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Bacteria and Bacterial Diseases

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

Benjamin Davido [a,b,*], Andrea R. Watson [c], Pierre de Truchis [a], Gianluca Galazzo [c], Aurelien Dinh [a,b], Rui Batista [d], Elisabeth M. Terveer [e], Christine Lawrence [f], Hugues Michelon [g], Marion Jobard [d], Azzam Saleh-Mghir [a,h], Ed J. Kuijper [e], Silvia Caballero [c]

[a] Maladies Infectieuses, Hôpital Universitaire Raymond-Poincaré, AP-HP Université Paris Saclay, 92380 Garches, France
[b] FHU PaCeMM, Hôpital Saint-Antoine, AP-HP Université Paris Centre, 75571 Paris Cedex 12, France
[c] Vedanta Biosciences Inc., Cambridge, MA 02139, USA
[d] Pharmacie Hospitalière, Hôpital Universitaire Cochin, AP-HP, 75014 Paris, France
[e] Department of Medical Microbiology, Netherlands Donor Feces Bank (NDFB) at Leiden University Medical Center, Leiden, the Netherlands
[f] Laboratoire de Microbiologie, Hôpital Universitaire Raymond-Poincaré, AP-HP Université Paris Saclay, 92380 Garches, France
[g] Pharmacie Hospitalière, Hôpital Universitaire Raymond-Poincaré, AP-HP Université Paris Saclay, 92380 Garches, France
[h] UMR1173, Université Versailles Saint-Quentin, 78000 Versailles, France

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SUMMARY

Objectives: We evaluated the effect of fecal microbiota transplantation (FMT) on the clearance of carbapenemase-producing Enterobacterales (CPE) carriage.
Methods: We performed a prospective, multi-center study, conducted among patients who received a single dose of FMT from one of four healthy donors. The primary endpoint was complete clearance of CPE carriage two weeks after FMT with a secondary endpoint at three months. Shotgun metagenomic sequencing was performed to assess gut microbiota composition of donors and recipients before and after FMT.
Results: Twenty CPE-colonized patients were included in the study, where post-FMT 20% (n = 4/20) of patients met the primary endpoint and 40% (n = 8/20) of patients met the secondary endpoint. Kaplan-Meier curves between patients with FMT intervention and the control group (n = 82) revealed a similar rate of decolonization between groups.

Microbiota composition analyses revealed that response to FMT was not donor-dependent. Responders had a significantly lower relative abundance of CPE species pre-FMT than non-responders, and 14 days post-FMT responders had significantly higher bacterial species richness and alpha diversity compared to non-responders ($p < 0.05$). Responder fecal samples were also enriched in specific species, with significantly higher relative abundances of *Faecalibacterium prausnitzii*, *Parabacteroides distasonis*, *Collinsella aerofaciens*, *Alistipes finegoldii* and *Blautia_A sp900066335* ($q < 0.01$) compared to non-responders.
Conclusion: FMT administration using the proposed regimen did not achieve statistical significance for complete CPE decolonization but was correlated with the relative abundance of specific bacterial taxa, including CPE species.

Introduction

Gastrointestinal (GI) colonization with multidrug-resistant organisms (MDROs) is a major risk factor for infection and mortality in high-risk patient populations.[1–5] In 2015, The European Centre for Disease Prevention and Control (ECDC) reported that infections with MDROs accounted for approximately 33,000 hospital deaths in Europe.[6] Additionally, in 2019, up to 1.27 million deaths were

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* Correspondence to: Service de Maladies Infectieuses et Tropicales, Hôpital Raymond-Poincaré, Garches 92380, France.
E-mail address: benjamin.davido@aphp.fr (B. Davido).

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attributable to antimicrobial resistance worldwide.[7] Of particular concern are carbapenemase-producing Enterobacterales (CPE), which have been classified by the World Health Organization as one of the most critical antibiotic-resistant pathogen groups for which new therapies are urgently needed.[8] The prevalence of CPE is increasing worldwide, and in France alone, for instance, the number of CPE cases increased from 248 to approximately 1704 (6-fold) from 2012 to 2018.[9] Likewise, a recent study conducted by the European Antimicrobial Resistance Surveillance Network (EARS-Net) revealed that the number of deaths attributable to carbapenem-resistant Enterobacterales in Europe increased by 4 to 6-fold between 2007 and 2015, especially in countries such as Italy, Greece, and Romania where CPE are highly prevalent.[6]

MDRO colonization has been shown to increase the risk of infection by 14% in a median follow-up period of 30 days.[10] Spontaneous decolonization of CPE is slow, ranging from 12% decolonization within 30 days in our hospital's dedicated ward for CPE-colonized patients under reinforced isolation precautions,[11] and up to 25% decolonization after 30 days among healthcare residents.[12] As a result, decolonization strategies including selective digestive decontamination (SDD) and fecal microbiota transplantation (FMT) have been implemented to accelerate MDRO decolonization and reduce infection risk.[13–16] While SDD against CPE significantly reduced infection risk following successful decolonization, it was associated with the development of antibiotic resistance and colonization recurrence.[13,14,17] These risks have limited the use of SDD for infection prevention and guidelines from the European Society for Clinical Microbiology and Infectious Diseases strongly recommend against the use of oral antibiotics for MDRO decolonization.[18] As an alternative, FMT has been shown to be a safer and more effective strategy for decolonization of CPE and other MDROs, with efficacy rates ranging from 20–90%[16] and a significant reduction in the number of MDRO infections following FMT treatment.[19–21]

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

Materials and methods

Setting

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

Participants

Patients ≥18 years with confirmed CPE colonization and able to provide informed consent were included in the study. CPE colonization was determined by at least three consecutive positive rectal swabs at weekly intervals, including the week prior to FMT. Exclusion criteria were immunosuppression (human immunodeficiency virus with CD4 < 200/mm3), immunosuppressive therapy (including corticosteroids > 60 mg/day for more than five days, chemotherapy, stem cell transplantation under immunosuppressant drugs and other inherited immunosuppressive disorder), antibiotic treatment at the time of FMT, previous infection with *Clostridioides difficile* treated with FMT, as well as individuals who were pregnant or breastfeeding, unable to provide informed consent, under tutorship or guardianship, homeless or without health insurance. Patients non-eligible for FMT intervention were assigned to the non-FMT cohort.

