An oral FMT capsule as efficient as an enema for microbiota reconstruction following disruption by antibiotics, as assessed in an in vitro human gut model.

Verdier Cécile
Universite Clermont Auvergne - Campus des Cezeaux
Denis Sylvain
Universite Clermont Auvergne
Gasc Cyrielle
Maat Pharma
Boucinha Lilia
Maat Pharma
Uriot Ophélie
Universite Clermont Auvergne
Delmas Dominique
Maat Pharma
Dore Joël
Institut Micalis
Le Camus Corentin
Maat Pharma
Schwintner Carole
Maat Pharma
Stephanie Blanquet-Doit (stephanie.blanquet@uca.fr)
https://orcid.org/0000-0003-0692-8726

Research

Keywords: gut microbiota, antibiotic, dysbiosis, FMT, enema, oral capsule, in vitro gut models

Posted Date: July 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-39859/v1

Abstract

Background

Fecal microbiota transfer (FMT) is an innovative treatment already successfully used in recurrent *Clostridioides difficile* infections. Researchers and clinicians are exploring its potential for treating other digestive or extra-intestinal pathologies where gut microbial dysbiosis has been evidenced, such as inflammatory bowel disease, obesity or cancers. Oral capsules were recently developed to address gaps of traditional routes of FMT such as enema or colonoscopy. Clinical studies are obviously the gold standard to evaluate FMT efficiency but remain limited by regulatory, ethics and cost constraints. The aim of the study was to use the *in vitro* human Artificial Colon ARCOL to compare the efficiency of two enema dosage (10 and 30 g) and a new oral caecum-release capsule in restoring gut microbiota composition and activity after treatment with ciprofloxacin, an antibiotic used in leukemia patients.

Results

By integrating the main physicochemical parameters of the human colon (pH, retention time, nutrient supply and anaerobiosis), ARCOL was shown to capture microbial diversity and inter-individual variability of stools from three healthy donors. Treatment with ciprofloxacin led to a state of marked dysbiosis with a sharp decrease in fermentation activities (production of gases and short chain fatty acids), a loss of microbial diversity and a shift in bacterial populations. All FMT treatments were able to speed-up the restoration of microbial profiles and functions, by decreasing the number of dysbiotic days from 12 to 7–8 depending on the FMT modes. Of note, the bacterial load showed no major influence on enema performance, and oral capsule was almost as efficient as enema even if the amount of administered bacteria was 100 times lower.

Conclusions

This study provides the first example of using an *in vitro* human colon model for evaluating autologous FMT efficiency and highlights the potential of oral capsule compared to a traditional enema formulation. This new mode of FMT administration combines efficiency with convenient and minimally invasive mode of administration. In accordance to 3R rules, gut models like ARCOL can be advantageously used to test FMT in preclinical phases as an alternative to *in vivo* assays.

Background

The digestive tract harbors the largest and most complex microbial community of the human body, the gut microbiota, which is mainly composed of thousands of bacterial species but also members of Archaea, Eukaryotes and viruses [1–3]. The highest bacterial density is found in the large intestine with up to 10^{12} cells per gram of intestinal content [2]. The gut microbiota plays a fundamental role for the

host under normal homeostatis, with an involvement in physiological, nutritional and immunological processes [4]. A large number of studies in animal models and humans have shown that a persistent imbalance in gut microbial community is associated with intestinal disorders such as inflammatory bowel diseases (IBD) or irritable bowel syndrome (IBS), or even extra-digestive pathologies like diabetes, obesity, cancer or neurological disorders. This alteration, named dysbiosis, has been described as coming with a loss of richness and diversity, a loss of keystone taxa, a shift in metabolic pathways and/or a bloom of pathobionts, like *Enterobacteriaceae* or *Clostridiaceae* [4, 5]. This raises a number of questions such as the cause and effect relationship between the disorder and the gut microbiota alteration, or whether a return to an equilibrium state would be sufficient to eradicate or alleviate symptoms, and/or how to return to a “healthy” state.

Fecal microbiota transfer (FMT) is the administration of fecal material from a healthy donor into the intestinal tract of a patient. FMT is performed in an attempt to restore an altered microbiome back to a healthy state and thus confer a health benefit. FMT was reported to be used in traditional Chinese medicine 1,700 years ago under the form of “yellow soup” to treat severe diarrhea [6, 7] and its first description in English literature was in 1958 to treat pseudomembranous colitis [8]. To date, FMT is a successful treatment option for patients with recurrent or refractory *Clostridioides difficil*e infection (rCDI), a major cause of antibiotic-associated diarrhea [9, 10]. However, FMT also has promising therapeutic value in several other disorders associated with gut microbial dysbiosis, such as IBD, IBS, cancer, acute myeloid leukemia, graft-versus-host disease, neurodegenerative disorders, autism, obesity and others [4, 10–12]. FMT can be divided into two types: autologous FMT (AFMT) using each patient’s own feces with stool collection before deleterious treatment, and allogenic FMT with the use of related or unrelated healthy donor stool [13, 14]. While AFMT has been effectively used in restoring intestinal microbiota composition disrupted by the use of antibiotics during allogeneic hematopoietic stem cell transplantation [15], allogenic FMT was found to be most efficient in case of rCDI [16]. Several routes of FMT administration are available, namely enema, colonoscopy, naso-gastric duodenal or jejunal infusion or oral capsule [6, 7, 17]. Oral capsules were recently developed to address gaps and limitations of the other modes of FMT administration. Oral capsules offer the least invasive, cheapest and most easily stored administered form, and eliminate several procedural risks encountered with the other ways of FMT treatment [14, 17]. In addition, Kao et al have shown that oral capsules are non-inferior to colonoscopy in rCDI treatment [18].

Of course, the gold standard to evaluate the effect of FMT treatment on gut microbial restoration and determine associated health benefits remains clinical studies. Nevertheless, this approach may be hampered by heavy regulations, ethical concerns and high experimental cost. As an alternative, FMT efficiency was evaluated in animal models, among them mainly conventional mice models but also human microbiota-associated or even DSS induced colitis mice models [19–22]. These models have been advantageously used to assess FMT effect after antibiotic treatment or chemotherapy [19], but also as an alternative strategy in IBD or management of metabolic disorders [20]. *In vivo* models present the great advantage to integrate host biological responses after FMT treatments, such as gain/loss of weight, inflammatory response, epithelial barrier integrity or even host cell receptor activation. However,

this *in vivo* approach in rodents remains limited by the high level of expertise needed to handle animals (especially human microbiota-associated mice) and the significant differences of both diet and digestive physiology, including gut microbiota, between animals and humans [23].

In line with the 3R rules aiming to minimize the number of animals used for research purpose and promote the development of alternative *in vitro* methods, we propose here for the first time the use of an *in vitro* model of the human colon as a relevant alternative to assess FMT treatments. In the present study, we used the ARtificial COLon (ARCOL) system. This *in vitro* model integrates the main physicochemical and microbial parameters of the human colonic environment: pH, temperature, retention time, supply of a nutritive medium reproducing the composition of ileal effluents, complex and metabolically active colonic microbiota and anaerobiosis maintained by the sole activity of this resident microbiota [24, 25]. The aim of this work was to use the ARCOL model to compare the efficiency of two enema dosage (10 g and 30 g) and a new AFMT capsule oral formulation to restore gut microbiota composition and activity after antibiotic (ATB) treatment with ciprofloxacin, an antibiotic used in leukemia patients.

Methods

Fecal sampling and FMT preparation

Fresh fecal samples were collected from three healthy adult human volunteers (a 27 years-old woman and two men aged 35 and 50 years) with no history of antibiotic treatment 3 months prior to the beginning of the study. Fecal samples were kept under anaerobiosis for a maximum of 6 h before treatment. Fecal inocula for the *in vitro* colon model were prepared using 55 g of fresh fecal samples under strict anaerobic conditions in a vinyl anaerobic chamber (Coy, Grass Lake, MI, USA). Stools were mixed with 500 mL of a 30 mM anaerobic sodium phosphate buffer (pH 6.5) supplemented with 1.9 mM cysteine and the suspension was filtered through a double layer of gauze. The fecal suspension was divided into 100 mL aliquots that were rapidly transferred into each of the five bioreactors simultaneously inoculated.

Filtrates of the same fecal suspensions were used by MaaT Pharma (Lyon, France) to prepare AFMT enema and capsules, according to its manufacturing operating system. FMT enema is a fecal-microbiota suspension for rectal administration stored in a special bag at -80°C (volume of 128-150 mL for 30 g enema and 43-50 mL for 10 g enema). 30 g and 10 g enema contain about 30×10^{11} and 10×10^{11} bacteria, respectively, with a bacterial viability superior to 50 %. FMT capsule (0.45 g) is a caecum-release capsule containing the freeze-dried form of the enema formulation. A capsule contains about 0.35×10^{10} bacteria, with a bacterial viability superior to 50 %. For FMT capsules, only the active ingredient (enema in its freeze-dried form) was introduced into the ARCOL model.

In vitro artificial colon system ARCOL

Human colonic conditions were simulated in the ARCOL model using MiniBio 500 mL my-Control bundles and Lucullus® Lite software from Applikon (Applikon, Delft, The Netherlands). Fermentations were conducted under continuous conditions. The *in vitro* system reproduces, based on *in vivo* data, the main physicochemical and microbial conditions encountered in a healthy human adult colon [24,26]. Briefly, at the beginning of the experiment, fecal suspension (100 mL) was added in the bioreactor containing 200 mL of nutritive medium while flushing with O_2-free N_2 gas. Afterwards, anaerobic conditions were maintained exclusively through the activity of the resident microbiota and by ensuring the airtightness of the system. Overproduced gas were collected in a gas sampling bag connected to the condenser. The temperature of the fermentation was fixed at 37°C and maintained inside the bioreactor using an incorporated panel heater. Colonic pH and redox potential were constantly recorded (Applikon, Delft, The Netherlands) and pH was adjusted to a value of 6.3 with an automatic addition of 2 M NaOH. The amount of NaOH consumed was recorded daily. After one day of batch fermentation, the nutritive medium containing various sources of carbohydrates, proteins, lipids, minerals and vitamins [27] to closely mimic the composition of human ileal effluents, was continuously introduced into the bioreactor at a speed of 0.21 mL/min. The fermentation medium was stirred at a constant speed of 400 rpm. Its volume was monitored using a level sensor and maintained at a constant value of 300 mL by automatic withdrawal of the fermentation medium, ensuring a mean retention time of 24 h.

