney U-Test was used to compare groups. The non-parametric Spearman's
159 correlation was performed to test the relationship between total ARGs and Proteobacteria
160 abundance. Statistical analyses were performed in GraphPad Prism v.9.0.3.

161 **Results**

162 **Participant characteristics**

163 A total 15/19 (79%) MET-2 for rCDI trial participants and 5/8 (62%) FMT for rCDI
164 study participants were included in the current study. Participants in both groups initially
165 received vancomycin, except for participant #1 in the FMT study who received fidaxomicin. The
166 median age of the MET-2 and FMT participants was 65 years and 67 years, respectively, and
167 female patients were more common in both groups (MET-2: 67%, 10/15, FMT: 100% (5/5). A
168 single FMT donor provided stool on multiple dates for preparation of FMT. Supplementary
169 Table 1 provides stool donation dates and to which recipients the donated stool was
170 administered. Participant #10 who received MET-2 failed initial MET-2 administration and was
171 retreated (stool samples before and after re-treatment are not included in this study). Participant
172 #3 of the FMT group failed the initial course of FMT and did not receive another course within
173 the study period. For the baseline and 1-month post-intervention analyses, data from 2 patients
174 from the MET-2 interventional group were excluded due to missing 1-month samples. These 2
175 patients were included for the longitudinal analyses.

176 **Proteobacteria abundance pre- and post-intervention**

177 Among included patients, median baseline Proteobacteria relative abundance for
178 participants who received MET-2 (n = 13) was 44% (range, 11% - 97%) and for participants who
179 received FMT (n = 5) was 55% (range, 17% - 93%) (Mann-Whitney p-value: 0.70). At baseline,
180 the most common and abundant Proteobacteria genera in the MET-2 and FMT groups were
181 *Klebsiella* which included *K. pneumoniae*, *K. oxytoca*, *K. variicola*, *K. michiganensis*, and *K.*
182 *quasipneumoniae*. Other species included *Escherichia coli*, *Enterobacter cloacae complex*, and
183 *Citrobacter* spp. (Figure 1A).

184 At 1-month post-intervention, the Proteobacteria relative abundance decreased (Figure
185 1B), to a median relative abundance of 0.01% (range, 0% - 38%) in the MET-2 group and 2.2%
186 (range, 0.5% - 3.7%) in the FMT group. Between baseline and 1-month post-MET-2/FMT there
187 was a decrease in the relative abundances of Proteobacteria (Figure 1C), γ -Proteobacteria
188 (Supplementary Figure 1), and Enterobacteriaceae (Figure 1D) (MET-2; p-values = 0.0005,
189 FMT; p-values = 0.06) with a median \log_2 fold Proteobacteria decrease of 11.8 and 4.9,
190 respectively. The absolute γ -Proteobacteria abundance decreased between baseline and 1-month
191 post-MET-2 (p-value = 0.006) but did not decrease post-FMT (p-value = 0.62) (Figure 1E). One
192 participant (participant 10), failed initial MET-2 therapy for rCDI [19] and was observed in the
193 current study to have a baseline Proteobacteria relative abundance of 17% that increased to 38%
194 by approximately 1 month post-MET-2 administration.

195 As a comparator to non-microbial therapies, a total of 6 individuals with CDI treated with
196 vancomycin were identified from published datasets [29,30]. The baseline Proteobacteria
197 abundance was 36% (range, 0.6% - 81%) where the most abundant family was
198 Enterobacteriaceae (Supplementary Figure 2A). At 1 month post-vancomycin treatment,
199 Proteobacteria relative abundance was 38% (range, 14% - 78%) (Supplementary Figure 2B).
200 There was no observed decrease in Proteobacteria or Enterobacteriaceae relative abundance
201 between baseline and 1-month post-vancomycin stool samples (Supplementary Figure 2C & D).