Data collection

The following data were collected from all study participants:

- Patient characteristics: age, sex, comorbidities, Charlson Comorbidity Index at admission, hospitalization 6 months prior to enrollment, duration of carriage, and travel abroad.
- Episodes of infection caused by CPE.
- Antibiotic administration and duration of treatment a month prior to CPE screening.
- CPE species ID and mechanism of resistance.
- Biological abnormalities, including white blood cell count, CRP, and albumin at the time of FMT.
- Functional autonomy scale based on the iso-resource group (IRG), a need-of-care scale ranging from 1 (i.e., fully dependent/bedridden) to 6 (i.e., fully independent) as previously described in nursing homes.[24]

Donor screening and FMT preparation

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

Study procedure

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

Microbiological methods

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

Outcomes measures

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

Metagenomic analysis

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

Statistical analysis

Results are described as n (%) or median [interquartile range (IQR)]. Analysis of decolonization following FMT was performed using GraphPad Prism v.8.0.2 (GraphPad Software Inc., La Jolla, CA). A Kaplan-Meier analysis using the Log-rank (Mantel-Cox) test was used to plot rate of decolonization, and statistical tests were performed using the parametric Student's *t-test* considering sample size (n < 30) or log-rank (Mantel-Cox test) for survival curves. Microbiota analyses were performed using R v4.2.2 and visualized using ggplot2. The R package vegan v2.6.4 "diversity" and "vegdist" functions were used to calculate Shannon diversity and Bray-Curtis dissimilarity indices, respectively, and the "specnumber" function was used to calculate species richness. The functional pathways selected for tests of association with recipient CPE clearance at day 14 were identified through principal component analysis (PCA) of all recipient samples and subsequent selection of the 5 pathways corresponding to the strongest PCA loadings. Statistical tests were performed using the Wilcoxon Rank-Sum test with false discovery rate (FDR) adjustment. Taxon associations with response were calculated using MaAslin2 v1.12.0 with default parameters, which ran a log-transformed linear model and performed FDR adjustment.

Study approval

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

Data availability

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

Results

Population characteristics

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

Decolonization following FMT

Fourteen days after FMT, 4 out of 20 patients (20%) tested negative for CPE and met the primary endpoint for the study. Thirty days post-FMT, 2 additional patients (30%) became negative for CPE followed by two others by day ninety, resulting in an overall CPE decolonization rate of 40% (8/20) three months post-transplant (secondary endpoint). The decolonization rate over time is shown as a percentage on a Kaplan-Meier curve between CPE cases in the FMT group versus

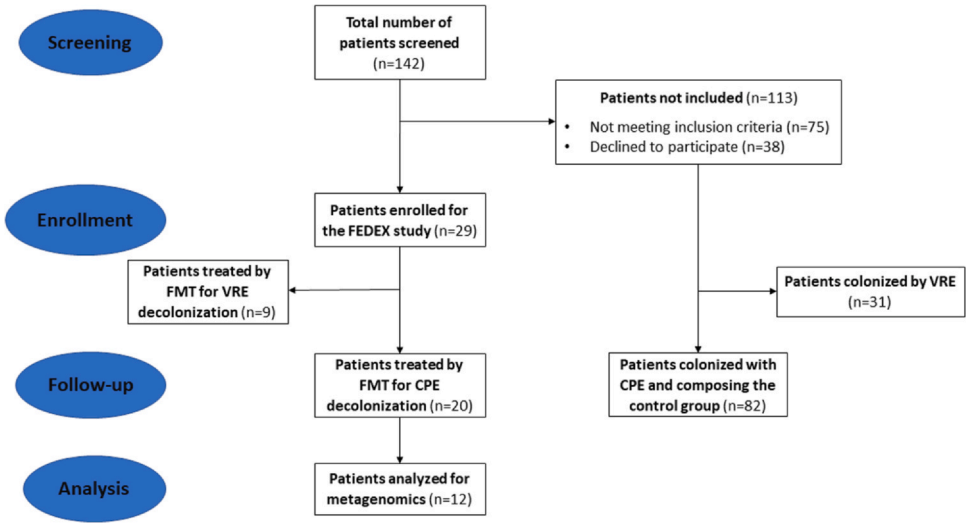

Fig. 1. STROBE Flowchart of the FEDEX study. Flowchart in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement http://www.strobestatement.org.

Table 1
Descriptive factors that may have contributed to decolonization using FMT at 1 month.

Variables	Success (n = 6)	Failure (n = 14)	p-value
Characteristics at baseline			
Age in years, median (IQR)	64.5 (59-71)	73.5 (70-83)	0.2
Hospitalization within previous 6 months, n (%)	5 (83)	12 (86)	0.9
Stay abroad, n (%)	3 (50)	10 (71)	0.6
Duration of carriage in days, median (IQR)	82.5 (61-115)	60 (46-119)	0.6
Previous antibiotic intake within the month, n (%)	4 (67)	10 (71)	0.9
- Duration of treatment in days, median (IQR)	2 (2-7)	7 (7-14)	0.5
Charlson Score, median (IQR)	5 (4-6)	5 (4-7)	0.5
GIR score, median (IQR)	3.5 (1.5-5.5)	5 (2.25-5)	0.6
Previously infected by an MDRO, n (%)	2 (33)	1 (7)	0.2
Biological abnormalities			
WBC count per mm^3, median (IQR)	10,600 (8775-11750)	8450 (6825-9175)	0.2
PMN count per mm^3, median (IQR)	7170 (6645-8190)	5235 (3750-6152)	0.1
Lymphocytes count per mm^3, median (IQR)	1670 (1147.5-1900)	1795 (1425-2387)	0.4
Monocytes count per mm^3, median (IQR)	815 (597-1115)	660 (487.5-812)	0.4
CRP level in mg/L, median (IQR)	25.5 (8-56)	16.5 (9.5-41)	0.9
Albumin level in g/L, median (IQR)	30 (29-31)	26.5 (24-32)	0.7
Type of microorganisms[a]			
E. coli, n (%)	4 (67)	5 (36)	0.3
K. pneumoniae, n (%)	4 (67)	11 (78)	0.6
E. cloacae, n (%)	1 (17)	0	0.3
Others, n (%)	0	1 (7)	0.9
Co-colonization, n (%)	4 (67)	6 (43)	0.6
Mechanism of resistance			
OXA48-type, n (%)	6 (100)	11 (78)	0.5
NDM1-type, n (%)	0	3 (21)	0.5
ESBL-producing co-colonization, n (%)	4 (67)	6 (43)	0.6
Antimicrobial therapy after FMT			
Antibiotic intake after FMT during follow-up, n (%)	1 (17)	5 (36)	0.6
- Duration of treatment in days, median (IQR)	7 (7-7)	7 (7-45)	0.7