Experimental design of *in vitro* fermentations

Five bioreactors running in parallel were inoculated with the fecal suspension from one donor (**Fig. 1**) and applied as follows: the first bioreactor was used as a control with no antibiotic treatment (control) while the second bioreactor was treated with ciprofloxacin (17850-5G-F, Sigma-Aldrich, Darmstadt, Germany), with an initial addition of 150 mg on day 6 followed by a continuous supply of 500 µg/mL in the nutritive medium up to day 12 (ATB control). The three other bioreactors were treated with ciprofloxacin as described for ATB control and then received: 27 mL of enema preparation at day 14 and day 15 (30 g enema), 9 mL of enema at day 14 and day 15 (10 g enema) or the content of 3 capsules per day for 7 consecutive days from day 14 to day 20 (capsule). The total amount of bacteria administered in the ARCOL model with FMT treatment is 1.2×10^{12}, 0.4×10^{12}, 1.47×10^{10} for 30 g enema, 10 g enema and capsule, respectively. The experiments were performed in triplicate (biological replicates named Run1, Run2 and Run3) with the fecal samples from each of the three healthy donors. During fermentations, samples were collected daily from the fermentative medium and the atmospheric phase for further analyses.

Antibiotic dosage

Ciprofloxacin concentrations in the fermentative medium were determined using a TurboFlow™ technology (TLX) coupled to Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) at the pharmacological and toxicological analytic unit (CREPTA) of Clermont-Ferrand university hospital.

Gut microbiota activity

Gas. Analysis of O_2, N_2, CO_2, CH_4 and H_2 found in the atmospheric phase of the bioreactor was performed using a 490 Micro-gas chromatograph (Agilent Technologies, USA) equipped with two columns, Molecular Sieve 5A and PoraPlot U, coupled with TCD detectors. Argon was used as the carrier gas. Gas composition was determined using calibration curves made from ambient air (78 % N_2, 21 % O_2, 0.04 % CO_2) and two gas mixtures A (5 % CO_2, 5 % H_2, 90 % N_2) and B (20 % CO_2, 80 % H_2). Results were expressed in relative percentages. The total volume of gases overproduced per day (in mL) was also measured by connecting a gas bag to the bioreactors.

Short chain fatty acids (SCFAs). Samples collected from the fermentative medium were centrifuged at 18,000 g for 15 min at 4°C and supernatants were filtered (0.45 µm). Concentrations of the three main SCFAs (acetate, propionate and butyrate) were determined using high performance liquid chromatography (HPLC) (Elite LaChrom, Merck HITACHI, USA) coupled with a diode-array detector. The HPLC column (ICSep ICE-COREGEL 87H3 9 µm 150 x 7.8 mm, Concise Separations, USA) and its guard column were maintained at 50°C. Sulfuric acid 0.008 N was used as mobile phase and SCFAs were separated at a flow rate of 0.6 mL/min. Data was analyzed by the EZChrom Elite software at 205 nm. SCFAs concentrations (expressed in mM or relative percentages) were calculated from calibration curves established with known concentrations of acetate, propionate and butyrate (0, 10, 25 and 40 mM).

Gut microbiota composition

Flow cytometry analysis. Concentrations of viable bacteria in the fermentation medium were determined by a live/dead analysis with flow cytometry. Samples were 10-fold diluted in sterile physiological water to reach 10^{-4} dilution factor. Bacteria were double-stained with the green-fluorescent DNA SYTO 9 dye labelling all bacteria and the red-fluorescent Propidium Iodide (PI) dye only penetrating and staining cells with damaged membranes (LIVE/DEAD™ BacLight™ Bacterial Viability and Counting Kit, for flow cytometry, Molecular probes, Whaltham, MA). Bacterial suspensions were thus incubated for 15 min at room temperature in the dark with 3.3 mM SYTO 9 and 0.375 mM PI and transferred into BD Trucount™ Tubes (BD Biosciences, San Jose, CA). Flow cytometry analysis was performed on a BD™ LSR II cytometer (BD Biosciences, San Jose, CA) and data were collected with BD FACSDiva™ software. Gating on forward-angle light scatter/side-angle light scatter was used in order to differentiate bacteria from the background. Then, the combined red and green fluorescence dot-plots were used to distinguish among the various subpopulations. Results were expressed as viable cells per mL of fermentative medium.

qPCR analysis. Total DNA was extracted from fermentative medium using the SmartExtract-DNA Extraction Kit (Eurogentec, Seraing, Belgium), according to the manufacturer's instructions. DNA quantity was evaluated with a NanoDrop™ 2000 (Thermo Scientific, Wilmington, Delaware USA). Samples were stored at -20°C prior to analysis. Total bacteria concentration was quantified by qPCR analysis performed on a Stratagene Mx3005P apparatus (Agilent, Waldbronn, Germany), using the Takyon Low ROX SYBR 2X MasterMix blue dTTP kit (Eurogentec, Seraing, Belgium). Each reaction was run in duplicate in a final volume of 10 µL with 5 µL of Master Mix, 0.45 µL of each primer (10 µM), 1 µL of DNA sample (10 ng/µL), and 3.1 µL of ultra-pure water. The following primers targeting 16S rRNA gene were used: BAC338R,

5’- ACTCCTACGGGAGGCAG-3’ and BAC516F, 5’-GTATTACCGCGGCTGCTG-3’, as described by Yu and colleagues [28]. Amplifications conditions consisted of 1 cycle at 95°C for 5 min followed by 40 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s. A final cycle of 5 min at 95°C was included. Standard curves were generated from genomic DNA extracted from ARCOL samples, standardized to 10 ng/µL and serially diluted from 10^{10} to 10^{0} gene copy/mL. Final results were expressed as copy/mL.

16S rRNA gene sequencing and bioinformatics analysis. Genomic DNA was extracted using the NucleoSpin Soil kit (Machery Nagel) and samples stored at -20°C before analysis. 16S rRNA gene sequencing was performed by Eurofins Genomics (Ebersberg, Germany). A sequencing library targeting the V3-V4 region of the 16S rRNA gene was constructed for each sample using the MyTaq HS-Mix 2X, Bioline, according to the manufacturer’s instructions. Libraries were then pooled in an equimolar mixture and sequenced in paired-end (2 x 300 bp) MiSeq V3 runs, Illumina. After amplicon merging using FLASH [29] and quality filtering using Trimmomatic [30], host sequence decontamination was performed with Bowtie2 [31]. Operational Taxonomic Unit (OTU) sequence clustering was performed with an identity threshold of 97 % using VSEARCH [32] and taxonomic profiling was then performed with the Silva SSU database Release 128 [33]. Taxonomic and diversity analyses were performed with R Statistical Software (R Core Team 2015, version 3.4.4) [34] using vegan and phyloseq packages. For fair comparison, the sequence number of each sample was randomly normalized to the same sequencing depth i.e. 50,000 amplicons per sample and normalized by total bacteria count based on qPCR results. Diversity measures correspond to the median value of 20 subsamplings per sample.

Dysbiosis criteria

Criteria selected to determine microbial dysbiosis periods in the ARCOL system were based on modifications of both gut microbiota activity and gut microbiota composition compared to stabilized conditions. For microbiota activity, the following parameters were selected: redox potential values, NaOH consumption, total gas production, CO_2 concentration, and SCFA concentrations. Regarding microbiota composition, the selected parameters were the following: total viable bacteria as determined by flow cytometry, total bacterial populations measured by qPCR, richness, Shannon and Bray Curtis indexes. In order to establish a dysbiotic period, each day of fermentation from day 6 for a treated bioreactor was compared to day 6 (corresponding to the end of stabilization phase) of the same bioreactor for all 16S rRNA gene analysis-related criteria (abundance and diversity indexes). For all the other criteria, each day of fermentation from day 6 for a treated bioreactor was compared to the same day of the control bioreactor. All the selected criteria and threshold values are summarized in **Table 1**.

Statistical analysis

Data were analyzed using a one-way repeated measure analysis of variance (ANOVA) followed by a Newman-Keuls multiple comparisons test. The statistical analysis was performed using GraphPad Prism software 8.0 (GraphPad Software, Inc., San Diego, CA). Results were expressed as means ± SEM (n=3). Differences were considered statistically significant when $P < 0.05$.

Results

Monitoring of in vitro **fermentations**

NaOH consumption. Microbial fermentation activities lead to organic acid production such as SCFA and a subsequent pH decrease in the bioreactor, resulting in NaOH consumption to maintain the pH at the setpoint value. Whatever the bioreactor, NaOH consumption was stable before ATB treatment (data not shown). Addition of ciprofloxacin led to an immediate interruption in NaOH consumption that persisted 3 to 4 days after the end of ATB treatment, depending on the runs. FMT treatments led to an earlier restart of NaOH consumption compared to ATB control, two days before for 30 g and 10 g enema and only one day before for oral capsule (in two out of the three replicates, Run3 being similar to ATB control).

Redox potential. Redox potential was also followed as an indicator of fermentation activities and anaerobiosis. Before ATB treatment, redox potential was stabilized around − 400 mV in all bioreactors (data not shown). Addition of ciprofloxacin led to an immediate change with a sharp increase in redox values (up to 0 mV). At the end of ATB treatment, redox potential slowly decreased to reach baseline values of the stabilization phase within 3 to 8 days depending on replicates. Enema treatment (both 30 g and 10 g) allowed an earlier return to baseline values (except for Run3), four days before ATB control in Run1 and two days before in Run2. For capsule, the effect was also donor dependent, with an earlier return to baseline in Run1 (four days before ATB control), no effect in Run2, and a slower return to stabilized state compared to ATB control in Run3.