202 **Antimicrobial resistance genes pre- and post-intervention**

203 Total ARGs were measured between baseline and 1-month post-intervention. The ARG
204 numbers at baseline were similar between interventions (Mann-Whitney p-value: 0.16), where
205 the median number of ARGs for participants who received MET-2 was 78 (range, 46 – 131) and
206 for participants who received FMT was 91 (range, 34 – 104). There was an observed decrease in

207 the total number of ARGs by 1 month after the MET-2 intervention, except for patient 10 where
208 the baseline ARG number was 90 and increased to 99 (Figure 2A). FMT was not associated with
209 a decrease in ARGs within 1-month of administration (Figure 2A). There was a strong positive
210 correlation (Spearman $r = 0.70$, $p < 0.0001$) between the total number of ARGs detected and
211 Proteobacteria relative abundance in the MET-2 interventional group (Supplementary Figure
212 3A), while there was a weak positive correlation (Spearman $r = 0.36$, $p = 0.31$) between the total
213 number of ARGs detected and Proteobacteria relative abundance in the FMT interventional
214 group (Supplementary Figure 3B).

215 The number of ARGs categorized by drug class-associated resistance for the baseline and
216 1-month post-MET2 or FMT interventions are shown in Figure 2B-H. At baseline in both
217 interventional groups, ARGs conferring resistance to fluoroquinolones (Figure 2E),
218 cephalosporins (Figure 2D), and tetracyclines (Figure 2H) were the most abundant. The number
219 of ARGs associated with resistance to the drug classes analyzed all decreased after MET-2
220 therapy with the exception of ARGs conferring resistance to glycopeptide antibiotics (Figure
221 2F). Clinically relevant vancomycin resistance genes (*vanA* or *vanB*) were not detected in 12/13
222 patients who received MET-2, while *vanA* was detected in patient 10 at baseline and at 1-month
223 post-MET-2 administration (Supplementary Figure 4A). The median *Enterococcus* relative
224 abundance at 1-month post-MET-2 administration was 0% (range, 0% - 21%), where patient 10
225 had an *Enterococcus* relative abundance of 21% suggesting that the increase in ARGs conferring
226 resistance to glycopeptides in the MET-2 group was not due to vancomycin-resistant
227 *Enterococcus* (Supplementary Figure 3B). FMT was not associated with a decrease in ARGs
228 associated with any drug class assessed (Figure 3B-H).

229 **Obligate anaerobes and butyrate-producers pre- and post-intervention**

230 Because of their significance in ARO colonisation resistance and intestinal epithelial
231 barrier and systemic immune function, we quantified obligate anaerobes and butyrate-producer
232 relative abundances. At baseline, the median obligate anaerobe relative abundances for the MET-
233 2 and FMT interventional groups were 5%; range, 0.5% - 47% and 20%; range, 1.1% - 45%,
234 respectively (Mann-Whitney p-value: 0.21). The median butyrate-producer relative abundances
235 for MET-2 and FMT interventional groups were 0.002%; range, 0% - 0.2% and 0.05%; range,
236 0% - 0.3%, respectively (Mann-Whitney p-value: 0.20). There was an observed increase in
237 obligate anaerobe (Figure 3A) (MET-2; p-value = 0.0005, FMT; p-value = 0.06) and butyrate-
238 producer (Figure 3B) (MET-2; p-value = 0.002, FMT; p-value = 0.06) relative abundances
239 between baseline and 1-month post-MET-2 and FMT.

240 **Microbiota response over time**

241 Proteobacteria, obligate anaerobe, and butyrate-producer abundances were plotted at
242 baseline, 0.5, 1, and 4 months or baseline, 1, 3, and 6 months post intervention for MET-2 (n =
243 15) and FMT (n = 5), respectively, to assess stability of the treatment-associated changes in
244 microbiome composition. The median Proteobacteria, obligate anaerobe, and butyrate-producer
245 relative abundances remained stable at the final sampling timepoints for both interventions
246 (Figure 4).

