

Fecal Microbiota Transplant in Cirrhosis Reduces Gut Microbial Antibiotic Resistance Genes: Analysis of Two Trials

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Antibiotic resistance leads to poor outcomes in cirrhosis. Fecal microbiota transplant (FMT) is associated with reduction in antibiotic resistance gene (ARG) burden in patients without cirrhosis; however, the impact in cirrhosis is unclear. We aimed to study the effect of capsule and enema FMT on ARG abundance in fecal samples, which were collected during two published FMT trials in patients with cirrhosis on rifaximin, lactulose, and proton pump inhibitors. ARGs were identified using metagenomics and mapped against the Comprehensive Antibiotic Resistance Database. Changes in ARG abundance were studied within/between groups. The capsule FMT trial involved a one-time FMT or placebo capsule administration with stool collection at baseline and week 4 postintervention. Antibiotics+enema FMT included preprocedure antibiotics followed by FMT enema versus standard-of-care (SOC). Stool was collected at baseline, postantibiotics, and day 7/15 postintervention. Both trials included 20 patients each. There was no safety/infection signal linked to FMT. In the capsule trial, beta-lactamase (OXY/LEN) expression decreased post-FMT versus baseline. Compared to placebo, patients who were post-FMT had lower abundance of vancomycin (VanH), beta-lactamase (ACT), and rifamycin ARGs; the latter was associated with cognitive improvement. No changes were seen within patients treated with placebo. In the antibiotics+enema trial for postantibiotics at day 7 versus baseline, there was an increase in vancomycin and beta-lactamase ARGs, which decreased at day 15. However, quinolone resistance increased at day 15 versus baseline. Between SOC and FMT, day 7 had largely lower ARG (CfxA beta-lactamase, VanW, and VanX) that continued at day 15 (cepA beta-lactamase, VanW). No changes were seen within the SOC group. **Conclusion:** Despite differences in routes of administration and preintervention antibiotics, we found that ARG abundance is largely reduced after FMT compared to pre-FMT baseline and non-FMT groups in decompensated cirrhosis. (*Hepatology Communications* 2021;5:258-271).

Patients with cirrhosis have an inordinate level of antibiotic exposure due to multiple hospitalizations, instrumentations, treatment of cirrhosis complications (such as variceal bleeding and peritonitis), as well as treatment of common urinary and respiratory infections.⁽¹⁾ This has led to an increasing diagnosis of multidrug resistant (MDR) and fungal infections that are difficult to anticipate and treat. Restricting antibiotic overuse is one important means of mitigating growing antibiotic resistance, but it is insufficient even if optimal guidelines are uniformly followed in patients with cirrhosis. Therefore, other

Abbreviations: AMR, antimicrobial resistance; ARG, antibiotic resistance gene; ARO, antibiotic resistance ontology; ATP, adenosine triphosphate; DSMB, Data Safety Monitoring Board; FMT, fecal microbiota transplant; HE, hepatic encephalopathy; MDR, multidrug resistant; PPI, proton pump inhibitor; RCT, randomized controlled trial; SAE, serious adverse event; SOC, standard of care.

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measures to reduce the antibiotic resistance gene (ARG) burden in cirrhosis are needed.

Fecal microbiota transplant (FMT) has been used extensively in patients with *Clostridioides difficile* infections, with excellent evidence of clinical resolution. There is also increasing evidence that the expression of ARGs is reduced in FMT recipients without cirrhosis being treated for *C. difficile* infections.⁽²⁾ Early phase clinical trials suggest that healthy donor FMT has the potential to correct intestinal dysbiosis and improve symptoms associated with advanced liver disease.^(3,4) Because our initial assessments were 16S based, the impact of FMT on ARGs in cirrhosis remain unclear.

Our aims were to determine the impact of healthy donor FMT on ARG expression in patients with decompensated cirrhosis through metagenomic analysis of the two published randomized controlled trials (RCTs).

Materials and Methods

We used stool samples collected from two prior RCTs in outpatients with cirrhosis who were on lactulose, rifaximin, and proton pump inhibitor (PPI) (supplement) throughout the trial. The FMT was derived from one stool sample from one donor per trial and selected for high abundance of *Lachnospiraceae* and *Ruminococcaceae* (Openbiome, Cambridge, MA) (Supporting Material).

CAPSULAR FMT

After institutional review board (IRB) approval, approval of an investigational new drug (IND) through the U.S. Food and Drug Administration, and informed

consent, eligible patients with cirrhosis and hepatic encephalopathy (HE) on lactulose, rifaximin, and PPI were randomized into placebo or FMT. Details of the eligibility criteria are in the Supporting Material. All patients gave stool at baseline; 15 capsules of FMT or placebo were then given orally, and stool was collected at day 30 postintervention (Fig. 1). We administered 15 capsules of FMT versus placebo once to each participant. The 15 capsules contained 4.125 g stool and are created using wet preparation of stool without lyophilization. Patients were followed for 6 months, and serious adverse events (SAEs) (emergency room visits or hospitalizations), specifically those related to infections, were evaluated and judged as related or not by the Data Safety Monitoring Board (DSMB).

The trial was registered at www.clinicaltrials.gov number NCT03152188 and was a phase 1 trial.

ANTIBIOTICS+ENEMA FMT

This RCT was performed after IND and IRB approval and patient consent. Patients were selected according to eligibility criteria mentioned in the Supporting Material.

Patients were randomized into receiving standard of care (SOC) versus 5 days of broad-spectrum antibiotics (metronidazole 500 mg 3 times a day [tid], amoxicillin 500 mg tid, and ciprofloxacin 500 mg twice daily) followed by 90 mL (27 g) of 10¹² bacteria-containing enema (Fig. 2A). Patients were then followed at day +1 and day +14 post-FMT. Patients with SOC did not receive antibiotics. The trial was registered at www.clinicaltrials.gov number NCT02636647 and performed as a phase 1 trial.

Potential conflict of interest: Dr. Sterling receives grants from Abbott, AbbVie, Roche, and Gilead; he is on the Data and Safety Monitoring Board for Pfizer and AskBio. The other authors have nothing to report.