Gas production

At the end of stabilization phase (day 6), gas composition of the atmospheric phase was the same in all tested conditions with approximately 95% of CO_2, 4% N_2, 1% H_2 and less than 1% O_2 (Fig. 2a to 2e). This confirms the ability of maintaining anaerobiosis inside bioreactors without flushing with CO_2 or N_2 during fermentation. These relative percentages remained constant throughout control experiments (Fig. 2a). As for NaOH consumption, addition of ciprofloxacin led to an immediate stop in gas overproduction that persisted four days after the end of ATB treatment (data not shown). This resulted in negative pressure in fermenters that required N_2 injection into bioreactors. ATB treatment was also associated with a change in gas composition (Fig. 2b to 2e). A sharp increase in relative percentages of N_2 was observed due to flushing (from 55 to 80%), as well as a lower but clear increase in H_2 (1–20%) and O_2 (5–10%) that cannot be linked to any gas leak. Consequently, CO_2 relative percentages decreased to 15–20% (Fig. 2b to 2e). For ATB control, a return to baseline profiles was seen only 10 days after the end of ciprofloxacin treatment, i.e. at day 22 (Fig. 2b). When FMT treatments were applied (Fig. 2c to 2e), gas production restarted faster (1 or 2 days before ATB control) for all modes of administration, except for oral capsule in Run3 (data not shown). Recovery of stabilized profiles (similar to that observed at day 6) was also obtained earlier for 30 g and 10 g enema treatment (day 19) or capsule (day 21) compared to ATB control (day 22).

SCFA production

Whatever the fermentations, SCFA profiles were stabilized at day 6 with relative percentages around 60%, 25% and 15% for acetate, propionate and butyrate, respectively (Fig. 2f to 2j), and a total concentration of 130–140 mM (Fig. 2k). In control experiment, these percentages remained similar throughout fermentation (Fig. 2f). Addition of ciprofloxacin induced a significant decrease ($P < 0.05$) in total SCFA concentrations to reach around 50 mM at the end of ATB treatment (Fig. 2k). ATB treatment also induced changes in SCFAs profiles (Fig. 2g to 2j), with a high increase in relative percentages of propionate (80–95% at day 12), associated with a decrease in acetate (5–15%) and butyrate (0–5%). These changes persisted at the end of ATB treatment since total SCFA concentrations returned to baseline value within 8 days (day 20, Fig. 2k) and two additional days (day 22) were needed to recover SCFA proportions similar to those observed at the end of stabilization (Fig. 2g). FMT treatment led to a sharp increase in total SCFA concentrations at day 15 for both enema treatment (around 280 mM), i.e. just after the first injection (Fig. 2k). High variability can be explained by a donor-dependent response, with a peak at day 15 for Run1, 4 to 5 times higher than for Run2 and Run3 (data not shown). Similarly, but to a lesser extent, capsule FMT led to an increase in total SCFA concentrations to reach a maximum of 150 mM at day 17 (Fig. 2k). All FMT treatments led to a clear reduction in the time needed to return to stabilized state (2–4 days before ATB control), with both total SCFA concentrations (Fig. 2k) and proportions (Fig. 2h to 2j) similar to baseline values at day 18. Of note, for oral capsule, stabilization of total SCFA concentrations occurred at a lower level (100 mM vs 130–140 mM).

Quantification of total bacteria

In control experiments, total bacteria stabilized around $6–7 \times 10^9$ 16S rRNA gene copies/mL, as determined by qPCR analysis (Fig. 3a to 3d). Microbial populations were mostly composed of viable cells since a similar population level was obtained by flow cytometry (Fig. 3e to 3 h). Total bacteria number and viable bacteria amount were both significantly impacted by ATB treatment with a regular decrease until day 12 in most bioreactors (up to 3–4 logs by qPCR and 2–3 logs by flow cytometry). Nevertheless, the impact of ciprofloxacin was not similar in all the experiments, with less impact on total and viable bacteria in Run2 for enema 30 g and capsule conditions (Fig. 3b and 3f). In average, return to baseline values for ATB control occurred progressively within 6 and 7 days after the end of ciprofloxacin administration, for total and viable bacteria, respectively (Fig. 3d and 3 h). Also, all FMT treatments allowed a return of viable bacteria concentrations to stabilized values 3 days before ATB control ($P < 0.05$, Fig. 3h). Nevertheless, a high variability was observed between the three replicates, especially for 30 g enema and capsule conditions. Notably, time to recover initial levels in Run3 was similar for FMT capsule compared to ATB control (Fig. 3c and 3 g).

Gut microbiota structure

Composition of fecal inoculum and after stabilization in ARCOL. Sequencing analysis of initial fecal suspensions at phylum level indicated that donor 1 and donor 2 (**Suppl. Figure 1, D0**, Run 1 and 2)

showed microbial profiles mainly composed of *Firmicutes* (70% and 65%, respectively) and *Bacteroidetes* (30% and 35%, respectively). Donor 3 (**Suppl. Figure 1 1c, D0**, Run 3) had a reverse *Firmicutes*/ *Bacteroidetes* ratio (35% / 60%), with a higher prevalence of *Proteobacteria* (3%). At family level, dominant taxa of fecal suspensions were as follows: *Ruminococcaceae* (40%), *Bacteroidaceae* (25%), *Lachnospiraceae* (20%) and *Veillonellaceae* (7%) for donor 1 (Fig. 4, Run 1); *Ruminococcaceae* (30%), *Prevotellaceae* (20%), *Lachnospiraceae* (20%), *Bacteroidaceae* (10%) and *Veillonellaceae* (10%) for donor 2 (Fig. 4, Run 2); and *Bacteroidaceae* (50%), *Ruminococcaceae* (25%), *Lachnospiraceae* (10%) and *Rikenellaceae* (6%) for donor 3 (Fig. 4, Run 3).

At the end of stabilization phase in ARCOL (**Suppl. Figure 1**, day 6), phyla profiles were quite similar for all runs with a large majority of *Bacteroidetes* (60–70%), followed by *Firmicutes* (20–40%) and *Proteobacteria* (1–3%). At family level (Fig. 4), profiles at day 6 were run-dependent, with close composition for Run1 and Run3, and clearly different profiles for Run2. Run1 and Run3 showed a high abundance of *Bacteroidaceae* (70–80% and 65–75%, respectively), followed by *Ruminococcaceae* (6% and 15%, respectively) and *Lachnospiraceae* (5% and 10%, respectively). Run1 also presented 4% of *Veillonellaceae.* For Run2, profiles are composed of stable abundances of *Ruminococcaceae* (30–40%), *Lachnospiraceae* (10–15%) and *Veillonellaceae* (1–3%), while *Prevotellaceae* and *Bacteroidaceae* were more variable between conditions (from 2 to 40%). Stabilized profiles remain almost constant for all control experiments during the 28 days fermentation (Fig. 4).

Impact of ATB treatment. Addition of ciprofloxacin led to marked changes in microbial profiles even at phylum level. For Run1 and Run3, at phylum level, main changes were characterized by an increase in *Firmicutes* abundance and a disappearance of *Proteobacteria* (**Suppl. Figure 1**). In Run1 only, *Verrucomicrobia* (up to 3%) appeared during ATB treatment. For Run2, opposite trends were observed with a decrease in *Firmicutes* abundance associated with a sharp increase in *Proteobacteria* (up to 90% in ATB control). At family level, ATB treatment had also a strong impact on microbial structure, with variations between runs and even between bioreactors for a single run. Main changes for Run1 were an increase in *Ruminococcaceae* and *Lachnospiraceae* together with a loss of *Veillonellaceae* and *Porphyromonodaceae* (Fig. 4). For Run2, *Prevotellaceae* and *Alcaligenaceae* abundances were mostly increased while those of *Bacteroidaceae* and *Porphyromonodaceae* declined. Lastly for Run3, according to bioreactors, main variations observed were a bloom of one or several families among the *Enterococcaceae, Planococcaceae, Clostridiaceae* or *Lachnospiraceae*. Perturbations of microbial profiles persisted after cessation of ATB treatment in control experiments (ATB control). Stabilization occurred only around day 25–27 but with different profiles compared to that of day 6, both at phylum (**Suppl. Figure 1**) and family (Fig. 4) levels.

Effect of FMT treatments. All FMT treatments induced a rapid clear shift (from day 15) in microbial profiles both at phylum (**Suppl. Figure 1**) and family levels (Fig. 4). Microbial abundances continued to evolve after this initial shift up to 5–8 days after the first FMT administration, to reach a new stable profile close to that observed at the end of stabilization phase. Of note, some taxa, such as *Veillonellaceae* in all runs, *Bacteroidales* S24-7 group in Run2 and *Alcaligenaceae* and

Porphyromonodaceae in Run3, which disappeared during ATB treatment reappeared only in FMT-treated bioreactors (but not in ATB control). On the contrary, some families found in control experiments, such as *Prevotellaceae* in Run3, were no more present after ATB treatment even in FMT-treated bioreactors (Fig. 4). Of interest, in Run2, *Prevotella 7*, the main genus from *Prevotellaceae* found at the end of stabilization phase, was replaced by *Paraprevotella* during ATB treatment, which disappeared again in favor of *Prevotella 7* when capsule and 30 g enema treatments were applied (data not shown).

Microbial richness and diversity

α-diversity. α-diversity was first evaluated by calculating sample richness at OTU level, i.e. the number of different OTUs reflecting species in a sample (Fig. 5, **panel A**). In control experiments, for the three donors (Run1, Run2 and Run3), richness stabilized around 150–200 OTUs all along fermentations. Administration of ciprofloxacin led to a rapid and pronounced decrease of richness index to reach less than 10 OTUs at the end of ATB treatment. At the end of ciprofloxacin injection in ATB control experiments, richness increased to stabilize at day 18–20 at lower values compared to control assays (around 80–100 OTUs). Of interest, FMT treatment allowed a return of richness values to baseline levels within 6–8 days after the end of ATB treatment. Shannon index was also calculated to get a better view of diversity and species distribution in the different samples (**Suppl. Figure 2**). In control experiments, Shannon index stabilized around 2–3 from day 6 to day 28. ATB treatment was associated to a sharp decrease of Shannon index down to 1. For ATB control, Shannon index regularly increased to reach baseline levels the last 2 days of experiments, except for Run3 where stabilized values remained lower than these of day 6 (around 2 vs 3). When FMT treatments were applied, Shannon index recovered baseline values within 2 to 6 days after the first administration for enema formulae and from 6 days for oral capsules.

