Table S1. Bacterial strains and primers included in this study.

Strains of *Lactobacillus* spp.	**Description**	**Reference**
L. casei LC-39	Infant fecal isolate	(1)
L. gasseri LG-3	Infant fecal isolate	(2)
L. rhamnosus LR-34	Infant fecal isolate	(1)
L. reuteri DSM 17938	Daughter strain derived from *L. reuteri* ATCC 55730, a Peruvian mother's breastmilk isolate	Biogaia AB; (3)
L. reuteri DSM 17938::*pocR*	Insertional mutation in the *pocR* gene, ErmR	(4)
L. reuteri DSM 17938::*gdh*	Insertional mutation in the *gdh* gene, ErmR	This study
L. reuteri ATCC PTA 6475	Isolate from Finnish mother's milk	BioGaia AB
L. reuteri ATCC PTA 6475::*pocR*	Insertional mutation in the *pocR* gene, ErmR	(5)
L. reuteri ATCC PTA 6475::*pocR* pJKS100	Contains an insertional mutation in the *pocR* gene and an empty *E. coli-L. reuteri* shuttle vector, ErmR, CmR	(5)

L. reuteri ATCC PTA 6475::*pocR* pJKS102	Insertional mutation in the *pocR* gene and a complementation vector containing the *pocR* gene, ErmR, CmR	(5)
L. reuteri ATCC PTA 6475::*gdh* (Also known as *L. reuteri* PRB94)	Insertional mutation in the *gdh* gene, ErmR	(6)
Pathogenic *C. difficile* strains	**Description**	**Source**
CD2015	NAP1/027 clinical isolate from CDI patient stool	(7)
R20291	NAP1/027 clinical isolate from CDI patient stool	(8)
VPI 10463	Ribotype 087, hyper-virulent, lab-adapted strain	(9)
630	Ribotype 012, lab-adapted, less virulent strain	(10)
Primers	**Primer sequence (5' – 3'), description**	**Source**
C. difficile 16S rRNA gene gene	F: TTGAGCGATTTACTTCGGTAAAGA R: CCATCCTGTACTGGCTCACCT	(11)
Universal 16S rRNA gene gene	F: GCAGGCCTAACACATGCAAGTC R: CTGCTGCCTCCCGTAGGAGT	(12)

ErmR = erythromycin resistant; CmR = chloramphenicol resistant

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12. **Castillo M, Martin-Orue SM, Manzanilla EG, Badiola I, Martin M, Gasa J.** 2006. Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by real-time PCR. Vet Microbiol **114:**165-170.

Table S2. Sample identifiers and barcode sequences.