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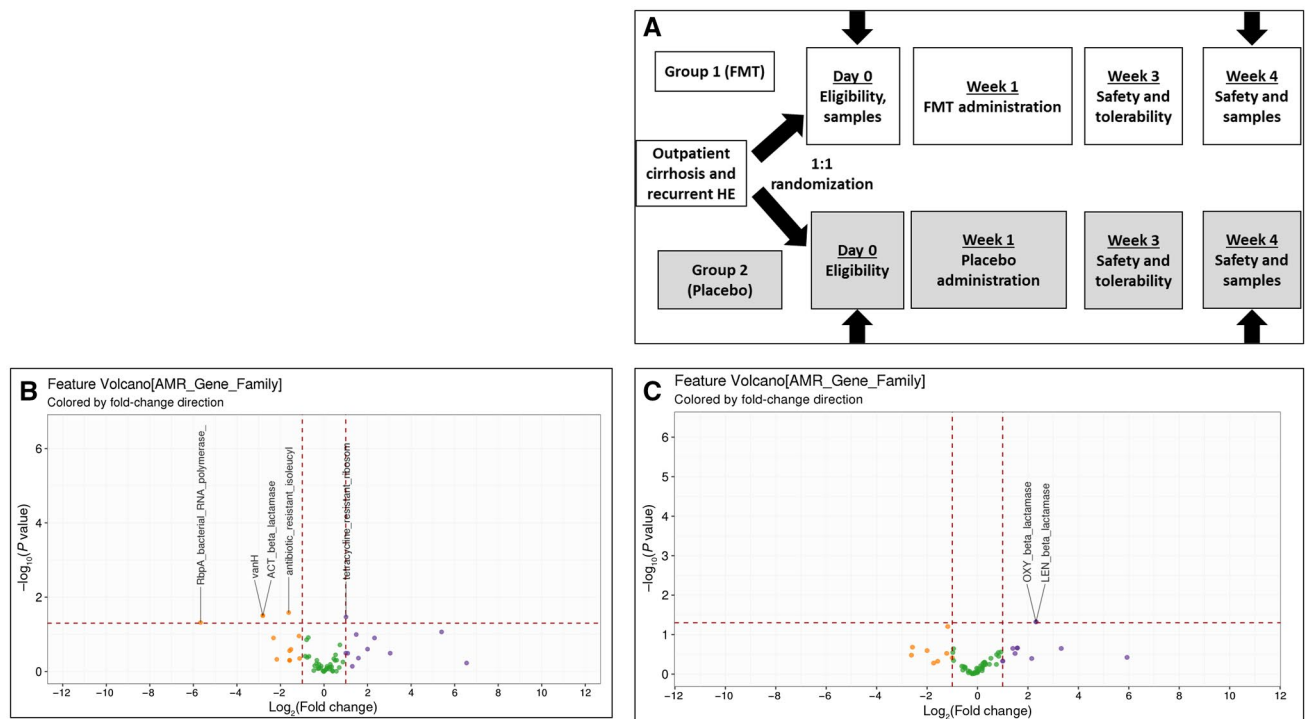


FIG. 1. Capsular FMT trial. (A) Schema of the trial design with solid arrows where stool samples were analyzed for ARGs. (B) Volcano plot of log₂-fold change and *P* values between post-placebo and post-FMT ARGs using Metastats. Orange shows higher post-placebo, purple shows higher post-FMT, green dots are relatively unchanged. (C) Volcano plot of log₂-fold change and *P* values between pre-FMT versus post-FMT ARGs using Metastats. Purple shows higher pre-FMT compared to post-FMT, orange shows the reverse, while green dots are unchanged ARGs. Abbreviations: ARG, antibiotic resistance genes; FMT, fecal microbiota transplant.

Similar to the trial above, patients were followed over 6 months, and SAEs, related or unrelated to FMT, and infections were evaluated and judged by the DSMB. Fecal samples were collected during all visits, as shown in Figs. 1 and 2; DNA was extracted and stored at -80°C until the analysis was performed.

METAGENOMICS

Microbial DNA extraction and sequencing

Metagenomic DNA from fecal samples was extracted using the MO BIO PowerFecal DNA Isolation Kit (Qiagen). Samples were processed in an automated high-throughput manner using the QiaCube DNA/RNA Purification System (Qiagen) with bead beating in 0.1-mm glass-bead plates. Isolated DNA was quantified and normalized using the Quant-iT Picogreen Double-Stranded DNA

Assay Kit. Shotgun metagenomic libraries were prepared with a procedure adapted from the Nextera Library Prep Kit (Illumina). Libraries were subsequently pooled and assessed using the Agilent Bioanalyzer. Sequencing was performed on either an Illumina NextSeq 550 (1 × 150 base pairs [bp]; NextSeq 500/550 High Output v2 kit) or an Illumina NovaSeq 6000 (1 × 100 bp; NovaSeq 6000 S2 Reagent Kit).

Metagenomic Analysis

Reads were processed and annotated using the BoosterShot in-house pipeline. Binary base call (Bcl) files were converted to fastq format using bcl2fastq (Illumina). Cutadapt⁽⁵⁾ was used for adapter and quality (final *Q* score >20) trimming. Reads shorter than 50 bp were filtered out using cutadapt, and all reads were trimmed to 100 bp before downstream alignment and annotation. Quality sequences were