β-diversity. Bray-Curtis similarity was calculated as a measure of β-diversity compared to day 6 values (end of stabilization phase for each bioreactor). Results obtained for the three replicates Run1, Run2 and Run3 are presented in Fig. 5 **panel B**. Whatever the replicates, results showed similar trends. For control experiments, Bray-Curtis similarity index versus day 6 remained stable during fermentations (except for Run3 where a slight decrease was observed). When no FMT treatment followed ciprofloxacin administration (ATB control), a clear shift from control experiments was observed with much lower values (from 0.2–0.3). Nonetheless, whatever the replicates (Run1, Run2 or Run3) FMT treatment induced a clear restoration of diversity since all the samples progressively went back to control values, except for 10 g enema and oral capsule in Run2 (indexes around 0.5 at the end of fermentations). For all other conditions, return to baseline values was observed between day 18 and day 20 for all FMT modes of administration.

Determination of dysbiotic periods

In order to assess FMT efficiency in restoring gut microbiota composition and activity, and the effect of mode of administration, the number of “dysbiotic days” was determined. This number of “dysbiotic days” was calculated for each variable listed in Table 1 and under each tested condition (ATB control, 30 g

enema, 10 g enema and capsule). Results were averaged for the three replicates (**Suppl. Table 1**) and graphically represented (Fig. 6). These results confirmed that ATB control experiments showed the highest number of dysbiotic days with an average of 12.1 days (**Suppl. Table 1**). FMT treatment clearly reduced dysbiotic periods with a similar 7.6 days for 30 g enema and 10 g enema and a non-significant higher value for oral capsules with 8.3 days. When further analyzing results at microbiota structure and activity levels, differential situations were observed depending on FMT mode of administration. Regarding microbial activity, capsule was the less efficient technique with 9.4 days followed by 10 g enema (8.7 days) and 30 g enema which showed the best score (7.7 days). This difference was mainly due to a high number of dysbiotic days for capsule in relation with acetate production (Fig. 6). Regarding microbial structure, different ranking was observed with 30 g enema being the less efficient (7.6 days), followed by capsule (6.9 days) and 10 g enema (6.1 days). The lower efficiency of 30 g enema resulted from a higher number of dysbiotic days in relation with microbial diversity indexes.

Discussion

In this study, we used for the first time an *in vitro* model of the human colon ARCOL to assess the efficiency of different modes of FMT treatments in restoring gut microbiota structure and metabolic activity after disruption by an antibiotic treatment. Addition of ciprofloxacin clearly induced *in vitro* a dysbiotic state of gut microbiota that can be efficiently reversed by all tested formulations, i.e. 30 g enema, 10 g enema and oral capsule.

FMT is currently used in patients as an effective therapeutic option for rCDI, but also has important potential in other intestinal and extra-intestinal diseases associated with gut microbial dysbiosis, including cancers and cancer therapies. FMT was considered for cancer management due to its beneficial effects on intestinal microbiota reconstruction, amelioration of bile acid metabolism, and modulation of immunotherapy efficacy [35]. Especially, patients suffering from acute myeloid leukemia and receiving chemotherapy are also often treated with antibiotics. This results in even more impaired intestinal microbiota, therefore increasing risk for infectious complications [36]. AFMT treatment was considered to limit the risk of such complications by promoting a return to non-disturbed intestinal microbiota. Nevertheless, there is limited clinical evidence to date on FMT efficiency in patients suffering from ALM and treated with antibiotics [37].

In the present study, we used for the first time an *in vitro* model of the human colon to test AFMT after antibiotic disturbance. The colon model used is the ARCOL system, set-up to mimic, based on *in vivo* data, not only physicochemical parameters of the adult human colon but also the microbial component [26, 27]. In order to reproduce antibiotic-induced microbial dysbiosis, ARCOL was treated with ciprofloxacin, a drug used in hospitals in acute myeloid leukemia patients. This antibiotic was also selected due to associated *C. difficile* infections and available information on its pharmacokinetic in humans, especially on its fecal excretion [38]. The dose (500 µg/mL) and the mode of administration (first initial input followed by a continuous supply during 7 days) were established considering hospital practices, ciprofloxacin metabolism (percentage of absorption in the human upper gut and estimated

antibiotic clearance in feces) and *in vitro* colon parameters (total volume and retention time). Ciprofloxacin concentrations were checked by antibiotic dosage throughout ARCOL experiments (500 µg/mL, data not shown). Our results clearly indicated that ciprofloxacin induced rapid changes in microbial composition and activity that intensified during antibiotic administration. These modifications clearly included a reduction of overall microbial richness and diversity and a loss in microbial fermentation capacities. In addition, in some replicates, a rise in dominance of bacterial species usually subdominant, including pathobionts such as *Enterobacteriaceae* and some *Clostridiaceae*, was observed. All these changes are indicative of a state of gut microbiota dysbiosis [4, 5]. Of note, at family level, effects of ciprofloxacin were widely donor-dependent in ARCOL, as previously observed in humans and mice [39]. Furthermore, in order to quantify this dysbiosis state in ARCOL, we calculated a dysbiosis index based on total number of dysbiotic days in bioreactors. To take into account all aspects related to dysbiosis definition [4, 5], this index integrates criteria related to both microbial structure (bacterial population relative abundances, microbial richness and diversity) and functions (related to main fermentation products such as gases and SCFAs).

Once the dysbiotic *in vitro* colon model was established, we evaluated the ability of various FMT formulations to restore gut microbial balance in ARCOL. Three different forms were tested, i.e. two dosages of enema and one oral capsule. Enema was until 1990 the method of choice for FMT and still remains frequently used at the hospital [14]. Enema is less invasive, easier to perform, and relatively less expensive than colonoscopy or upper gastro-intestinal routes. Two doses were tested, 30 g enema which is commonly used in clinical practices and 10 g enema to comparatively assess the efficiency of a lower microbial quantity. The effect of these two formulations was compared to that of a new caecum-releasing capsule containing a freeze-dried form of the enema formulation. Oral capsule is the most recently developed mode of stool delivery, the first formulation being described in 2014 [40, 41]. Capsules which are esthetically pleasing, convenient and minimally invasive are preferred by patients, and present the benefit to be the cheapest FMT mode of administration [18, 40, 41]. Enemas were directly introduced into the ARCOL model since in humans they are delivered to the distal colon using a canula. The oral capsule tested was specifically designed to disrupt only at the end of human small intestine. In a preliminary study, we checked the integrity of capsules in ileal effluents of the human TNO gastrointestinal TIM system [24, 27] (data not shown). As they remain intact up to the end of small intestine *in vitro*, capsules content was directly added as a suspension in this work to the ARCOL system.

Our data revealed that all tested forms were able to speed up return to a stable state compared to ATB control, thereby reinforcing the resilience of the ecosystem. FMT treatments had an immediate impact on gut microbiota structure while there was a lag for recovery of a stable microbial activity. This was due to necessary adaptation of newly added bacteria. Interestingly, the lowest enema dosage (10 g) was as efficient as the highest one (30 g) and reduced in a similar way the number of dysbiotic days (− 4.5 days). To our knowledge, no previous study had already investigated a dose effect for FMT enema. These *in vitro* results suggest that bacterial load may be reduced without any deleterious effect while restoring microbial balance after antibiotic-induced dysbiosis. The oral capsule proved almost as efficient as enema forms (− 3.8 dysbiotic days), even if the amount of administered bacteria was 100 times lower

and required a longer administration period (1 week compared to two days). In previous studies, two approaches were mainly developed for FMT oral capsules, first freezing at -80 °C with glycerol, then more recently freeze-drying with various cryoprotectants, as performed in the present study [41–43]. Interestingly, Jiang and colleagues (2017)[42] showed in mice that there was no difference in efficiency of frozen and lyophilized capsules in rCDI treatment, and that products can be stored up to 7 months without losing microbiota composition and therapeutic efficacy. Oral capsules were also designed for delivery in various luminal segments of the gastro-intestinal tract. A comparative study between two capsules preparations, with either gastric or colonic delivery, showed that the colonic-release form tended to be the most effective in case of rCDI, particularly in restoring Bacteroidetes phylum and increasing gut microbial diversity [41]. This is in accordance with the strategy favored in this study testing a caecum-release oral formulation. To date, few studies in humans have already compared the efficiency of oral capsules (frozen or lyophilized) for rCDI treatment to more traditional FMT routes such as colonoscopy or enema. In line with our results, available data indicates that oral capsules are as effective as traditional modes of administration, especially to restore bacterial diversity [18, 43]. Lastly, Allegretti and colleagues (2019)[41] have shown in humans that the lower dose treatment with colonic release capsules (10 capsules in a single administration) was equally effective to cure rCDI as the higher treatment-dose (30 capsules). This is fully in accordance with our results on enema forms.

This study has revealed the potential of *in vitro* gut models for the assessment of efficacy of various formulations for FMT treatments. Such approach appears as a relevant alternative to *in vivo* assays in preclinical phases and *in vitro* results could appropriately document technical files ahead of clinical trials. Although they document very well the ecological dynamics in terms of structure and function, these models are hindered by their inability to fully reproduce the overall processes occurring *in vivo*, particularly hormonal and nervous control, feedback mechanisms, local immune system and host-bacterial mutualism. Particularly, patients receiving FMT are often subjected to diverse stresses that cannot be integrated *in vitro* but may widely influence gut physiology and microbiota restoration. Yet for obvious regulatory, ethics and cost reasons, *in vitro* colon models are advantageous over *in vivo* assays due to their flexibility, accuracy and reproducibility [44]. As a stable microbiome can be maintained in bioreactors over a long timeframe, the effect of successive treatments can also be tested. In addition, gut models can be adapted to perform colon-segment specific research [45, 46] and several bioreactors can be inoculated with the same fecal sample to perform parallel control and treatments. Lastly, inter-individual gut microbiota variability can also be taken into account by performing replicates with fecal samples from different donors. In the present study, ARCOL was inoculated with stools from healthy adult donors, but we can easily imagine to extend its potentialities in FMT assessment by using fecal samples from selected age groups, such as infants [47–49] or elderly subjects [50, 51], or from patients suffering from cancers, IBD or metabolic disorders [52, 53].