247 **Discussion**

248 In the current post-hoc analysis of adult participants with rCDI colonised with
249 Proteobacteria ($\geq 10\%$ relative abundance), we observed that MET-2 had similar effects to FMT
250 for decreasing gut microbiota Proteobacteria relative abundance and increasing obligate anaerobe
251 and butyrate-producer relative abundances by 1-month post-intervention. Absolute abundance of
252 γ -Proteobacteria, measured by qPCR, decreased in the MET-2 interventional group. We did not

253 observe a decrease in the absolute abundance of γ -Proteobacteria in the FMT group suggesting
254 that non-significant trend towards decreasing Proteobacteria relative abundance may not be due
255 to low sample size. Additionally, we observed that the decreased Proteobacteria and increased
256 obligate anaerobes and butyrate-producers observed at 1-month was similar to that at 4 and 6
257 months for MET-2 and FMT administration, respectively. Lastly, MET-2 and not FMT was
258 associated with decreases in ARG numbers in the gut microbiome by 1-month post-MET-2.

259 To our knowledge, this is the first study to assess the effects of a therapeutic microbial
260 consortium on Proteobacteria and ARGs in the gut microbiome of patients being treated for
261 rCDI. The results observed in the MET-2 interventional group is similar to other studies using
262 metagenomic sequencing to assess the effects of FMT on the gut microbiota composition of
263 patients with rCDI [38–40]. In a sub-study of an open-label, multicentre, clinical trial of
264 RBX2660, a liquid suspension of donor stool for rCDI treatment, Langdon and colleagues [40]
265 found that RBX2660 was associated with a decrease in Enterobacteriaceae by 2 months post-
266 therapy with a microbiota response that remained relatively stable until the final time point of
267 approximately 6 months.

268 Although MET-2 was associated with a large decrease in Proteobacteria relative
269 abundance (\log_2 fold decrease of 11.8 in the absence of rCDI recurrence), Proteobacteria relative
270 abundance decreased below 20–30% in both interventional groups by 1-month post-therapy.
271 Given that relative abundances of 20–30% have been previously associated with risks such as
272 bacteremia in allogeneic hematopoietic cell transplant patients [5] and patients in a long-term
273 acute care hospital [41], it is uncertain whether the larger decreases in Proteobacteria abundance
274 observed in the MET-2 recipients is associated with additional benefit over simply decreasing
275 relative abundance below this risk-associated threshold.

276 We observed that MET-2 was associated with a decrease in ARGs by 1-month which is
277 similar to published reports using FMT for this indication [38–40]. In our analysis, we did not
278 observe ARG decreases in the 5 patients with rCDI who received FMT. We did not sequence the
279 donor material from the FMT group, so could not ascertain if the ARGs are being introduced by
280 the FMT. However, in previous studies, even after extensive screening of donor stool, FMT
281 administration was the source of an antibiotic resistant *E. coli* bacteremia [17], while Leung and
282 colleagues [39] have reported that FMT may be a source of clinically-relevant ARGs.

283 Our study has multiple limitations, first this was a post-hoc analysis of two separate
284 cohorts that were designed to test the effects of MET-2 or FMT for rCDI treatment. It would be
285 inappropriate to make direct comparisons between the effects of MET-2 and FMT on our
286 measured outcomes, so we aimed to instead understand if MET-2 had similar effects to FMT.
287 Next, there is no placebo-control group to account for spontaneous decolonisation of
288 Proteobacteria and ARGs. However, our analyses of published datasets of patients with CDI pre-
289 and post-vancomycin therapy suggest that it is possible that Proteobacteria can remain colonized
290 or increase in some cases 1-month after vancomycin. Others have also reported persistence of
291 ARGs in the gut microbiome acquired post-antibiotic therapy for up to 2 years [42]. Our results
292 were of the metagenome only and did not include culture-based measurements of ARO
293 colonisation. Although, we observed a multiple-log decrease in Proteobacteria and ARGs, these
294 results may not be associated with complete decolonisation of the gastrointestinal tract or host.
295 Lastly, we did not link microbiome changes to any clinical outcomes. Interestingly, Ianiro and
296 colleagues [15] found that the incidence of bloodstream infection was lower in patients who
297 received FMT for rCDI compared to patients who received standard antibiotics, and our results
298 are consistent with putative mechanisms by which this may occur, through both decreasing

299 pathogen burden and increasing the number of anaerobes associated with colonisation and
300 infection-resistance.