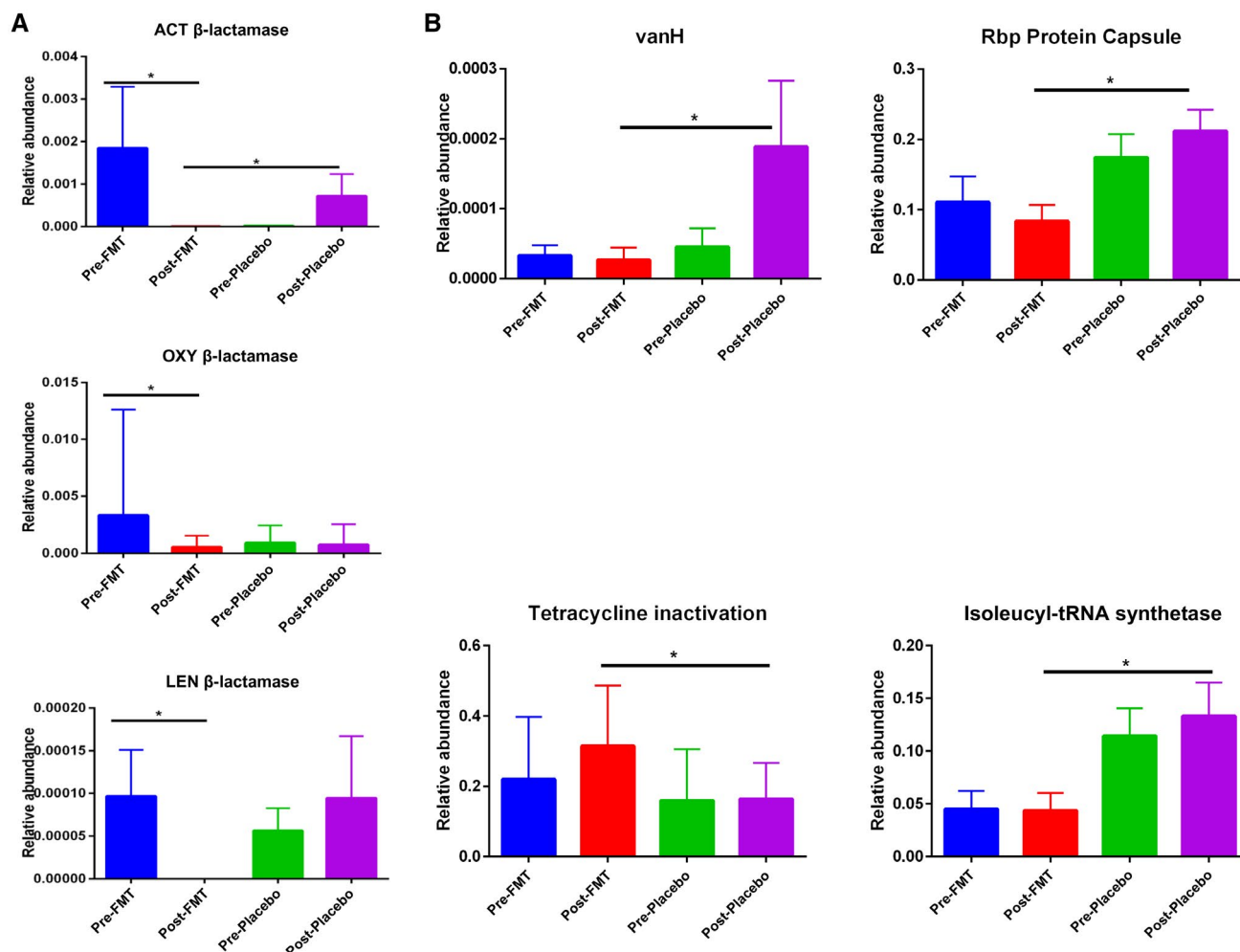


FIG. 2. Changes in specific AMR gene family relative abundances during the capsular FMT trial. (A) Beta-lactamases, (B) other ARGs. All relative gene expression abundances are presented as mean \pm SEM and compared using within and between groups. $*P < 0.05$ using Wilcoxon, analysis of variance, or Kruskal-Wallis test as appropriate. Pre indicates before placebo or FMT; post indicates 4 weeks postintervention. Abbreviation: tRNA, transfer RNA. Abbreviations: ARG, antibiotic resistance genes; FMT, fecal microbiota transplant.

then aligned at 97% identity to a curated database (Venti) containing all representative genomes in the Reference Sequence database⁽⁶⁾ for bacteria and additional manually curated strains using the BURST optimal aligner.⁽⁷⁾ Ties in alignment were broken by minimizing the overall number of unique Operational Taxonomic Units (OTUs). For taxonomic assignment, each input sequence was assigned the lowest common ancestor that was consistent across at least 80% of all reference sequences tied for best hit. Counts were normalized to the species-level average genome length. OTUs accounting for less than one millionth of all species-level genomic markers were discarded as

were those with either less than 0.01% of their unique genome or less than 1% of the whole genome covered by reads in any sample.

BIOINFORMATICS

Metagenomic analyses were performed at Diversigen (www.diversigen.com), and reads after quality trimming were mapped against antimicrobial resistance (AMR) accessions available in the Comprehensive Antibiotic Resistance Database (CARD) (<https://card.mcmaster.ca/>).⁽⁸⁾ CARD includes well-characterized peer-reviewed resistance determinants and associated

antibiotics and is updated monthly. It has data on 88 pathogens, 9,560 chromosomes, 21,362 plasmids, 102,181 whole-genome shotgun assemblies, and 222,011 alleles. The outputs are organized by the antibiotic resistance ontology (ARO) and AMR gene detection models.

Comparisons within and between groups were performed using BiomMiner, which leverages several pipelines for analysis, including Metastats, which we used for these analyses, and includes correction for multiple testing.^(9,10) Baseline was compared to post-FMT and post-FMT versus after the placebo in the capsular FMT trial. Baseline was compared to post-antibiotics, post-FMT day 7 and day 15, and comparisons between post-FMT and post-SOC at days 7 and 15 in the antibiotics+enema FMT trial. Similar comparisons were also performed within the placebo and SOC groups. Log₂-fold change and *P* values were analyzed.

We compared the common and distinct features between each donor and the pre-FMT baseline using Biomminer. Common features denote the top 20 features that are common between the donor and post-FMT based on scaled abundance. Distinct features denote the top 20 features that are distinct between the two groups based on scaled abundance. Individual ARGs that differentiated between and within groups were also evaluated in the donor sequences.

Results

CLINICAL OUTCOMES

As published, there were 20 participants in each trial.^(3,4) In the capsule FMT trial, 20 patients were randomized 1:1 to placebo or FMT. The baseline characteristics were similar with respect to demographics (mean ± SD) (age FMT, 63.3 ± 4.2 vs. placebo, 64.2 ± 6.2 years), sex (8 of 10 were men in each group), Model for End-Stage Liver Disease (MELD) score (FMT, 9.6 vs. placebo, 10.9), and HE episodes and characteristics. During the follow-up period, a lower proportion of patients assigned to capsular FMT required hospitalizations compared to the placebo group (median, 1 vs. 9; *P* = 0.03), but this did not extend to HE-related hospitalizations. We found infections in 3 patients in the placebo group and 2 patients assigned to FMT. Pneumonia, cellulitis,

and infectious gastroenteritis occurred in the placebo-treated group, while incident urinary tract infection (UTI) with pansensitive *Klebsiella pneumoniae* and a pneumonia were the causes of infections in the FMT-assigned groups. These two infections were considered unrelated to FMT by the DSMB.

In the antibiotics+enema FMT trial, again, 20 patients were randomized 1:1 to placebo or FMT. All subjects were men and had similar (mean ± SD) age (FMT, 64.5 ± 5.1 vs. SOC, 62.9 ± 9.8 years), MELD score (FMT, 12.0 ± 2.9 vs. SOC, 13.2 ± 3.7), and HE status. Groups assigned to antibiotics+FMT had a lower rate of total SAEs (FMT, 1 vs. SOC, 7; *P* = 0.02) and HE-related SAEs (FMT, 0 vs. SOC, 5; *P* = 0.03) over the follow-up period. None of the patients assigned to FMT developed an infection over the follow-up, but 2 of the patients in the placebo group did; one infection was a presumed viral gastroenteritis and the other was a pneumonia in which the organism was not isolated.