Conclusion

This study provides the first example of application of a human colon *in vitro* model for evaluating FMT efficiency. The three formulations tested, i.e. 30 g enema, 10 g enema and a new caecum-release oral

capsule, showed similar effects by clearly reducing time to restore gut microbiota structure and activity. Due to regulatory, ethic, cost and technical advantages, *in vitro* gut models such as ARCOL can be advantageously used in preclinical phases as an alternative to *in vivo* assays in animals. Their potential may be extended to evaluate efficiency of different formulations for both AFMT and allogenic FMT, when targeting various age groups and intestinal or extra-digestive pathologies.

Abbreviations

AFMT
Autologous fecal microbiota transfer
ARCOL
Artificial colon
ATB
Antibiotic
FC
Flow cytometry
FMT
Fecal microbiota transfer
HPLC
High performance liquid chromatography
IBD
Inflammatory bowel diseases
IBS
Irritable bowel syndrome
rCDI
refractory *Clostridioides difficile* infection
SCFA
Short chain fatty acids

Declarations

Availability of data and materials

All sequencing data were deposited to the National Center for Biotechnology Information Sequence Read Archive under accession number PRJNA642894.

Ethics approval

This study being a non-interventional study with no addition to usual clinical care, according to the French Health Public Law (CSP Art L 1121 – 1.1), the protocol does not require approval from an ethic committee.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funding by Maat Pharma company.

Contributions

CV and SD did laboratory work, performed sampling and analyzed the data. CV and UO wrote the first draft of the manuscript. SBD designed the study and provided support for data analysis and writing the manuscript. DD, CLC, JD and CS assisted in the study design and provided constructive feedback on the manuscript. CG and LB performed sequencing and bioinformatics analysis. All authors reviewed and approved the final version of the manuscript.

References

1. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science. 2005;308:1635–8.
2. Hugon P, Lagier J-C, Colson P, Bittar F, Raoult D. Repertoire of human gut microbes. Microb Pathog. 2017;106:103–12.
3. Walker AW, Duncan SH, Louis P, Flint HJ. Phylogeny, culturing, and metagenomics of the human gut microbiota. Trends Microbiol. 2014;22:267–74.
4. Malard F, Gasc C, Plantamura E, Doré J. High gastrointestinal microbial diversity and clinical outcome in graft-versus-host disease patients. Bone Marrow Transplant. 2018;53:1493–7.
5. Vangay P, Ward T, Gerber JS, Knights D. Antibiotics, Pediatric Dysbiosis, and Disease. Cell Host Microbe. 2015;17:553–64.
6. Zhang F, Cui B, He X, Nie Y, Wu K, Fan D, et al. Microbiota transplantation: concept, methodology and strategy for its modernization. Protein Cell. 2018;9:462–73.
7. Wang J-W, Kuo C-H, Kuo F-C, Wang Y-K, Hsu W-H, Yu F-J, et al. Fecal microbiota transplantation: Review and update. J Formos Med Assoc Taiwan Yi Zhi. 2019;118(Suppl 1):23–31.
8. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958;44:854–9.

9. Khan MY, Dirweesh A, Khurshid T, Siddiqui WJ. Comparing fecal microbiota transplantation to standard-of-care treatment for recurrent Clostridium difficile infection: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol. 2018;30:1309–17.
10. DuPont HL, Jiang Z-D, DuPont AW, Utay NS. Abnormal Intestinal Microbiome in Medical Disorders and Potential Reversibility by Fecal Microbiota Transplantation. Dig Dis Sci. 2020;65:741–56.
11. Gupta S, Allen-Vercoe E, Petrof EO. Fecal microbiota transplantation: in perspective. Ther Adv Gastroenterol. 2016;9:229–39.
12. Ooijevaar RE, Terveer EM, Verspaget HW, Kuijper EJ, Keller JJ. Clinical Application and Potential of Fecal Microbiota Transplantation. Annu Rev Med. 2019;70:335–51.
13. DeFilipp Z, Hohmann E, Jenq RR, Chen Y-B. Fecal Microbiota Transplantation: Restoring the Injured Microbiome after Allogeneic Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2019;25:e17–22.
14. Ramai D, Zakhia K, Ofosu A, Ofori E, Reddy M. Fecal microbiota transplantation: donor relation, fresh or frozen, delivery methods, cost-effectiveness. Ann Gastroenterol. 2019;32:30–8.
15. Taur Y, Coyte K, Schluter J, Robilotti E, Figueroa C, Gjonbalaj M, et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. Sci Transl Med. 2018;10.
16. Kelly CR, Khoruts A, Staley C, Sadowsky MJ, Abd M, Alani M, et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. Ann Intern Med. 2016;165:609–16.
17. Heath RD, Cockerell C, Mankoo R, Ibdah JA, Tahan V. Fecal microbiota transplantation and its potential therapeutic uses in gastrointestinal disorders. North Clin Istanb. 2018;5:79–88.
18. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. JAMA. 2017;318:1985–93.
19. Le Bastard Q, Ward T, Sidiropoulos D, Hillmann BM, Chun CL, Sadowsky MJ, et al. Fecal microbiota transplantation reverses antibiotic and chemotherapy-induced gut dysbiosis in mice. Sci Rep. 2018;8:6219.
20. Wei Y-L, Chen Y-Q, Gong H, Li N, Wu K-Q, Hu W, et al. Fecal Microbiota Transplantation Ameliorates Experimentally Induced Colitis in Mice by Upregulating AhR. Front Microbiol. 2018;9:1921.
21. Wrzosek L, Ciocan D, Borentain P, Spatz M, Puchois V, Hugot C, et al. Transplantation of human microbiota into conventional mice durably reshapes the gut microbiota. Sci Rep. 2018;8:6854.
22. Zhou J, Zhou Z, Ji P, Ma M, Guo J, Jiang S. Effect of fecal microbiota transplantation on experimental colitis in mice. Exp Ther Med. 2019;17:2581–6.
23. Hartung T. Thoughts on limitations of animal models. Parkinsonism Relat Disord. 2008;14(Suppl 2):81–3.

24. Blanquet-Diot S, Denis S, Chalancon S, Chaira F, Cardot J-M, Alric M. Use of artificial digestive systems to investigate the biopharmaceutical factors influencing the survival of probiotic yeast during gastrointestinal transit in humans. Pharm Res. 2012;29:1444–53.
25. Thévenot J, Etienne-Mesmin L, Denis S, Chalancon S, Alric M, Livrelli V, et al. Enterohemorrhagic Escherichia coli O157:H7 survival in an in vitro model of the human large intestine and interactions with probiotic yeasts and resident microbiota. Appl Environ Microbiol. 2013;79:1058–64.
26. Thévenot J, Cordonnier C, Rougeron A, Le Goff O, Nguyen HTT, Denis S, et al. Enterohemorrhagic Escherichia coli infection has donor-dependent effect on human gut microbiota and may be antagonized by probiotic yeast during interaction with Peyer's patches. Appl Microbiol Biotechnol. 2015;99:9097–110.
27. Cordonnier C, Thévenot J, Etienne-Mesmin L, Denis S, Alric M, Livrelli V, et al. Dynamic In Vitro Models of the Human Gastrointestinal Tract as Relevant Tools to Assess the Survival of Probiotic Strains and Their Interactions with Gut Microbiota. Microorganisms. 2015;3:725–45.
28. Yu Y, Lee C, Kim J, Hwang S. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol Bioeng. 2005;89:670–9.
29. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinforma Oxf Engl. 2011;27:2957–63.
30. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinforma Oxf Engl. 2014;30:2114–20.
31. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.
32. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016;4:e2584.
33. Silva SSU. database Release 128 [Internet]. [cited 2020 May 25]. Available from: https://www.arb-silva.de/documentation/release-128/.
34. R: The R Project for Statistical Computing [Internet]. [cited 2020 May 25]. Available from: https://www.r-project.org/.
35. Chen D, Wu J, Jin D, Wang B, Cao H. Fecal microbiota transplantation in cancer management: Current status and perspectives. Int J Cancer. 2019;145:2021–31.
36. Rashidi A, Kaiser T, Shields-Cutler R, Graiziger C, Holtan SG, Rehman TU, et al. Dysbiosis patterns during re-induction/salvage versus induction chemotherapy for acute leukemia. Sci Rep [Internet]. 2019 [cited 2020 Apr 1];9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6465677/.
37. Lee MSL, Ramakrishna B, Moss AC, Gold HS, Branch-Elliman W. Successful treatment of fulminant Clostridioides difficile infection with emergent fecal microbiota transplantation in a patient with acute myeloid leukemia and prolonged, severe neutropenia. Transpl Infect Dis Off J Transplant Soc. 2020;22:e13216.