301 In conclusion, MET-2 has similar effects to FMT for decreasing intestinal Proteobacteria
302 and ARGs, while increasing obligate anaerobes and beneficial butyrate-producers. Our results
303 observed in the MET-2 interventional group require validation in a large placebo-controlled
304 prospective trial that includes outcomes of clinical significance.

305

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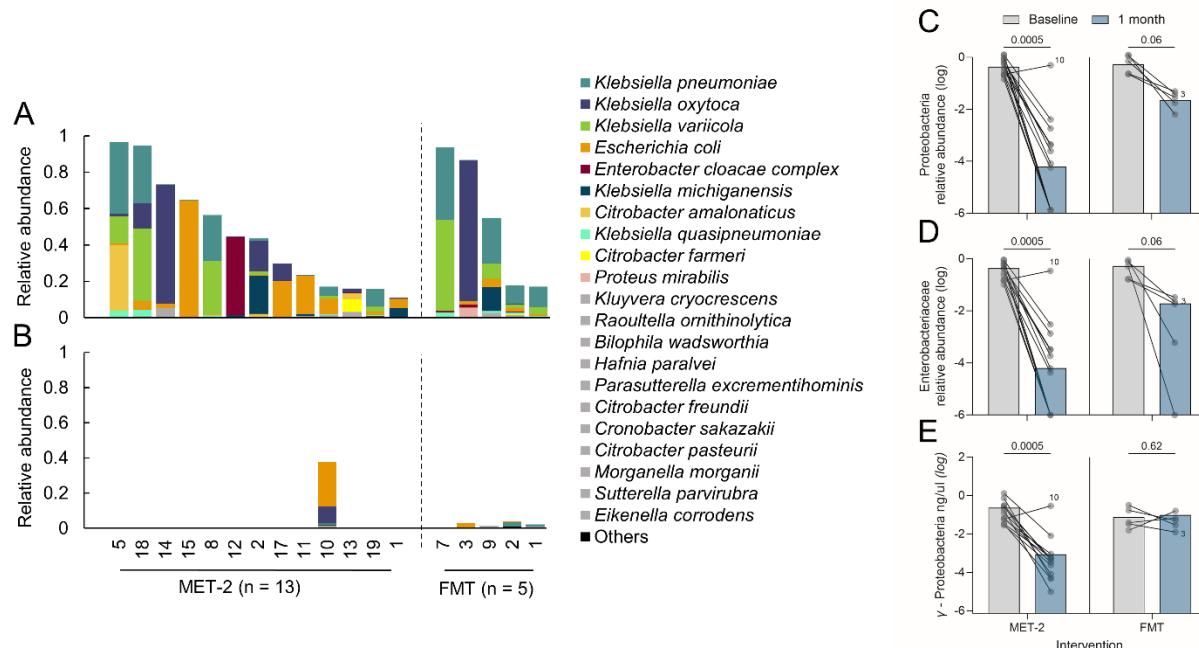
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447 **Figures**



448

449 **Figure 1.** Proteobacteria relative abundances in participants who received MET-2 (n = 13) or
450 FMT (n = 5). Proteobacteria are summarized at the species-level in the baseline (**A**) and 1-month
451 post-intervention stool samples (**B**). **A-B**, Species contributing <1% relative abundance in at least
452 one sample were aggregated as “Others”. Species contributing <5% relative abundance, but $\geq 1\%$
453 in at least one sample are in grey. **C**, The log-scale Proteobacteria relative abundance between
454 baseline and 1-month post-intervention. **D**, The log-scale Enterobacteriaceae relative abundance
455 between baseline and 1-month post-intervention. **E**, The log-scale γ -Proteobacteria absolute
456 abundance (ng/ μ l, limit of detection (log): -6.14 ng/ μ l) between baseline and 1-month post-
457 intervention. Dots represent individual patients with lines connecting the same patients measured
458 at different time points. Participant 10 and participant 3 are highlighted as individuals who failed
459 initial MET-2 or FMT therapy, respectively. Medians are plotted with p-values displayed above

460 each interventional group. Pairwise analysis performed using Wilcoxon matched-pairs signed
461 rank test.