ARG CHANGES

We present gene abundances in terms of their antibiotic resistance ontology (ARO) acronyms.

Capsular FMT

A comparison of baseline versus post-FMT within the FMT group showed there was significantly higher OXY-beta-lactamase and LEN-beta-lactamase gene abundance at baseline, which was not found after FMT (Figs. 1 and 2 Supporting Table S1).

Recipients who had received the placebo demonstrated significantly higher levels of rifamycin RNA polymerase-binding protein RbpA (RbpA)-bacterial RNA polymerase, vancomycin (VanH), ACT-beta-lactamase, and antibiotic-resistant isoleucyl-transfer RNA synthetase as well as lower tetracycline-resistant ribosomal protection protein ARG compared to patients post-FMT (Table 1; Supporting Table S2). Because this RbpA-bacterial RNA polymerase can be related to rifaximin and cognitive impairment, we correlated end-of-study Stroop OffTime+OnTime values between groups. A high OffTime+OnTime value indicates poor cognition, and we found this improved in the FMT capsule group only.⁽³⁾ We found ARG expression in the group that had received the

TABLE 1. CHANGES IN ARGs IN THE CAPSULE FMT TRIAL

Antibiotic Resistance Genes in Capsule FMT Group	Pre-FMT vs. Post-FMT		Post-FMT vs. After Placebo	
	Higher Pre-FMT	Higher Post-FMT	Higher Post-FMT	Higher After Placebo
Beta-lactamase	-OXY-beta-lactamase-LEN-beta-lactamase	-	-	ACT-beta-lactamase
Vancomycin resistance	-	-	-	VanH
Others	-	-	-	- Rifamycin RbpA-bacterial RNA polymerase - Antibiotic-resistant isoleucyl-transfer RNA synthetase

placebo was positively correlated with Stroop outcomes ($r = 0.88$; $P < 0.001$) but not with the post-FMT group ($r = -0.2$; $P = 0.71$). The correlation was also seen at baseline in the placebo group ($r = 0.47$; $P = 0.04$).

No changes were found between baseline FMT and baseline placebo groups and between before versus after the placebo.

Antibiotics+Enema FMT

Details of the antibiotics+enema FMT are shown in Figs. 3 and 4.

WITHIN THE FMT GROUP

Comparing ARGs before versus after antibiotics, the specific DNA content after antibiotics was too low to be quantified. Therefore, an analysis of the ARGs could not be performed.

Comparing baseline with post-FMT day 7, there was a significant increase in VanH, BlaZ beta-lactamase, adenosine triphosphate (ATP)-binding cassette antibiotic efflux pump, and sulfonamide resistance genes on post-FMT day 7 compared to baseline. The reverse pattern was seen with CfxA beta-lactamase and tetracycline-inactivation enzyme genes, which were higher at baseline compared to post-FMT day 7 (Table 2; Supporting Table S3).

At post-FMT day 15, there was a decrease in VanX, VanS, VanY, and ATP-binding cassette antibiotic efflux pump expression and higher defensin-resistant MprF relative to their expression after post-FMT day 7 (Table 2; Supporting Table S4).

On post-FMT day 15, there was a significant increase in CTX-M and SRT beta-lactamases, fosfomycin, and porin and quinolone resistance genes compared to baseline, while there was a decrease in PDC beta-lactamase expression (Table 2; Supporting Table S7).

BETWEEN FMT AND SOC GROUPS

Post-FMT day 7 showed higher BlaZ beta-lactamase and AA-6 but lower CfxA beta-lactamase, VanW, VanX, and tetracycline inactivation enzymes compared to day 7 SOC (Table 3; Supporting Table S5).

At day 15, Llm 23S ribosomal RNA methyltransferase, lincosamide nucleotidyl-transferase, cepA beta-lactamase, aminocoumarin-resistant parY, and VanW genes were lower post-FMT compared to day 15 SOC (Table 3; Supporting Table S5). No changes were seen within the SOC group or at baseline between the SOC and FMT groups.

COMPARISON OF DONOR ARGs TO BASELINE FMT VALUES

Distinct AROs in the oral capsule donor were not found in the post-FMT common AROs, indicating that none of the AROs from the donor were found post-FMT (Fig. 5A-D). In addition, distinct AROs in the enema donor were not found in the post-FMT common AROs, indicating that none of the AROs from the donor were established in the FMT recipients (Fig. 6A-D). Individual AROs detected in the respective donors are shown in Supporting Tables S8 and S9. In the capsule study, the donor had tetracycline resistance protein genes but none of the other genes. For the enema study, BlaZ beta-lactamase and AAC(6') were present in the donor but none of the other genes that differentiated between and within groups were seen in the donor.

Discussion

There is a growing worldwide epidemic of antibiotic resistance in patients with cirrhosis and other chronic

diseases. In cirrhosis, it is exacerbated by the impaired mucosal and systemic immune response, frequent exposures to health care, and compromised gut barrier. The

consequences of drug resistance in cirrhosis can be dire, with precipitation of acute on chronic liver failure, delisting from the liver transplant list, and death. Current