38. Krueger WA, Ruckdeschel G, Unertl K. Influence of intravenously administered ciprofloxacin on aerobic intestinal microflora and fecal drug levels when administered simultaneously with sucralfate. Antimicrob Agents Chemother. 1997;41:1725–30.
39. Zimmermann P, Curtis N. The effect of antibiotics on the composition of the intestinal microbiota - a systematic review. J Infect. 2019;79:471–89.
40. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. JAMA. 2014;312:1772–8.
41. Allegretti JR, Fischer M, Sagi SV, Bohm ME, Fadda HM, Ranmal SR, et al. Fecal Microbiota Transplantation Capsules with Targeted Colonic Versus Gastric Delivery in Recurrent Clostridium difficile Infection: A Comparative Cohort Analysis of High and Lose Dose. Dig Dis Sci. 2019;64:1672–8.
42. Jiang Z-D, Alexander A, Ke S, Valilis EM, Hu S, Li B, et al. Stability and efficacy of frozen and lyophilized fecal microbiota transplant (FMT) product in a mouse model of Clostridium difficile infection (CDI). Anaerobe. 2017;48:110–4.
43. Jiang Z-D, Jenq RR, Ajami NJ, Petrosino JF, Alexander AA, Ke S, et al. Safety and preliminary efficacy of orally administered lyophilized fecal microbiota product compared with frozen product given by enema for recurrent Clostridium difficile infection: A randomized clinical trial. PloS One. 2018;13:e0205064.
44. Payne AN, Zihler A, Chassard C, Lacroix C. Advances and perspectives in in vitro human gut fermentation modeling. Trends Biotechnol. 2012;30:17–25.
45. Fehlbaum S, Chassard C, Poeker SA, Derrien M, Fourmestraux C, Lacroix C. Clostridium difficile colonization and antibiotics response in PolyFermS continuous model mimicking elderly intestinal fermentation. Gut Pathog. 2016;8:63.
46. Van den Abbeele P, Grootaert C, Marzorati M, Possemiers S, Verstraete W, Gérard P, et al. Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and Clostridium cluster IX. Appl Environ Microbiol. 2010;76:5237–46.
47. Davidovics ZH, Michail S, Nicholson MR, Kociolek LK, Pai N, Hansen R, et al. Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection and Other Conditions in Children: A Joint Position Paper From the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr. 2019;68:130–43.
48. Gurram B, Sue PK. Fecal microbiota transplantation in children: current concepts. Curr Opin Pediatr. 2019;31:623–9.
49. Pham VT, Chassard C, Rifa E, Braegger C, Geirnaert A, Rocha Martin VN, et al. Lactate Metabolism Is Strongly Modulated by Fecal Inoculum, pH, and Retention Time in PolyFermS Continuous Colonic Fermentation Models Mimicking Young Infant Proximal Colon. mSystems. 2019;4.
50. Fehlbaum S, Chassard C, Haug MC, Fourmestraux C, Derrien M, Lacroix C. Design and Investigation of PolyFermS In Vitro Continuous Fermentation Models Inoculated with Immobilized Fecal

Microbiota Mimicking the Elderly Colon. PLOS ONE Public Library of Science. 2015;10:e0142793.

51. Cheng Y-W, Fischer M. The Present Status of Fecal Microbiota Transplantation and Its Value in the Elderly. Curr Treat Options Gastroenterol. 2017;15:349–62.
52. Vermeiren J, Van den Abbeele P, Laukens D, Vigsnaes LK, De Vos M, Boon N, et al. Decreased colonization of fecal Clostridium coccoides/Eubacterium rectale species from ulcerative colitis patients in an in vitro dynamic gut model with mucin environment. FEMS Microbiol Ecol. 2012;79:685–96.
53. Bussolo de Souza C, Roeselers G, Troost F, Jonkers D, Koenen ME, Venema K. Prebiotic effects of cassava bagasse in TNO's in vitro model of the colon in lean versus obese microbiota. J Funct Foods. 2014;11:210–20.

Tables

Table 1: Selected criteria used to determine dysbiosis periods in ARCOL system

Criteria	Cut-off levels for differences	Control condition
Gut microbiota activity		
Redox potential	≥ ± 200 mV	d-Day in treated bioreactor compared to d-Day in control experiment
NaOH consumption **Total gas production**	Stop NaOH consumption Stop gas production	
CO_2 concentrations	≥ ± 10 %	
SCFA concentrations (acetate, propionate and butyrate)	≥ ± 25 % for each SCFA	
Gut microbiota composition		
Total viable bacteria-FC	≥ ± 1 log	d-Day in treated bioreactor compared to d-Day in control experiment
Total bacteria-qPCR	≥ ± 1 log	
Richness	≥ ± 20 %	d-Day compared to day 6 in treated bioreactor
Shannon	≥ ± 20 %	
Bray-Curtis	≥ 0.5	

ATB: antibiotic, FC: Flow cytometry, FMT: Faecal microbiota transfer, SCFA: Short Chain Fatty Acid

Figures

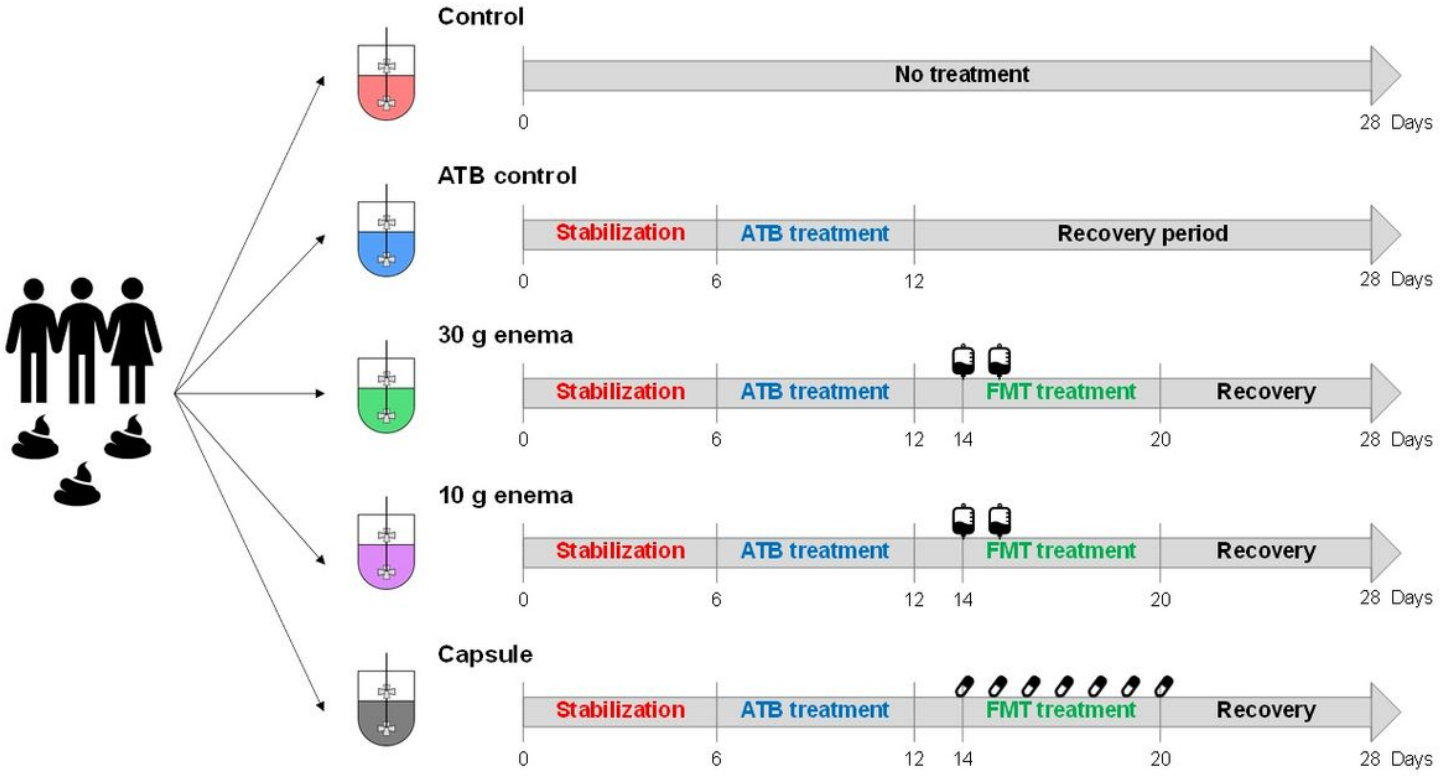

Figure 1

Experimental design of in vitro fermentations in the ARCOL system Five bioreactors were inoculated with the same faecal suspension and ran in parallel for 28 days. No treatment was applied in control condition (control, red bioreactor). After 6 days of microbiota stabilization, the other four bioreactors were treated with 500 µg/mL ciprofloxacin for 6 days to induce gut microbiota dysbiosis. One of them received no FMT treatment (ATB control, blue fermenter). After 2 days of antibiotic wash-out, FMT treatment was performed in the last three bioreactors with different modes of administration: 30 g enema (green bioreactor), 10 g enema (pink bioreactor) or oral capsule (grey bioreactor). Enema treatments were administered at day 14 and day 15. Oral capsules were given three times per day for seven days from day 14 to day 20. The recovery period was defined as days of fermentation after cessation of FMT treatment. Experiments were performed in triplicate with the feces from three different healthy adult donors (Run1, Run2 and Run3). ATB: antibiotic; FMT: faecal microbiota transfer