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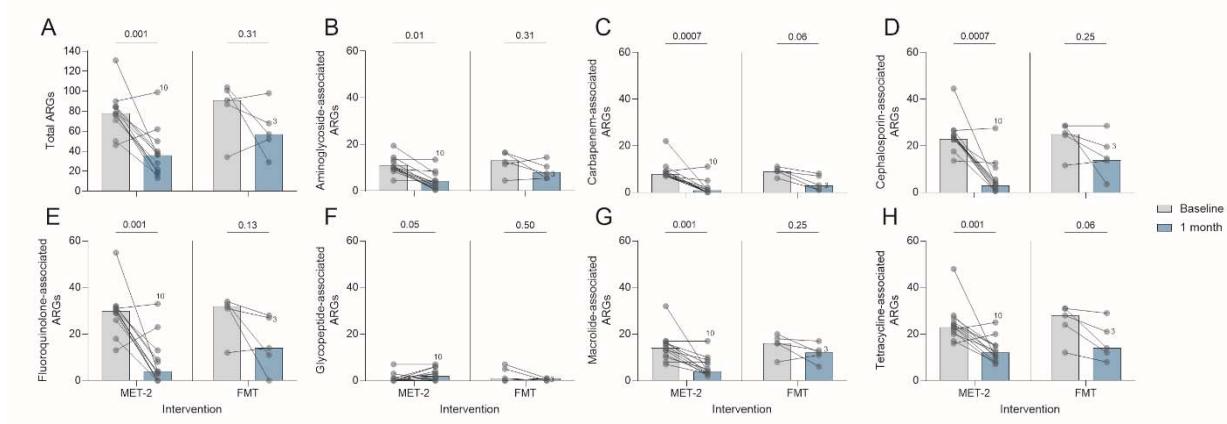
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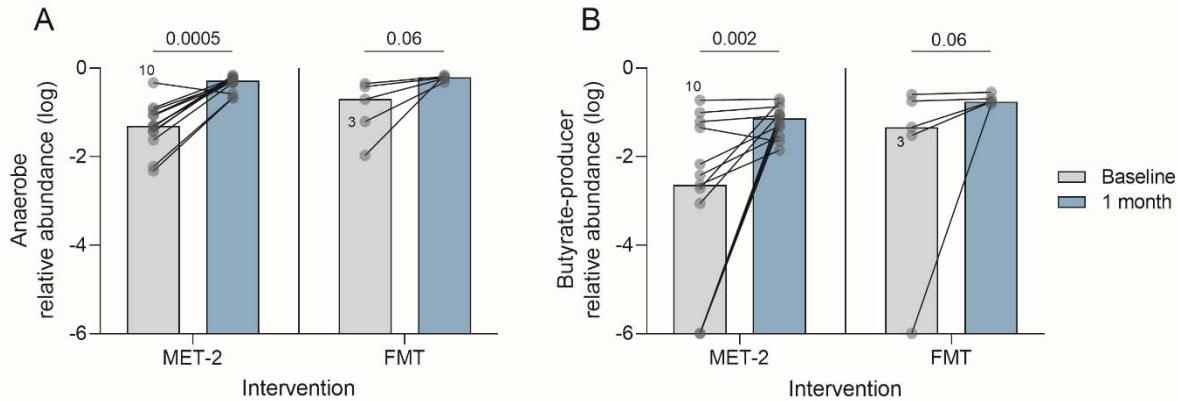
480 **Figure 2. A,** Antimicrobial resistance genes (ARGs) in participants who received MET-2 (n =
481 13) or FMT (n = 5) between baseline and 1-month post-intervention. **B-H,** ARGs categorized by
482 drug class-associated resistance in participants who received MET-2 (n = 13) or FMT (n = 5)
483 between baseline and 1-month post-intervention. Drug classes analyzed include aminoglycosides
484 (B), carbapenems (C), cephalosporins (D), fluoroquinolones (E), glycopeptides (F), macrolides
485 (G), and tetracyclines (H). Dots represent individual patients with lines connecting the same
486 patients measured at different time points. Participant 10 and participant 3 are highlighted as
487 individuals who failed initial MET-2 or FMT therapy, respectively. Medians are plotted with p-
488 values displayed above each interventional group. Pairwise analysis performed using Wilcoxon
489 matched-pairs signed rank test. and p-values are plotted. Each dot represents an individual with
490 the baseline and 1-month time points included.