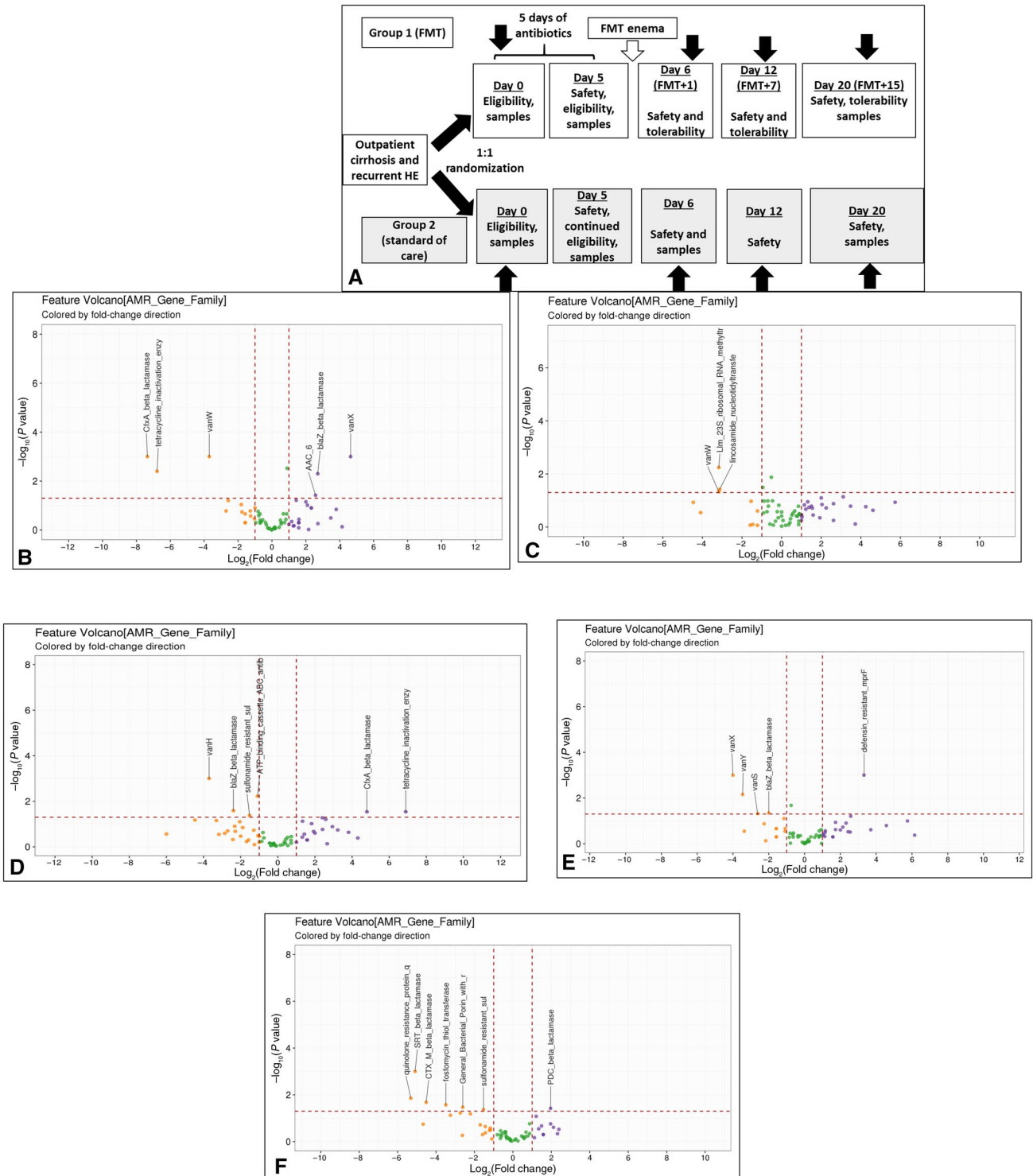


FIG. 3. Antibiotics+enema FMT trial. (A) Schema of the trial design with solid arrows where stool samples were analyzed for ARGs. (B) Volcano plot of log₂-fold change and *P* values between day 7 post-SOC and post-FMT ARGs using Metastats. Orange shows higher day 7 post-SOC, purple shows higher day 7 post-FMT, green dots are relatively unchanged. (C) Volcano plot of log₂-fold change and *P* values between day 14 post-SOC versus post-FMT ARGs using Metastats. Orange shows higher post-SOC versus post-FMT. (D) Volcano plot of log₂-fold change and *P* values between baseline versus post-day 7 FMT ARGs using Metastats. Purple shows higher baseline compared to day 7 post-FMT, orange shows the reverse, green dots are unchanged ARGs. (E) Volcano plot of log₂-fold change and *P* values between post-FMT day 7 versus day 15 ARGs using Metastats. Purple shows higher day 7 compared to day 15 FMT, orange shows the reverse, green dots are unchanged ARGs. (F) Volcano plot of log₂-fold change and *P* values between baseline versus post-day 15 ARGs using Metastats. Purple shows higher baseline compared to day 15 FMT, orange shows the reverse, green dots are unchanged ARGs. Abbreviations: ARG, antibiotic resistance genes; FMT, fecal microbiota transplant.

strategies of restricting antibiotic use are not being fully implemented, and newer strategies to counter the epidemic antibiotic resistance in cirrhosis are needed.⁽¹⁾

Our data span the capsular and enema routes of FMT with/without pre-FMT antibiotics in the setting of decompensated cirrhosis on rifaximin and PPI. This is representative of the most challenging cirrhosis population, which would also be at greatest risk of negative outcomes if a resistant infection were discovered.^(11,12) The data found that ARGs reflected carriage of potential pathobionts belonging to *Enterobacteriaceae* and *Enterococcaceae* even in outpatients included in these trials who were relatively asymptomatic. This builds on prior studies where members of these taxa are found in greater relative abundance as disease progresses and can result in infections.⁽¹³⁻¹⁵⁾ We found encouraging trends toward beta-lactamase and vancomycin-resistance reduction after FMT, regardless of the mode of administration, even in this advanced population.

FMT and antibiotic resistance has the potential to be a double-edged sword. While the current experience with FMT in cirrhosis has a track record of safety,^(3,4,16,17) there remains the potential for transferring MDR organisms in this sick and advanced population.⁽¹⁸⁾ The protocols followed by OpenBiome rigorously tested for these organisms in potential donors. This was complemented by our findings comparing baseline and post-FMT ARG carriage in donors compared to recipients, who did not show any donor-related ARGs related to beta-lactamases or vancomycin-resistance genes that were transferred. On the other hand, in studies across countries in patients without cirrhosis, FMT has been associated in ARG reduction regardless of whether it was used for *C. difficile* infection or primarily for modifying ARGs.⁽¹⁹⁻²¹⁾

Capsular FMT induced a significant reduction post-FMT compared to baseline in the preexisting LEN beta-lactamase genes, which confer resistance to

all beta-lactams. This FMT route also reduced OXY-beta-lactamases, which can act as extended-spectrum beta-lactamase only when induced at high levels. Both OXY and LEN are found in *Klebsiella* spp. This accompanying reduction in ACT beta-lactamases, which are cephalosporinases found in *Escherichia* and *Klebsiella* spp. through plasmid and chromosomal expression post-FMT versus after receiving the placebo, also underlines this potential reduction in pathobiont-related ARG burden. *Escherichia* and *Klebsiella* spp. remain major causes of UTI and spontaneous bacterial peritonitis, two of the most common infections in cirrhosis.