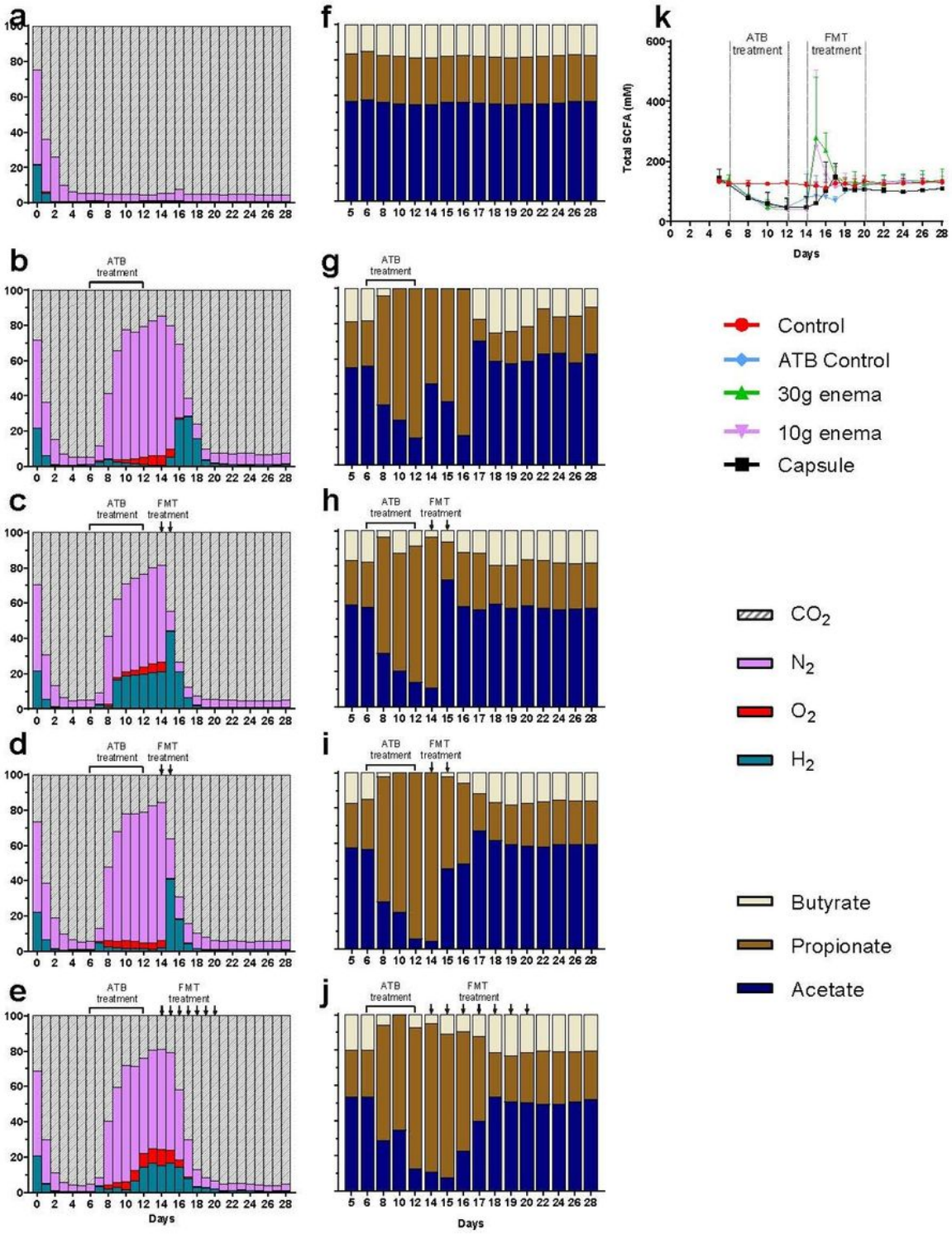

Figure 2

Effect of FMT treatment on gas and SCFA production Experiments were performed as described in Figure 1 and different conditions were applied: no treatment (control, graph a and f), ciprofloxacin (ATB control, graph b and g), ciprofloxacin and 30 g enema (graph c and h), ciprofloxacin and 10 g enema (graph d and i), and ciprofloxacin with oral capsules (graph e and j). Gas composition was determined by gas chromatography and results expressed as mean relative percentages (left panel a to e, n=3). Main SCFAs

(acetate, propionate and butyrate) were analysed by high performance liquid chromatography. Results were either expressed as mean relative percentages (right panel f to j, n=3) or as total SCFA concentrations ± SEM (in mM, graph k). ATB: antibiotic; FMT: faecal microbiota transfer

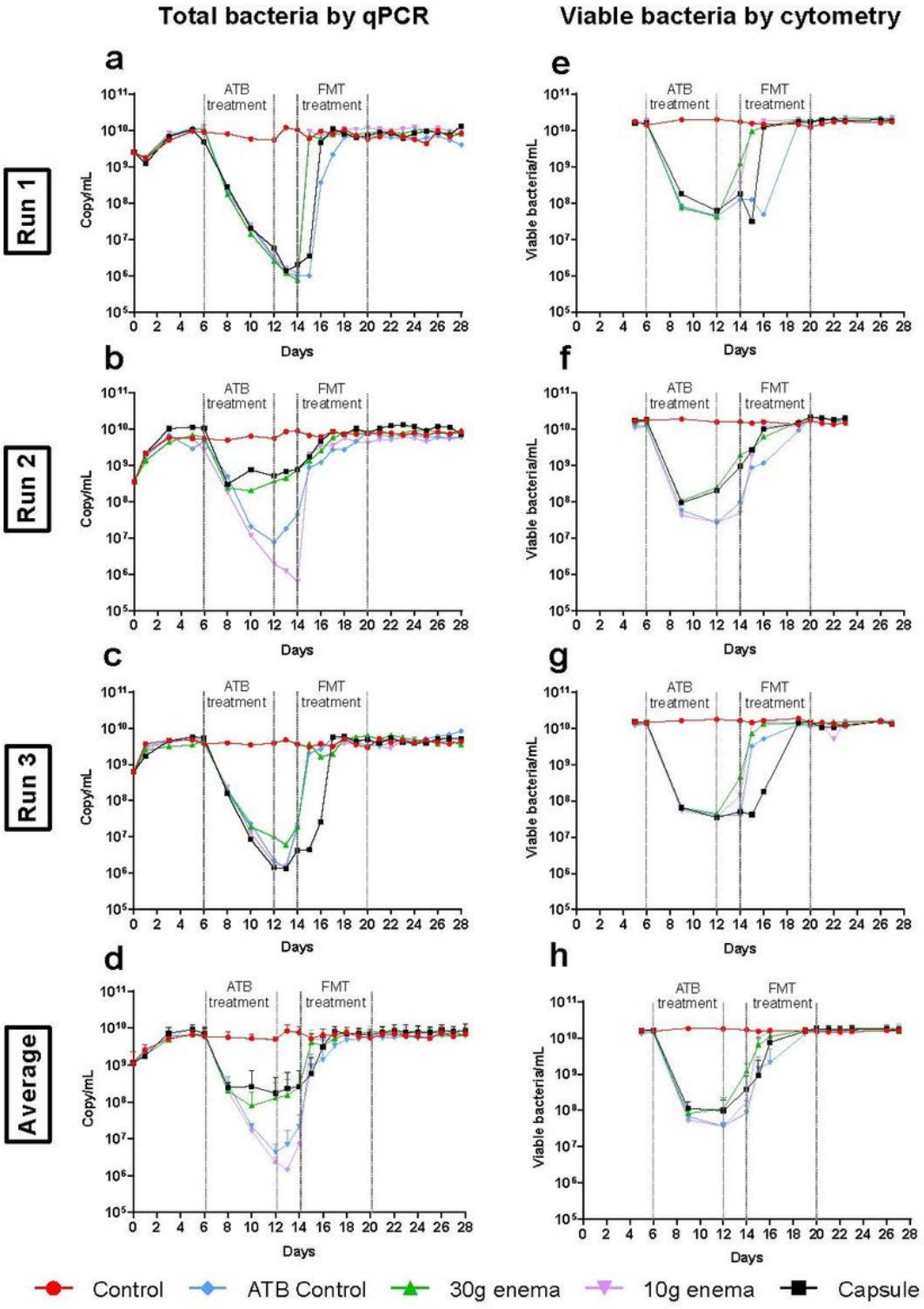

Figure 3

Effect of FMT treatment on total bacteria Experiments were performed as described in Figure 1 in triplicate with fecal samples from three healthy adult donors (Run1, Run2 and Run3). Different conditions

were applied: no treatment (control, red), ciprofloxacin (ATB control, blue), ciprofloxacin and 30 g enema (green), ciprofloxacin and 10 g enema (purple), and ciprofloxacin with oral capsules (black). Total bacteria was determined by qPCR analysis and expressed as numbers of 16S rRNA gene copies/mL in Run1 to Run3 (right panel, e to h) or in mean number of copies/mL ± SEM (left panel, graph h, n=3). Total viable bacteria was determined by live/dead flow cytometry analysis and expressed as number of viable cells/mL in Run1 to Run3 (left panel, a to c) or in mean viable cells/mL ± SEM (left panel, graph d, n=3). ATB: antibiotic; FMT: faecal microbiota transfer

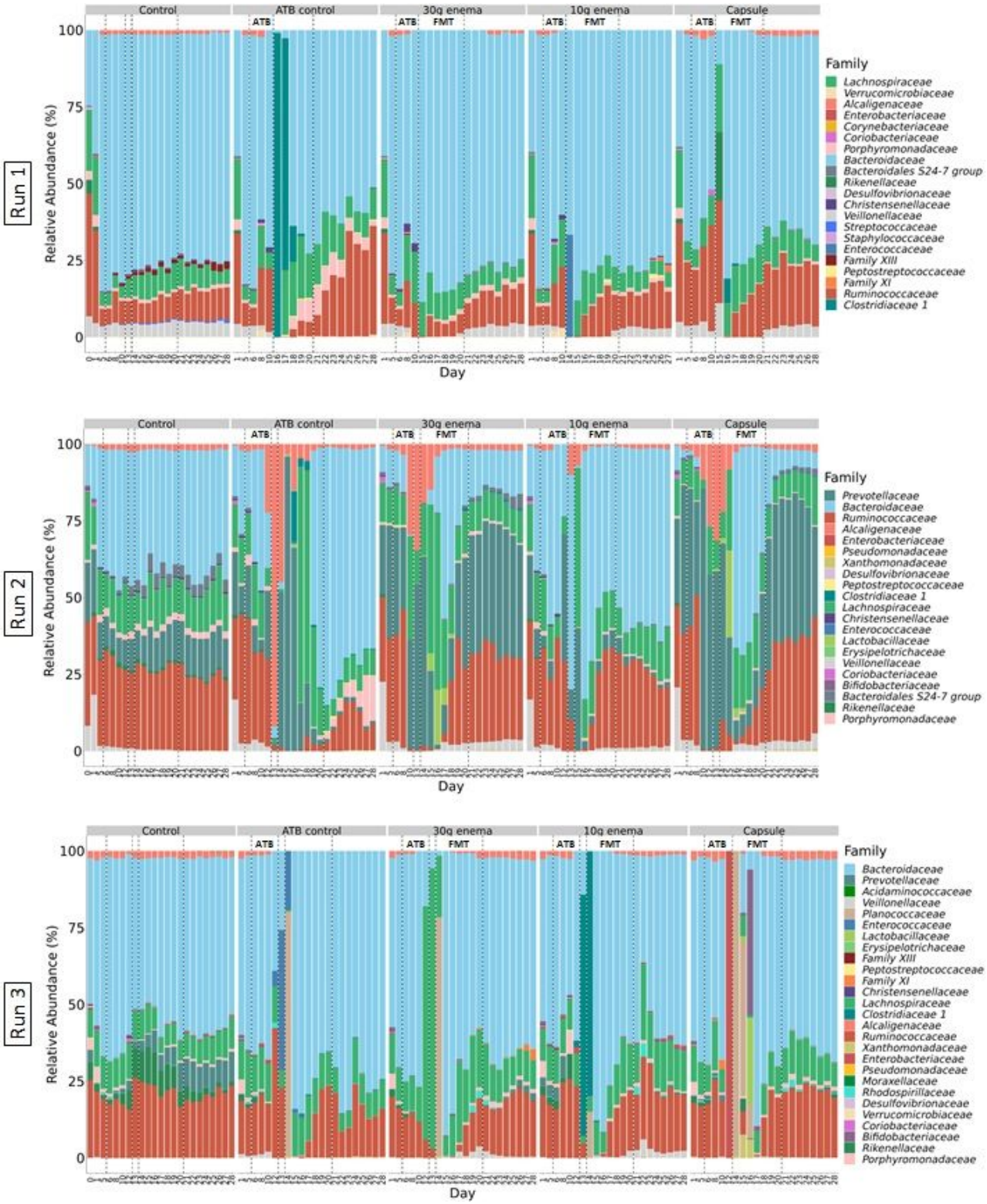

Figure 4

Effect of FMT treatment on microbial composition at family level Experiments were performed as described in Figure 1 in triplicate with fecal samples from three healthy adult donors (Run1, Run2 and Run3). Different conditions were applied: no treatment (control), ciprofloxacin (ATB control), ciprofloxacin and 30 g enema, ciprofloxacin and 10 g enema, and ciprofloxacin with oral capsules. Microbial composition was determined at family level by 16S rRNA gene sequencing and expressed as relative abundances. ATB: antibiotic; FMT: faecal microbiota transfer.