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496 **Figure 3.** Obligate anaerobes (A) and butyrate-producer (B) log-scale relative abundances in
497 participants who received MET-2 (n = 13) or FMT (n = 5) between baseline and 1-month post-
498 intervention. Dots represent individual patients with lines connecting the same patients measured
499 at different time points. Participant 10 and participant 3 are highlighted as individuals who failed
500 initial MET-2 therapy. Medians are plotted with p-values displayed above each interventional
501 group. Pairwise analysis performed using Wilcoxon matched-pairs signed rank test.

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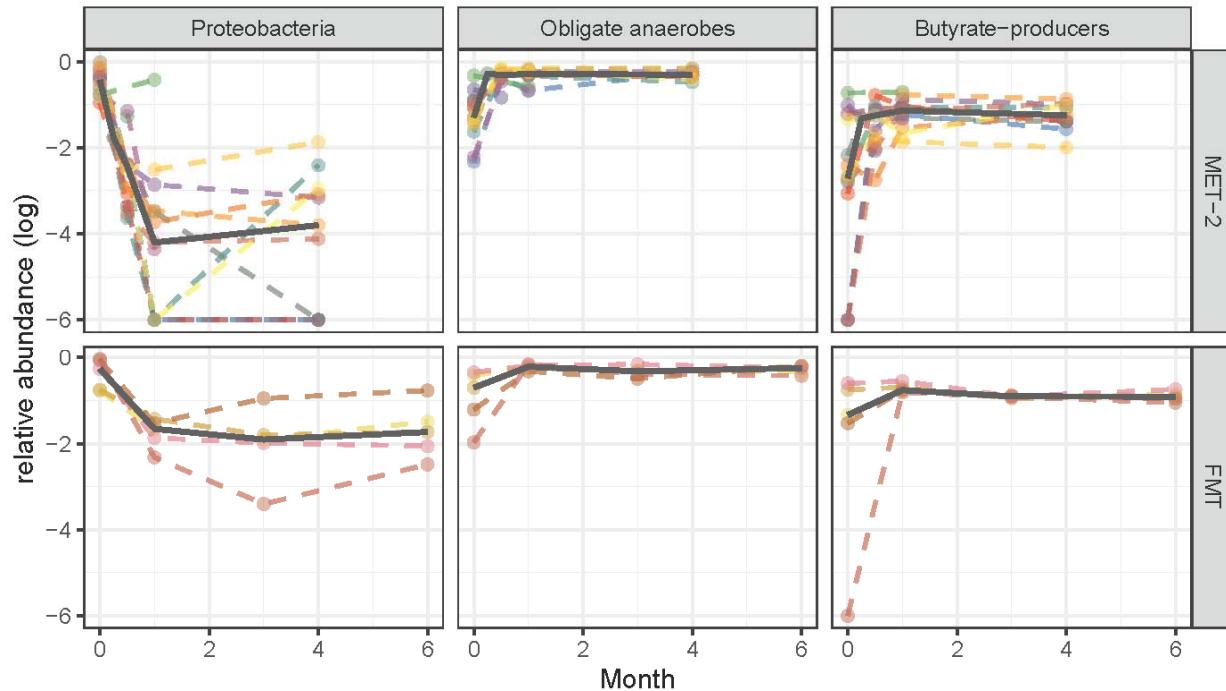
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511 **Figure 4.** Proteobacteria, obligate anaerobes, and butyrate-producer log-scale relative
512 abundances in participants who received MET-2 ($n = 15$) or FMT ($n = 5$) measured over time in
513 months. Dots represent individual patients with dashed lines connecting the same patients
514 measured at different time points. The solid line represents the median across time points.

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