[a] Total percentage was > 100% considering some patients carried 2 types of microorganisms.

spontaneous CPE decolonization in our historical non-FMT cohort [11] (Fig. 2). Patients in the comparator arm (n = 82) had clinical profiles similar to patients in the FMT group (see supplementary Table) but were not considered for FMT either because they decided not to participate in the study or because they failed to meet the inclusion criteria, including medical transfer in our hospital for FMT intervention. Although the percentage of patients with CPE carriage decreased within the first 14 days after intervention, this difference was not statistically significant (p = 0.1) and was ultimately similar between the groups (p = 0.9, Fig. 2). Furthermore, there was no statistically significant difference in decolonization efficacy between the four FMT donors used in the first half of the study (n = 2/12) and the fifth FMT donor used thereafter (n = 2/8) (p = 0.99). It is worth noting that the 2 responders in the second half of the study were also colonized with ESBL-producing Enterobacterales and had a history of recurrent urinary tract infection (UTI) caused by these organisms. They did not have any further UTI episodes for up to 12 months following the transplant. To the best of our knowledge, no patient in the control group had a previous medical history of recurrent UTI. Finally, there were no complications during the FMT procedure, and no serious adverse events were reported at follow-up, except for mild transient GI complaints (n = 4).

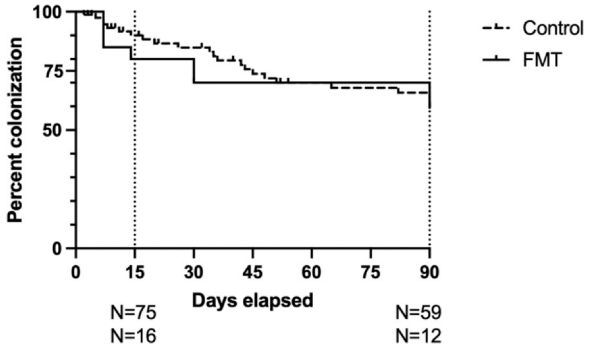

Fig. 2. Kaplan-Meier representation for decolonization between spontaneous decolonization (control group, n = 82) and FMT intervention (n = 20). Dashed lines indicate primary and secondary endpoints.

Microbiota analyses

Metagenomic sequencing was performed on fecal samples collected before and after FMT for 12 patients and their 3 corresponding donors. Fecal material derived from Donor 1, Donor 2, and Donor 5 was used to treat one, three, and eight patients in this sub-cohort, respectively. Four of 12 patients (33%) met the primary endpoint for decolonization. We analyzed the gut microbiota composition and taxonomic diversity in responders and non-responders at baseline and 1 week, 2 weeks, 1 month, and 2–3 months after fecal transplantation. We found that the number of detected bacterial species, as measured by species richness, was significantly higher in responders than in non-responders 2 weeks and 1 month after FMT and was comparable to the bacterial richness observed in the donors (Fig. 3A). Consistent with these findings, the gut microbiota alpha diversity, measured by the Shannon index, was significantly higher in responders compared to non-responders at 2 weeks, 1 month and 2–3 months post-transplant (Fig. 3B). These observations suggested that engraftment of likely donor-derived taxa was more prevalent in responders than in non-responders. To evaluate this further, we measured the Euclidean distance between recipient samples and the centroid of their corresponding donor samples, calculated using the first 2 principal co-ordinates of a Bray-Curtis dissimilarity matrix, and found the median distance to be shorter among responders than non-responders at all timepoints following transplant with the greatest distance at 1-month post-FMT (p = 0.152 after FDR adjustment) (Fig. 4). This finding demonstrated a trend where the microbiota of patients that decolonized CPE was more similar to the microbiota of their donors compared to patients that failed to respond.