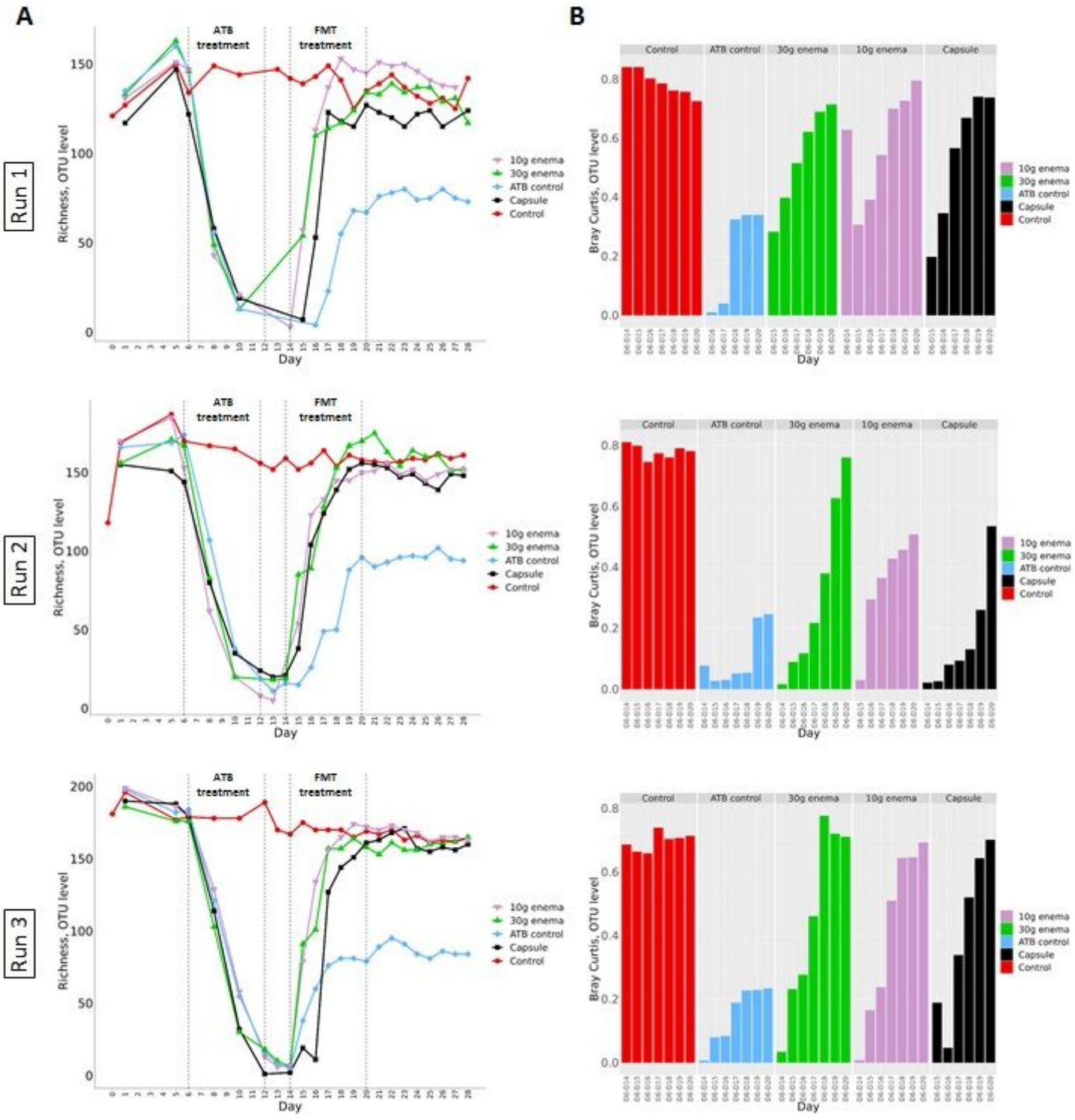

Figure 5

Effect of FMT treatment on richness and Bray-Curtis indexes at OTU level Experiments were performed as described in Figure 1 in triplicate with fecal samples from three healthy adult donors (Run1, Run2 and Run3). Different conditions were applied: no treatment (control, red), ciprofloxacin (ATB control, blue), ciprofloxacin and 30 g enema (green), ciprofloxacin and 10 g enema (purple), and ciprofloxacin with oral capsules (black). Richness (panel A) and Bray-Curtis (panel B, compared to day 6 values) indexes were determined at OTU level after 16S-rRNA gene sequencing. Bray-Curtis values represent similarity of samples versus day 6. ATB: antibiotic; FMT: faecal microbiota transfer.

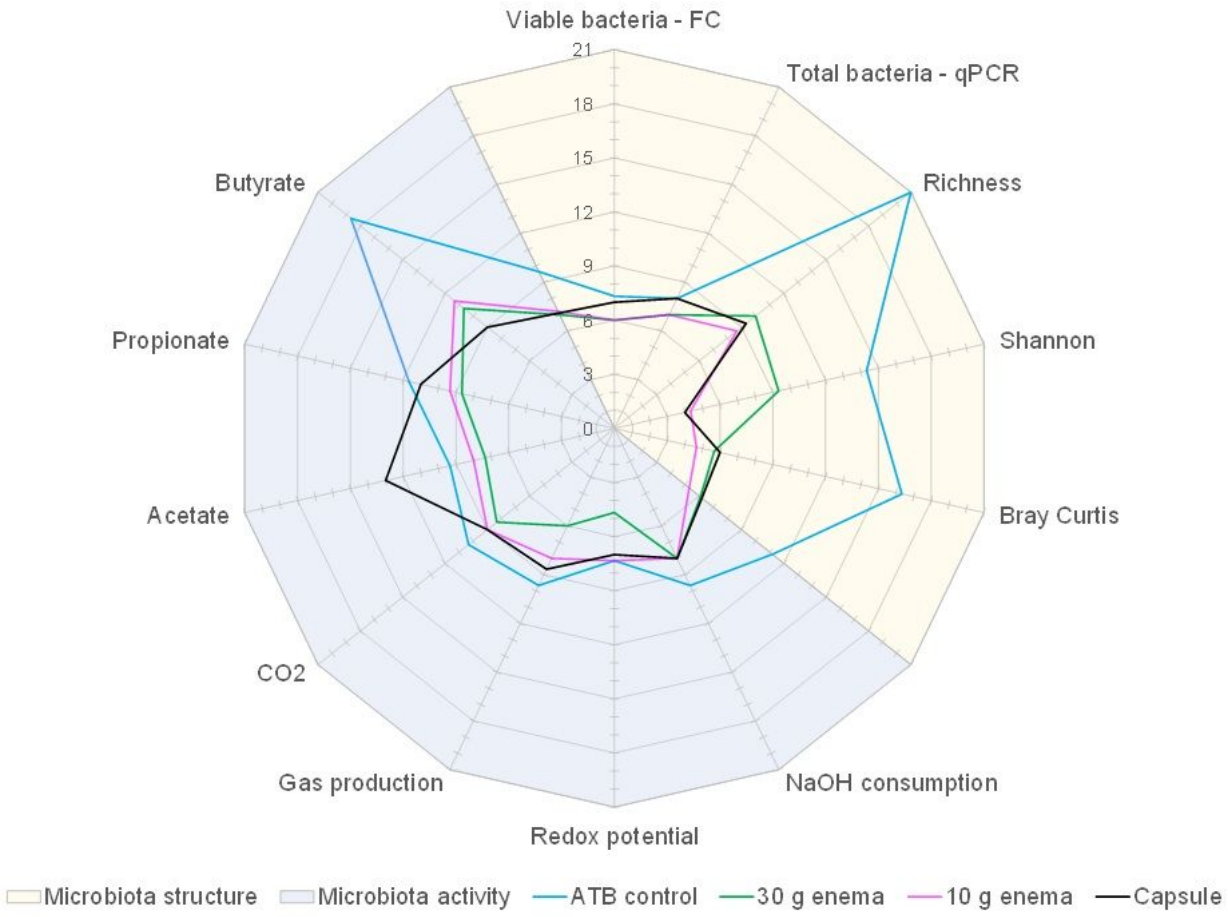

Figure 6

Effect of FMT treatment on the duration of dysbiosis Experiments were performed as described in Figure 1 and different conditions were applied: no treatment (control, red), ciprofloxacin (ATB control, blue), ciprofloxacin and 30 g enema (green), ciprofloxacin and 10 g enema (purple), and ciprofloxacin with oral capsules (black). For each tested condition, the number of dysbiotic days was determined compared to the appropriate control using criteria described in Table 1 (in relation with both microbiota structure and activity) and expressed as mean number of dysbiotic days (n=3). ATB: antibiotic, FC: flow cytometry, FMT: faecal microbiota transfer.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure1.pptx
- Supplementaryfigure2.pptx