In addition, there was an increase in VanH expression in the placebo group over time that was not found in the FMT group. VanH strengthens the *E. faecium* cell wall against vancomycin through synthesis of D-lactate. Because vancomycin-resistant *Enterococci* are associated with poor outcomes in patients with and without cirrhosis, this potential reduction is encouraging.^(22,23) These were accompanied by relatively low-level nonspecific ARGs for mupirocin, which is rarely used. It is also interesting that resistance to rifamycin antibiotics, such as rifaximin, which all patients were on, also decreased post-FMT compared to placebo. Because cognition improved post-FMT but not after the placebo, it is possible that reduction in rifaximin resistance by the FMT could have played a role in potentially enhancing the impact of rifaximin; this has also been corroborated by the correlation between Stroop tests and relative expression of this ARG in placebo at baseline and study end.⁽³⁾ It is interesting that tetracycline resistance through proteins that bind to the 30S ribosomal subunit and prevent the action were higher post-FMT. These genes are often inherent in healthy microbiota and could reflect soil and food-related changes.⁽²⁴⁾ Tetracycline-related genes were indeed higher in the donors as well; however, because tetracycline is not a commonly used antibiotic in cirrhosis, the potential clinical significance of this finding is unclear.

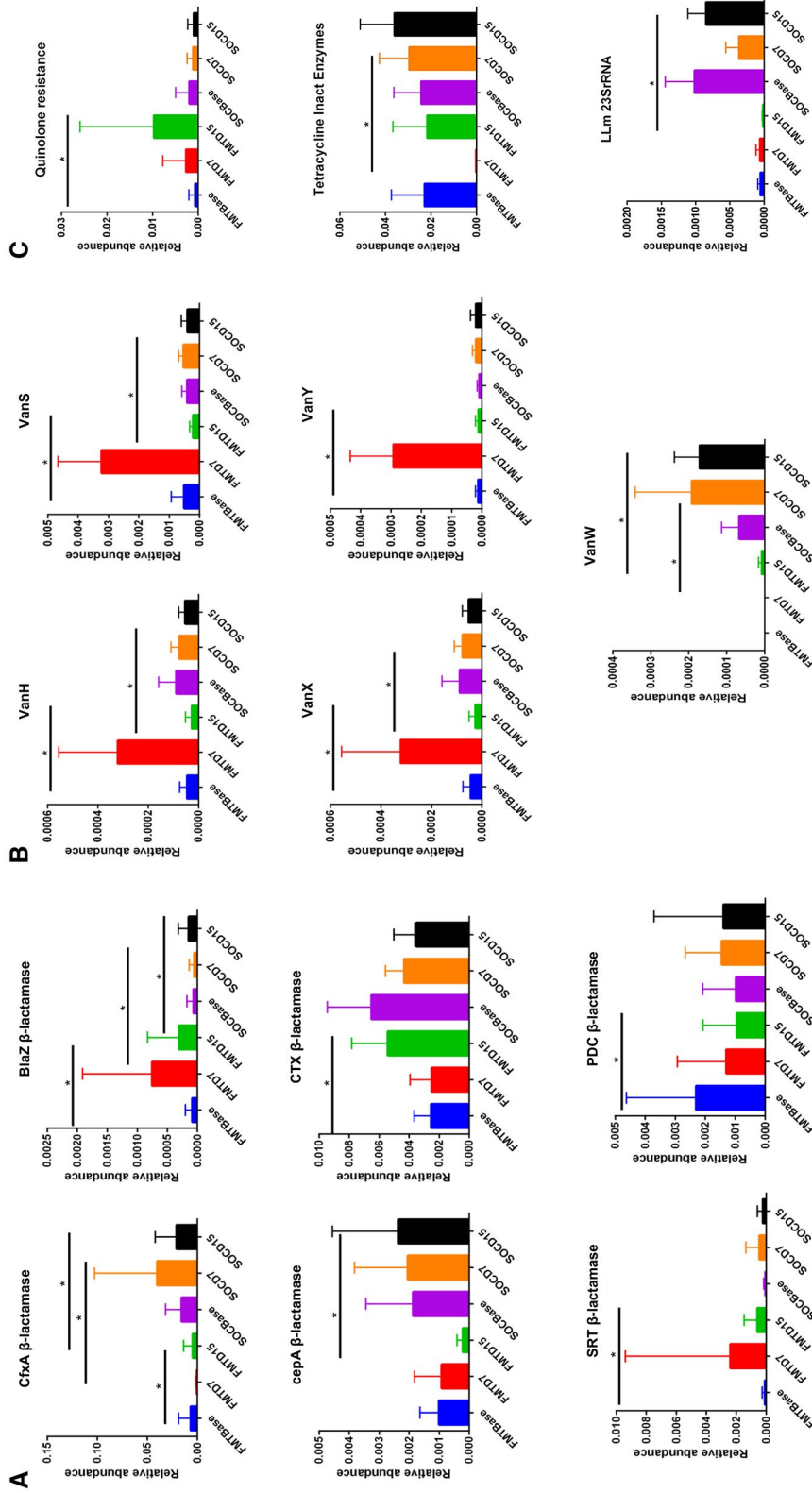


FIG. 4. Changes in specific AMR gene family relative abundances during the antibiotics+enema FMT trial. (A) Beta-lactamases, (B) vancomycin-related ARGs, (C) other ARGs. All relative gene expression abundances are presented as mean \pm SEM and compared using within and between groups. * $P < 0.05$, using Wilcoxon, analysis of variance, or Kruskal-Wallis as appropriate. Abbreviations: ARG, antibiotic resistance genes; FMT, fecal microbiota transplant; FMTBase, baseline; FMTD7, 7 days after FMT; FMTD15, 15 days after FMT; Inact, inactivation; rRNA, ribosomal RNA; SOCBase, standard of care baseline; SOCD7, 7 days after SOC; SOCD15, 15 days after SOC.