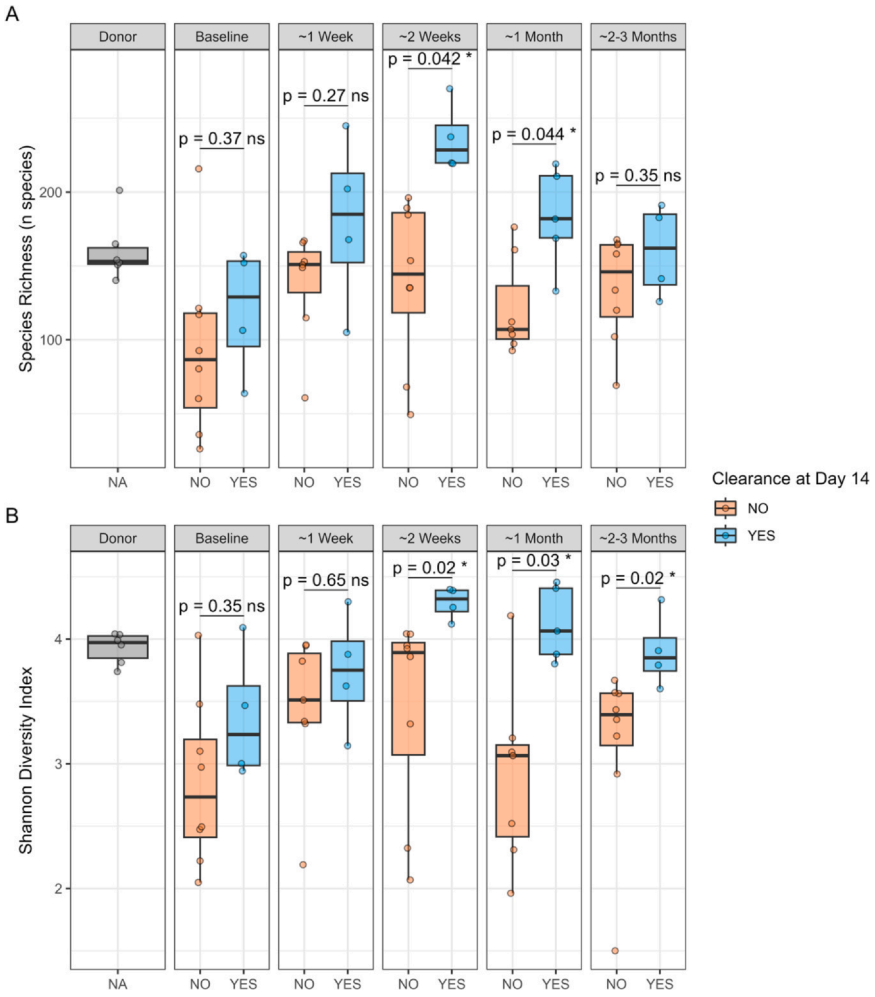

Fig. 3. Gut microbiota analysis between responders and non-responders. Box and whisker plots indicating the (A) species richness and (B) Shannon diversity index of donor samples (gray), samples from patients with CPE clearance on day 14 (teal), and patients without CPE clearance on day 14 (orange). The median (± interquartile range) is plotted for each group at the indicated timepoints. Whiskers extend to the lowest and highest values no greater than 1.5x the interquartile range from the closest hinge. Patients with CPE clearance on day 14 have significantly higher species richness and/or Shannon diversity index than patients without CPE clearance on day 14 at 2 weeks, 1 month, and 2–3 months post-FMT (Wilcoxon Rank-Sum test with FDR adjustment).

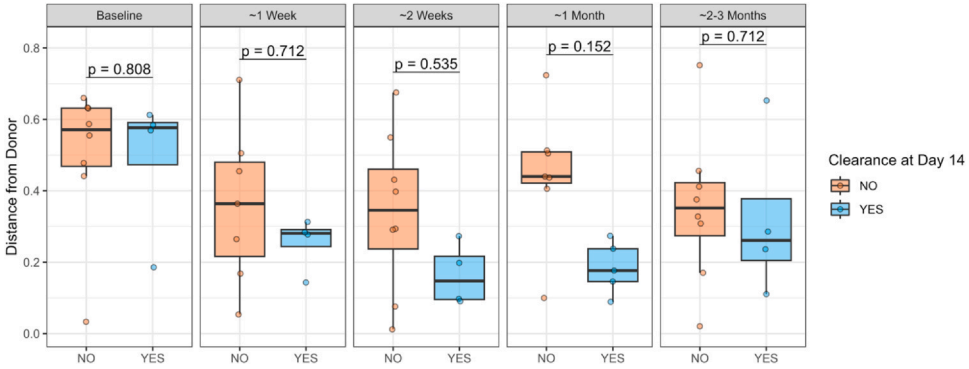

Fig. 4. Microbiota distance between FMT recipients and donors. Box and whisker plots indicating the Euclidean distance of samples from patients with CPE clearance on day 14 (teal), and patients without CPE clearance on day 14 (orange) to the centroid of their corresponding donor samples, calculated using the first 2 principal co-ordinates of a Bray-Curtis dissimilarity matrix between samples. The median (± interquartile range) is plotted for each group at the indicated timepoints. Whiskers extend to the lowest and highest values no greater than 1.5x the interquartile range from the closest hinge. While not statistically significant, patients with CPE clearance on day 14 have a lower mean distance from their donors than patients without CPE clearance at all post-FMT timepoints (Wilcoxon Rank-Sum test with FDR adjustment).

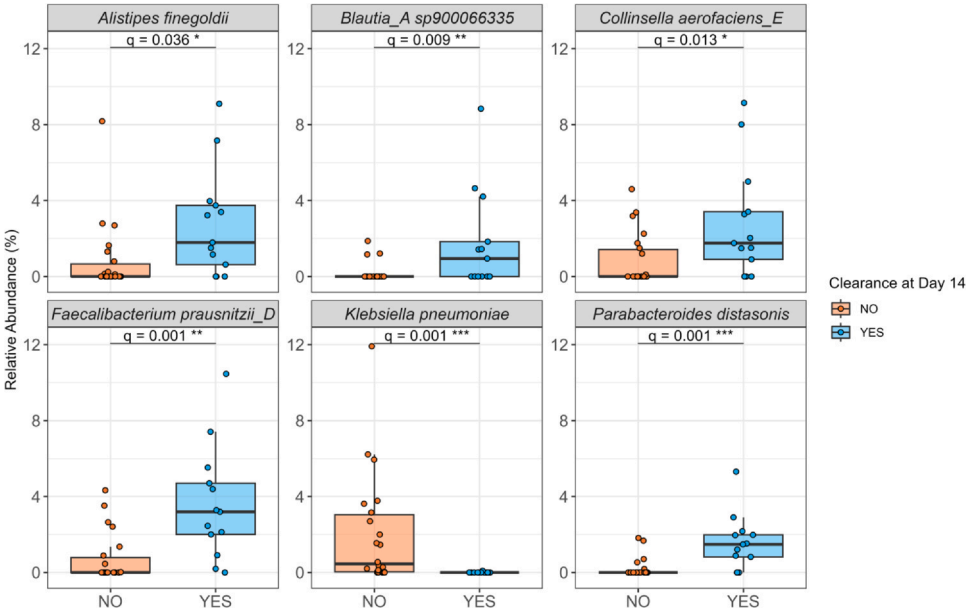

Fig. 5. Bacterial taxa associated with response. Box and whisker plots of the percent relative abundance for the indicated species in all samples from patients who ultimately had CPE clearance on day 14 (teal), and all samples from patients who did not ultimately have CPE clearance on day 14 (orange). The median (± interquartile range) is plotted for each species in each group. Whiskers extend to the lowest and highest values no greater than 1.5x the interquartile range from the closest hinge. The displayed taxa were significantly altered between patients with and without CPE clearance at day 14 across all samples (MaAsLin2 log-transformed linear model with FDR adjustment).

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

Discussion

Consistent with findings previously reported by our group [22,23] and others,[17] our results showed that a single dose of FMT administered via enteric feeding tube has limited efficacy to decolonize CPE. Nevertheless, the efficacy of FMT against MDROs is variable and successful decolonization of CPE or ESBL producing Enterobacterales following FMT administration has been reported.[29,30] Factors contributing to the variability in FMT efficacy in such indication include number of FMT doses, donor selection, FMT preparation, route of FMT administration, and pre-treatment regimen.[16,17,29,31] Indeed, it should be considered that the route of administration used in the present study, as well as the protocol of FMT involving a single dose, may have negatively influenced the decolonization process. As a result, there is an urgent need to standardize the FMT process in this population for more consistent results. Despite the variability in efficacy, FMT is still a safer option than decolonization strategies

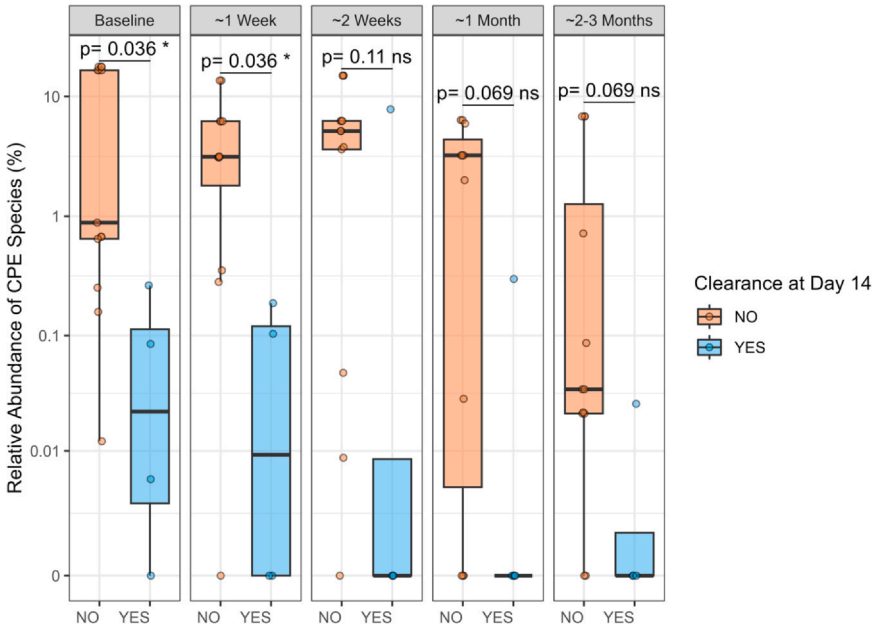

Fig. 6. Abundance of CPE species pre- and post-FMT. Box and whisker plots indicating the cumulative percent relative abundance of CPE species in samples from patients with CPE clearance on day 14 (teal) and patients without CPE clearance on day 14 (orange). The median (± interquartile range) is plotted for each group at the indicated timepoints. Whiskers extend to the lowest and highest values no greater than 1.5x the interquartile range from the closest hinge. Patients with CPE clearance on day 14 have significantly lower CPE relative abundance than patients without CPE clearance on day 14 at baseline and 1-week post-FMT (Wilcoxon Rank-Sum test with FDR adjustment).

involving targeted antibiotics, where the emergence of antibiotic-resistant isolates and colonization recurrence have been observed.[13,14] Importantly, there was no colonization recurrence after 1 month among responders in the FEDEX study.

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

Furthermore, 2/2 non-immunocompromised patients with recurrent UTI due to ESBL-producing Enterobacterales did not experience any UTI episodes for up to 12 months after FMT administration. One 33-year-old individual had previously been admitted twice for prostatitis within the 12 months prior to intervention and had required two courses of carbapenems (with the last injection occurring 3 months before the FMT). The other individual was a 57-year-old female with recurrent UTI due to neurogenic bladder, and had historically presented with pyelonephritis every 1–2 months treated by cefoxitin using outpatient parenteral antimicrobial therapy (with the last injection occurring 2 months before the FMT).

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

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

In our present work, we showed that among responders, *Faecalibacterium prausnitzii*, *Parabacteroides distasonis*, *Collinsella aerofaciens*, *Alistipes finegoldii,* and *Blautia_A sp900066335* had significantly higher relative abundances and were therefore associated with successful CPE decolonization post-FMT. *Faecalibacterium* species have been previously reported to be correlated with Enterobacterales suppression likely due to their ability to produce metabolites, specifically short chain fatty acids (SCFA), that can inhibit the growth of Gram-negative pathogens.[4,39] It is noteworthy that Woodworth *et al.* [35] also described specific taxa, including *Alistipes* and *Faecalibacterium* species, associated with the decolonization of ESBL-producing Enterobacterales in a small sample size trial (n = 11). Likewise, Bilinski *et al.* [30] described microbial

signatures associated with decolonization using FMT that pertained to *Barnesiella*, *Bacteroides*, and *Butyricimonas* species. Additionally, Leo *et al.* reported that decolonization was associated with the presence of *Bifidobacterium* strains that were enriched in individuals who had cleared multidrug-resistant Enterobacterales one month after the return from travel to tropical regions.[40]

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

The microbiota of non-responders exhibited a higher relative abundance of *Klebsiella pneumoniae*, which represented ≤12% of the patients' microbiota, compared to responders where *Klebsiella pneumoniae* levels were nearly undetectable. Importantly, we found that the relative abundance of CPE organisms before FMT was much lower in responders than in non-responders, suggesting that CPE colonization levels may be correlated with FMT response, defined by complete elimination of carriage. This finding, however, does not suggest that patients with high levels of CPE colonization will not respond to FMT or other microbiome-based therapies. In fact, these individuals may benefit from multiple rounds of FMT treatment as demonstrated by studies where administration of more than 2 FMT doses led to successful MDRO decolonization after a single dose failed to do so.[29,31,35] However, while multiple FMT doses may be a potential approach to lower CPE levels enough to reduce infection risk and limit transmission, in the absence of encapsulated FMT undergoing multiple rounds of FMT via nasoduodenal tube may increase the risk for serious adverse events, including bacteremia and perforations.[41] Moreover, an important medical consideration for MDRO-colonized patients is that CPE carriers are often placed under contact isolation in a dedicated ward resulting in higher healthcare costs and treatment delays.[42,43] Therefore, FMT administration for MDRO decolonization could be a viable intervention for CPE carriers undergoing surgery as well as serve as a measure for outbreak control.[44] In line with this, Saidaini *et al.* showed that in a case-control study in MDRO-colonized patients, FMT administration accelerated discharge of patients that experienced decolonization (n = 8/10) compared to those in the control group.[45] In contrast, our findings showed no difference in terms of decolonization (up to 90 days) between the patients who underwent FMT and our control group consisting of 82 individuals.