TABLE 2. CHANGES IN ARGs IN THE ANTIBIOTICS+ENEMA FMT TRIAL WITHIN-GROUP COMPARISONS

Antibiotic Resistance Genes Within FMT Group	Baseline vs. FMT Day 7		Post-FMT Day 7 vs. Day 15		Baseline vs. FMT Day 15	
	Higher Baseline	Higher Post-FMT Day 7	Higher Post-FMT Day 7	Higher Post-FMT Day 15	Higher Baseline	Higher Post-FMT Day 15
Beta-lactamase	CfxA beta-Lactamase	BlaZ beta-Lactamase	-	-	PDC beta-Lactamase	CTX beta-Lactamase SRT beta-Lactamase
Vancomycin resistance	-	VanH	-VanX -VanS -VanY	-	-	-
Others	Tetracycline Inactivation enzymes	- ATP-binding cassette antibiotic efflux pump - Sulfonamide resistance	ATP-binding cassette antibiotic efflux pump	defensin-resistant MprF	- Quinolone resistance protein - Sulfonamide resistance - General bacterial porin	-

The complicated nature of the antibiotics+enema FMT trial included additional broad-spectrum antibiotics and their potential impact and the subsequent FMT. There was significant reduction in microbial DNA after the antibiotics, which may have enhanced engraftment of the donor microbiota following the FMT enema. Over the duration of the trial, there was evolution of the ARGs compared to baseline and to the SOC group at days 7 and 14. CfxA beta-lactamase was relatively high at baseline before antibiotics or FMT, and it continued to be higher compared to day 7 in the FMT group. CfxA beta-lactamase was lower in FMT day 7 samples compared to SOC. This gene has been described in oral bacteria and *Pseudomonas*, all of which are relevant and increased in the stool with PPIs in cirrhosis.⁽²⁵⁻²⁷⁾ The opposite trend was seen with BlaZ beta-lactamase, which is found across multiple pathobionts belonging to *Staphylococcus* spp.⁽²⁸⁾ This was likely introduced by the pre-FMT antibiotics. However, it decreased after day 14 compared to day 7 FMT and compared to day 15 SOC, indicating that donor engraftment likely reduced the expression at day 15 postenema. The relative increase in expression of vancomycin resistance genes found in *E. fecalis* and *E. faecium* as well as ATP-binding cassette antibiotic efflux pump at day 7 post-FMT compared to baseline and day 14 post-FMT is likely due to antibiotics.⁽²⁹⁾ Interestingly, despite antibiotics and FMT, vancomycin resistance expression was still lower than in the SOC group at both time points and none of these were related to the donor metagenome.

Compared to day 7 within the FMT group and day 15 within the SOC group, the day 15 post-FMT group had reduced expression of CepA (cephalosporinases in *B. fragilis*), VanW (*E. fecalis*), lincosamide (includes clindamycin), and aminocoumarin resistance gene expression. This broad swathe of potentially beneficial reduction in ARGs is in line with the overall benefit induced by FMT, which also restored bile acid and short-chain fatty acid production capacity after it was reduced by the antibiotics.⁽³⁰⁾

The use of preprocedure broad-spectrum antibiotics complicates the interpretation because compared to baseline, patients on post-FMT day 15 continued to show high quinolone resistance and other forms of beta-lactamases, which were not sourced from the donor. The pre-FMT antibiotics were meant to enhance engraftment but could have also encouraged

TABLE 3. CHANGES IN ARGs IN THE ANTIBIOTICS+ENEMA FMT TRIAL BETWEEN-GROUP COMPARISONS

Antibiotic Resistance Genes Between SOC and FMT Groups	Day 7 Post-FMT vs. Post-SOC		Day 15 Post-FMT vs. Post-SOC	
	Higher Post-FMT	Higher Post-SOC	Higher Post-FMT	Higher Post-SOC
Beta-lactamase	BlaZ beta-lactamase	CfxA beta-lactamase	–	cepA beta-lactamase
Vancomycin resistance	–	vanW, vanX	–	VanW
Others	AAC-6'	Tetracycline inactivation enzymes	–	- Llm 23S ribosomal RNA methyltransferase - lincosamide nucleotidyl-transferase - aminocoumarin-resistant parY

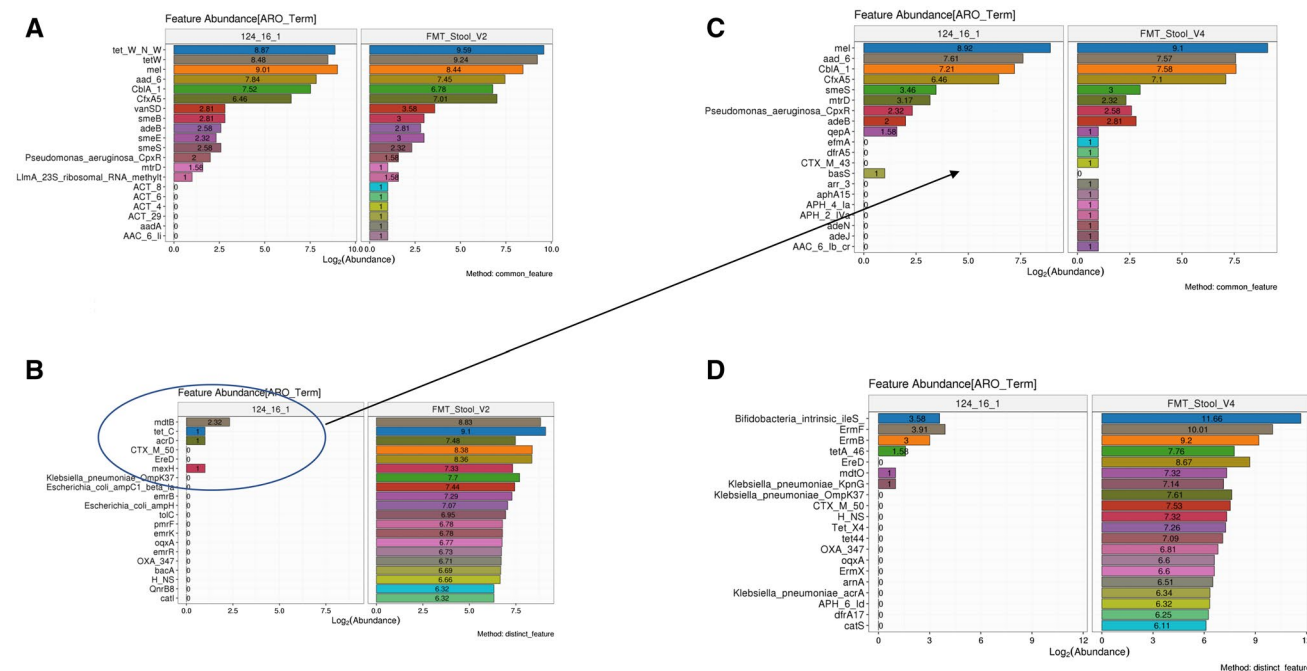


FIG. 5. Comparison of donor AROs with post-FMT AROs in the capsule trial. (A-D) Common and distinct features of ARG abundance at baseline and post-FMT in the capsule FMT trial. Donor number was 124_16_1, and V2 is baseline while V4 is post-FMT. (A) ARGs that are common at baseline; (B) ARGs that are distinct at baseline between the donor and the recipients; (C) ARGs that are common at post-FMT; (D) ARGs that are distinct post-FMT between the donor and the recipients. The arrow shows that none of the ARGs that were distinct at baseline were those that ended being common post-FMT. Abbreviations: ARG, antibiotic resistance genes; FMT, fecal microbiota transplant.

higher expression of quinolone and certain beta-lactamases genes that were incompletely reduced by FMT. On the other hand, FMT resulted in a consistently lower vancomycin ARG burden.

These data extend prior studies of FMT and ARG reduction into the advanced cirrhosis realm and demonstrate that a wide range of ARGs are targeted with healthy donor FMT.⁽¹⁹⁾ This remains an unmet need that could potentially be targeted by FMT in cirrhosis.⁽³¹⁾

This study is limited by small sample size, the advanced nature of cirrhosis in our patients, and continued use of PPIs and rifaximin. In addition, the two trials are different in design with the route of administration and preprocedure antibiotics, which can impact engraftment. Preprocedure broad-spectrum antibiotics likely induced ARG expression, which, when combined with the baseline values, added to the dysbiosis. This was reduced with respect to vancomycin, but quinolone resistance and some beta-lactamases were incompletely

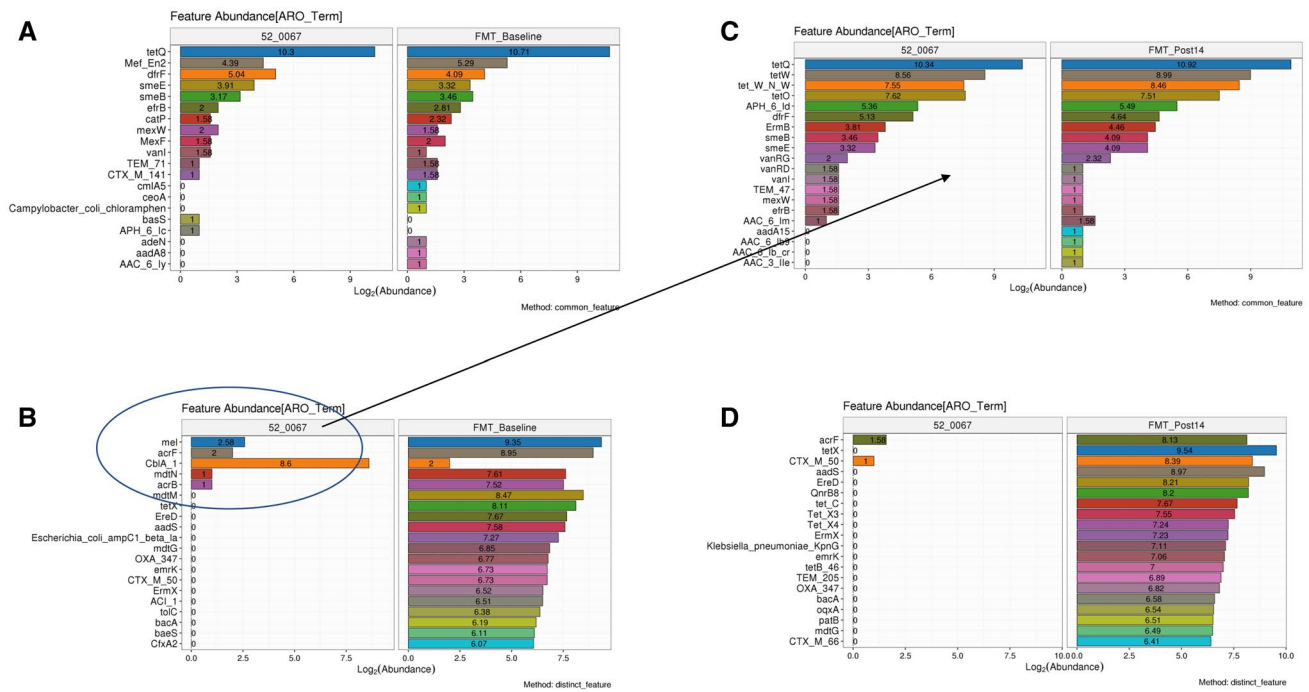


FIG. 6. Comparison of donor AROs with post-FMT AROs in the enema trial. (A-D) Common and distinct features of ARG abundance at baseline and post-FMT in the antibiotics+enema FMT trial. Donor number was 52_0067. (A) ARGs that are common at baseline; (B) ARGs that are distinct at baseline between the donor and the recipients; (C) ARGs that are common at post-FMT; (D) ARGs that are distinct post-FMT between the donor and the recipients. The arrow shows that none of the ARGs that were distinct at baseline were those that ended being common post-FMT. Abbreviations: ARG, antibiotic resistance genes; FMT, fecal microbiota transplant.

suppressed after FMT using enema. We only used one administration, and further studies are needed to evaluate if multiple administrations can reduce this burden. This again points toward the negative impact and need to restrict indiscriminate antibiotic use in this vulnerable population.⁽¹¹⁾ This was further highlighted by a largely consistent suppression of ARGs in the capsular FMT trial, which did not include preprocedure antibiotics.

Despite the difference in trial design, the patients included were relatively homogeneous and the population reflects the advanced cirrhosis population that is often prone to life-threatening infections. The data here may not be applicable to populations earlier on in the disease process or to patients with cirrhosis from other sites, given the relatively localized nature of antibiotic resistance patterns. The two studies had differing designs, including route of FMT and preprocedure antibiotics and timing of stool collection. However, major changes in beta-lactamase and vancomycin resistance genes post-FMT and the relative homogeneity of the population could improve the generalizability of these data. Although we did not isolate pathogens,

prior metagenomic studies have linked specific ARGs to the presence of these pathobionts.⁽³²⁾ While the gut is a reservoir for drug resistance, the determinants of progression from colonization to infection are not always clear.⁽³³⁾ However, carriage of ARGs remains a major risk factor for development of those specific infections, especially in those with impaired immune response.⁽³⁴⁻³⁷⁾ Patients with cirrhosis have an altered immune response and are prone to these infections; therefore, even though none of our patients developed proven MDR-associated infections, potential reduction in this burden may be of value.^(38,39)

We conclude in this metagenomic analysis that there is a relative reduction in antibiotic resistance gene expression after capsule or enema FMT in patients with decompensated cirrhosis. Vancomycin resistance ARGs were more likely to be suppressed by the FMT, while some beta-lactamase and quinolone resistance genes could be enhanced by preprocedure antibiotics. Further trials dedicated toward defining FMT as a potential approach to reduce antibiotic resistance in cirrhosis are needed.

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