In 2019, a multicenter, randomized controlled study was conducted to evaluate the efficacy of a single dose of FMT against decolonization of multidrug-resistant Enterobacterales in 39 patients.[17] Prior to FMT, patients in the intervention group received a 5-day antibiotic course which markedly reduced MDRO levels. At 1-month, the percentage of patients that remained positive for MDRO colonization was 61.9% and 79% in the FMT and control groups, respectively. The low efficacy rate reported could be attributed to poor bacterial viability in the fecal transplants following antibiotic pre-treatment for gram-negative bacteria. Therefore, further study of microbiota conditioning regimens, including pre-treatment with specific antibiotic classes and length of antibiotic course is needed in other indications beyond CDI. Bowel lavage may be a more effective pre-treatment option based on the high decolonization rates reported.[31] Similarly, patients in the FEDEX study received a bowel prep without antibiotics. Furthermore, studies have shown that administration of antibiotics within 7 days post-FMT is the most common cause of decolonization failure.[23,31,44] Although our results are not statistically significant due to the limited number of patients studied, they support this finding (17% of antibiotic use within 30 days post-FMT in the responder group versus 36% in the non-responder group).

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

Finally, the main limitation of testing for decolonization in the clinic is that the current methods fail to take into account pathogen load and decolonization is defined by the absence of MDRO detection. Although testing over weeks/months may not be sufficient to show persistent eradication, MDRO elimination within the first 1–3 months after FMT may have long-term benefits, as demonstrated by the FMT recipient who decolonized and remained UTI-free for over a year. Furthermore, previous reports have shown that pathogen eradication is not needed to provide clinical benefit suggesting that keeping MDRO levels below a certain threshold is key for limiting extraintestinal infections with the colonizing organism.[21] Finally, other underlying factors involved in decolonization, including antibiotic administration and diet, may impact the gut microbiome composition and create a temporary state of dysbiosis where colonization recurrence with either the same or a different MDRO strain may occur.

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

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

Trial Registration

The trial was registered in 2014 under NCT03029078 and is available at https://clinicaltrials.gov/.

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Author contributions

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

Declaration of Competing Interest

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

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Declaration of interests

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106216.

References

1. Vehreschild MJGT, Hamprecht A, Peterson L, Schubert S, Hantschel M, Peter S, et al. *A multicentre cohort study on colonization and infection with ESBL-producing Enterobacteriaceae in high-risk patients with haematological malignancies. J Antimicrob Chemother* 2014;**69**(12):3387–92. https://doi.org/10.1093/jac/dku305
2. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, et al. *Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis* 2012;**55**(7):905–14. https://doi.org/10.1093/cid/cis580
3. McConville TH, Sullivan SB, Gomez-Simmonds A, Whittier S, Uhlemann AC. *Carbapenem-resistant Enterobacteriaceae colonization (CRE) and subsequent risk of infection and 90-day mortality in critically ill patients, an observational study. PLoS One* 2017;**12**(10):e0186195. https://doi.org/10.1371/journal.pone.0186195
4. Magruder M, Sholi AN, Gong C, Zhang L, Edusei E, Huang J, et al. *Gut uropathogen abundance is a risk factor for development of bacteriuria and urinary tract infection. Nat Commun* 2019;**10**(1):5521. https://doi.org/10.1038/s41467-019-13467-w
5. Ferstl PG, Filmann N, Heilgenthal EM, Schnitzbauer AA, Bechstein WO, Kempf VAJ, et al. *Colonization with multidrug-resistant organisms is associated with in increased mortality in liver transplant candidates. PLoS One* 2021;**16**(1):e0245091. https://doi.org/10.1371/journal.pone.0245091
6. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. *Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis* 2019;**19**(1):56–66. https://doi.org/10.1016/S1473-3099(18)30605-4
7. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. *Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet* 2022;**399**(10325):629–55. https://doi.org/10.1016/S0140-6736(21)02724-0
8. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. *Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis* 2018;**18**(3):318–27. https://doi.org/10.1016/S1473-3099(17)30753-3
9. Colomb-Cotinat M, Soing-Altrach S, Leon A, Savitch Y, Poujol I, Naas T, et al. *Emerging extensively drug-resistant bacteria (eXDR) in France in 2018. Med Mal Infect* 2020;**50**(8):715–22. https://doi.org/10.1016/j.medmal.2020.01.011
10. Willems RPJ, van Dijk K, Vehreschild MJGT, Biehl LM, Ket JCF, Remmelzwaal S, et al. *Incidence of infection with multidrug-resistant Gram-negative bacteria and vancomycin-resistant enterococci in carriers: a systematic review and meta-regression analysis. Lancet Infect Dis* 2023;**23**(6):719–31. https://doi.org/10.1016/S1473-3099(22)00811-8
11. Davido B, Moussiegt A, Dinh A, Bouchand F, Matt M, Senard O, et al. *Germs of thrones – spontaneous decolonization of Carbapenem-Resistant Enterobacteriaceae (CRE) and Vancomycin-Resistant Enterococci (VRE) in Western Europe: is this myth or reality? Antimicrob Resist Infect Control* 2018;**7**(1):100. https://doi.org/10.1186/s13756-018-0390-5
12. Bar-Yoseph H, Hussein K, Braun E, Paul M. *Natural history and decolonization strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-analysis. J Antimicrob Chemother* 2016;**71**(10):2729–39. https://doi.org/10.1093/jac/dkw221
13. Machuca I, Gutiérrez-Gutiérrez B, Pérez Cortés S, Gracia-Ahufinger I, Serrano J, Madrigal MD, et al. *Oral decontamination with aminoglycosides is associated with lower risk of mortality and infections in high-risk patients colonized with colistin-resistant, KPC-producing Klebsiella pneumoniae. J Antimicrob Chemother* 2016;**71**(11):3242–9. https://doi.org/10.1093/jac/dkw272
14. Oren I, Sprecher H, Finkelstein R, Hadad S, Neuberger A, Hussein K, et al. *Eradication of carbapenem-resistant Enterobacteriaceae gastrointestinal colonization with nonabsorbable oral antibiotic treatment: a prospective controlled trial. Am J Infect Control* 2013;**41**(12):1167–72. https://doi.org/10.1016/J.AJIC.2013.04.018
15. Tascini C, Sbrana F, Flammini S, Tagliaferri E, Arena F, Leonildi A, et al. *Oral gentamicin gut decontamination for prevention of KPC-producing Klebsiella pneumoniae infections: relevance of concomitant systemic antibiotic therapy. Antimicrob Agents Chemother* 2014;**58**(4):1972–6. https://doi.org/10.1128/AAC.02283-13
16. Bilsen MP, Lambregts MMC, van Prehn J, Kuijper EJ. *Faecal microbiota replacement to eradicate antimicrobial resistant bacteria in the intestinal tract – a systematic review. Curr Opin Gastroenterol* 2022;**38**(1):15–25. https://doi.org/10.1097/MOG.0000000000000792
17. Huttner BD, de Lastours V, Wassenberg M, Maharshak N, Mauris A, Galperine T, et al. *A 5-day course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant Enterobacteriaceae: a randomized clinical trial. Clin Microbiol Infect* 2019;**25**(7):830–8. https://doi.org/10.1016/j.cmi.2018.12.009
18. Tacconelli E, Mazzaferri F, de Smet AM, Bragantini D, Eggimann P, Huttner BD, et al. *ESCMID-EUCIC clinical guidelines on decolonization of multidrug-resistant Gram-negative bacteria carriers. Clin Microbiol Infect* 2019;**25**(7):807–17. https://doi.org/10.1016/J.CMI.2019.01.005
19. Crum-Cianflone NF, Sullivan E, Ballon-Landa G. *Fecal microbiota transplantation and successful resolution of multidrug-resistant-organism colonization. J Clin Microbiol* 2015;**53**(6):1986–9. https://doi.org/10.1128/JCM.00820-15
20. Stripling J, Kumar R, Baddley JW, Nellore A, Dixon P, Howard D, et al. *Loss of vancomycin-resistant Enterococcus Fecal dominance in an organ transplant patient with Clostridium difficile colitis after fecal microbiota transplant. Open Forum Infect Dis* 2015;**2**(2):ofv078. https://doi.org/10.1093/ofid/ofv078
21. Ghani R, Mullish BH, McDonald JAK, Ghazy A, Williams HRT, Brannigan ET, et al. *Disease prevention not decolonization: a model for fecal microbiota transplantation in patients colonized with multidrug-resistant organisms. Clin Infect Dis* 2021;**72**(8):1444–7. https://doi.org/10.1093/cid/ciaa948
22. Davido B, Batista R, Michelon H, Lepainteur M, Bouchand F, Lepeule R, et al. *Is faecal microbiota transplantation an option to eradicate highly drug-resistant enteric bacteria carriage? J Hosp Infect* 2017;**95**(4):433–7. https://doi.org/10.1016/j.jhin.2017.02.001
23. Dinh A, Fessi H, Duran C, Batista R, Michelon H, Bouchand F, et al. *Clearance of carbapenem-resistant Enterobacteriaceae vs vancomycin-resistant enterococci carriage after faecal microbiota transplant: a prospective comparative study. J Hosp Infect* 2018(4):481–6. https://doi.org/10.1016/j.jhin.2018.02.018
24. Fries BE, Schneider DP, Foley WJ, Gavazzi M, Burke R, Cornelius E. *Refining a case-mix measure for nursing homes: resource utilization groups (rug-iii). Med Care* 1994;**32**(7):668–85. https://doi.org/10.1097/00005650-199407000-00002

25. Sokol H, Galperine T, Kapel N, Bourlioux P, Seksik P, Barbut F, et al. *Faecal microbiota transplantation in recurrent Clostridium difficile infection: recommendations from the French Group of Faecal microbiota Transplantation. Dig Liver Dis* 2016;**48**(3):242–7. https://doi.org/10.1016/j.dld.2015.08.017
26. Caballero S, Kim S, Carter RA, Leiner IM, Sušac B, Miller L, et al. *Cooperating commensals restore colonization resistance to vancomycin-resistant Enterococcus faecium. Cell Host Microbe* 2017;**21**(5):592–602.e4. https://doi.org/10.1016/j.chom.2017.04.002
27. Caballero S, Carter R, Ke X, Sušac B, Leiner IM, Kim GJ, et al. *Distinct but spatially overlapping intestinal niches for vancomycin-resistant Enterococcus faecium and carbapenem-resistant Klebsiella pneumoniae. PLoS Pathog* 2015;**11**(9):1–20. https://doi.org/10.1371/journal.ppat.1005132
28. Minot SS, Krumm N, Greenfield NB. *One Codex: a sensitive and accurate data platform for genomic microbial identification. BioRxiv* 2015:027607. https://doi.org/10.1101/027607
29. Singh R, de Groot PF, Geerlings SE, Hodiamont CJ, Belzer C, Berge IJMT, et al. *Fecal microbiota transplantation against intestinal colonization by extended spectrum beta-lactamase producing Enterobacteriaceae: a proof of principle study. BMC Res Notes* 2018;**11**(1):190. https://doi.org/10.1186/s13104-018-3293-x
30. Bilinski J, Grzesiowski P, Sorensen N, Madry K, Muszynski J, Robak K, et al. *Fecal microbiota transplantation in patients with blood disorders inhibits gut colonization with antibiotic-resistant bacteria: results of a prospective, single-center study. Clin Infect Dis* 2017(3):364–70. https://doi.org/10.1093/cid/cix252
31. Biliński J, Grzesiowski P, Muszyński J, Wróblewska M, Mądry K, Robak K, et al. *Fecal microbiota transplantation inhibits multidrug-resistant gut pathogens: preliminary report performed in an immunocompromised host. Arch Immunol Ther Exp* 2016;**64**(3):255–8. https://doi.org/10.1007/s00005-016-0387-9
32. Niki M, Hirai I, Yoshinaga A, Ulzii-Orshikh L, Nakata A, Yamamoto A, et al. *Extended-spectrum β-lactamase-producing Escherichia coli strains in the feces of carriers contribute substantially to urinary tract infections in these patients. Infection* 2011;**39**(5):467–71. https://doi.org/10.1007/s15010-011-0128-2
33. Tariq R, Pardi DS, Tosh PK, Walker RC, Razonable RR, Khanna S. *Fecal microbiota transplantation for recurrent Clostridium difficile infection reduces recurrent urinary tract infection frequency. Clin Infect Dis* 2017:1745–7. https://doi.org/10.1093/cid/cix618
34. Vendrik KEW, de Meij TGJ, Bökenkamp A, Ooijevaar RE, Groenewegen B, Hendrickx APA, et al. *Transmission of antibiotic-susceptible Escherichia coli causing urinary tract infections in a fecal microbiota transplantation recipient: consequences for donor screening? Open Forum Infect Dis* 2022;**9**(7):ofac324. https://doi.org/10.1093/ofid/ofac324
35. Woodworth MH, Conrad RE, Haldopoulos M, Pouch SM, Babiker A, Mehta AK, et al. *Fecal microbiota transplantation promotes reduction of antimicrobial resistance by strain replacement. Sci Transl Med* 2023;**15**(720):eabo2750. https://doi.org/10.1126/SCITRANSLMED.ABO2750
36. Tourret J, Willing BP, Dion S, MacPherson J, Denamur E, Finlay BB. *Immunosuppressive treatment alters secretion of ileal antimicrobial peptides and gut microbiota, and favors subsequent colonization by uropathogenic Escherichia coli. Transplantation* 2017;**101**(1):74–82. https://doi.org/10.1097/TP.0000000000001492
37. Gibson CM, Childs-Kean LM, Naziruddin Z, Howell CK. *The alteration of the gut microbiome by immunosuppressive agents used in solid organ transplantation. Transpl Infect Dis* 2021;**23**(1):e13397. https://doi.org/10.1111/TID.13397
38. Gabarre P, Loens C, Tamzali Y, Barrou B, Jaisser F, Tourret J. *Immunosuppressive therapy after solid organ transplantation and the gut microbiota: bidirectional interactions with clinical consequences. Am J Transpl* 2022;**22**(4):1014–30. https://doi.org/10.1111/AJT.16836
39. Sorbara MT, Dubin K, Littmann ER, Moody TU, Fontana E, Seok R, et al. *Inhibiting antibiotic-resistant Enterobacteriaceae by microbiota-mediated intracellular acidification. J Exp Med* 2019;**216**(1):84–98. https://doi.org/10.1084/jem.20181639
40. Leo S, Lazarevic V, Gaïa N, Estellat C, Girard M, Matheron S, et al. *The intestinal microbiota predisposes to traveler's diarrhea and to the carriage of multidrug-resistant Enterobacteriaceae after traveling to tropical regions. Gut Microbes* 2019;**10**(5):631–41. https://doi.org/10.1080/19490976.2018.1564431
41. Baxter M, Colville A. *Adverse events in faecal microbiota transplant: a review of the literature. J Hosp Infect* 2016;**92**(2):117–27. https://doi.org/10.1016/J.JHIN.2015.10.024
42. Tackin A, Lawrence C, Godin E, Verheye JC, Davido B. *Management in a dedicated ward of patients colonized with highly resistant bacteria: is it worth it? Med Mal Infect* 2020;**50**(5):454–5. https://doi.org/10.1016/j.medmal.2020.01.007
43. van Dijk MD, Voor In 't Holt AF, Polinder S, Severin JA, Vos MC. *The daily direct costs of isolating patients identified with highly resistant micro-organisms in a non-outbreak setting. J Hosp Infect* 2021;**109**:88–95. https://doi.org/10.1016/j.jhin.2020.12.013
44. Davido B, Batista R, Fessi H, Salomon J, Dinh A. *Impact of faecal microbiota transplantation to eradicate vancomycin-resistant enterococci (VRE) colonization in humans. J Infect* 2017;**75**(4):376–7. https://doi.org/10.1016/j.jinf.2017.06.001
45. Saïdani N, Lagier JC, Cassir N, Million M, Baron S, Dubourg G, et al. *Faecal microbiota transplantation shortens the colonisation period and allows re-entry of patients carrying carbapenamase-producing bacteria into medical care facilities. Int J Antimicrob Agents* 2019;**53**(4):355–61. https://doi.org/10.1016/j.ijantimicag.2018.11.014
46. Kazerouni A, Wein LM. *Exploring the efficacy of pooled stools in fecal microbiota transplantation for microbiota-associated chronic diseases. PLoS One* 2017;**12**(1):e0163956. https://doi.org/10.1371/journal.pone.0163956
47. Cold F, Baunwall SMD, Dahlerup JF, Petersen AM, Hvas CL, Hansen LH. *Systematic review with meta-analysis: encapsulated faecal microbiota transplantation – evidence for clinical efficacy. Ther Adv Gastroenterol* 2021;**14**:17562848211041004. https://doi.org/10.1177/17562848211041004/SUPPL_FILE/SJ-DOCX-2-TAG-10.1177_17562848211041004.DOCX
48. Orenstein R, Hecht G, Harvey A, Tillotson G, Khanna S. *Two-year durability of REBYOTA™ (RBL), a live biotherapeutic for the prevention of recurrent Clostridioides difficile infections. Open Forum Infect Dis* 2023;**10**(9):ofad456. https://doi.org/10.1093/ofid/ofad456