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0714.REUT1B.A115.1	CGACGAGTACT	0714.REUT1B.C814.1	AGTGTAGTAGT
0714.REUT1B.A116.1	ACAGACTATAT	0714.REUT1B.C815.1	GTGTACGACGT
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0714.REUT1B.A121.1	AGTACGAGAGT	0714.REUT1B.C819.1	ACTACGTCTCT
0714.REUT1B.A123.1	CGTACGTCGAT	0714.REUT1B.C821.1	CAGTACGTACT
0714.REUT1B.A210.1	ACAGACAGCGT	0714.REUT1B.C823.1	ACACTCATACT
0714.REUT1B.A212.1	ACACTACTCGT	0714.REUT1B.C910.1	ACGATGAGTGT
0714.REUT1B.A214.1	AGACTCGACGT	0714.REUT1B.C912.1	ACGTAGATCGT
0714.REUT1B.A215.1	CGATACTACGT	0714.REUT1B.C914.1	ATAGTATACGT
0714.REUT1B.A216.1	ACAGAGACTCT	0714.REUT1B.C915.1	ACACAGTGAGT
0714.REUT1B.A219.1	ACGAGTAGACT	0714.REUT1B.C916.1	ACACGACGACT
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0714.REUT1B.A223.1	CTACTCGTAGT	0714.REUT1B.C921.1	CGACGACGCGT
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0714.REUT1B.A312.1	ACGACACGTAT	0714.REUT1B.D1010.1	ACGCGAGAGAT
0714.REUT1B.A314.1	AGTACGAGAGT	0714.REUT1B.D1012.1	ACTACGTCTCT
0714.REUT1B.A315.1	CGTACGTCGAT	0714.REUT1B.D1014.1	CAGTACGTACT
0714.REUT1B.A316.1	ACAGCTCGTGT	0714.REUT1B.D1015.1	ACACTCATACT
0714.REUT1B.A319.1	ACGCGTCTAGT	0714.REUT1B.D1016.1	ACACGTAGTAT
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0714.REUT1B.B412.1	ACGAGTAGACT	0714.REUT1B.D1210.1	ACACGACGACT
0714.REUT1B.B414.1	AGTACTACTAT	0714.REUT1B.D1212.1	ACTATACGAGT
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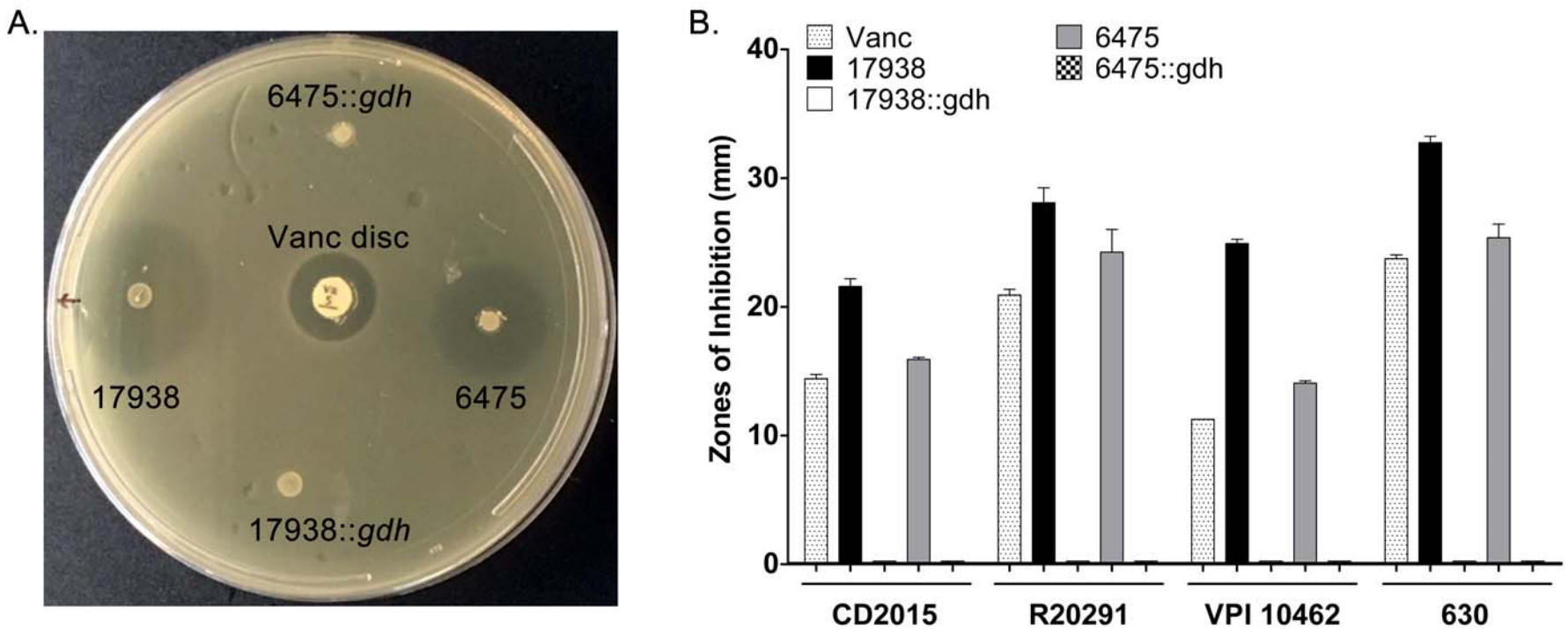

Figure S1. Reuterin-dependent *C. difficile* inhibition requires the glycerol dehydratase (*gdh*) gene. Spots of *L. reuteri* strains and a vancomycin disc (5 ug) were overlain with *C. difficile* to assess the effect of the inactivated *gdh* gene on *C. difficile* growth. Clear zones of inhibition were measured (mm). A) Representative image of the pathogen overlay assay showing clear zones where *C. difficile* CD2015 was inhibited. Wildtype *L. reuteri* 17938 and 6475 and isogenic *gdh* insertion mutants (17938::gdh and 6475:gdh) are shown. B) Bar graph representing zone of inhibition measurements (mm). Results represent the mean +/- S.E.M., n=3.

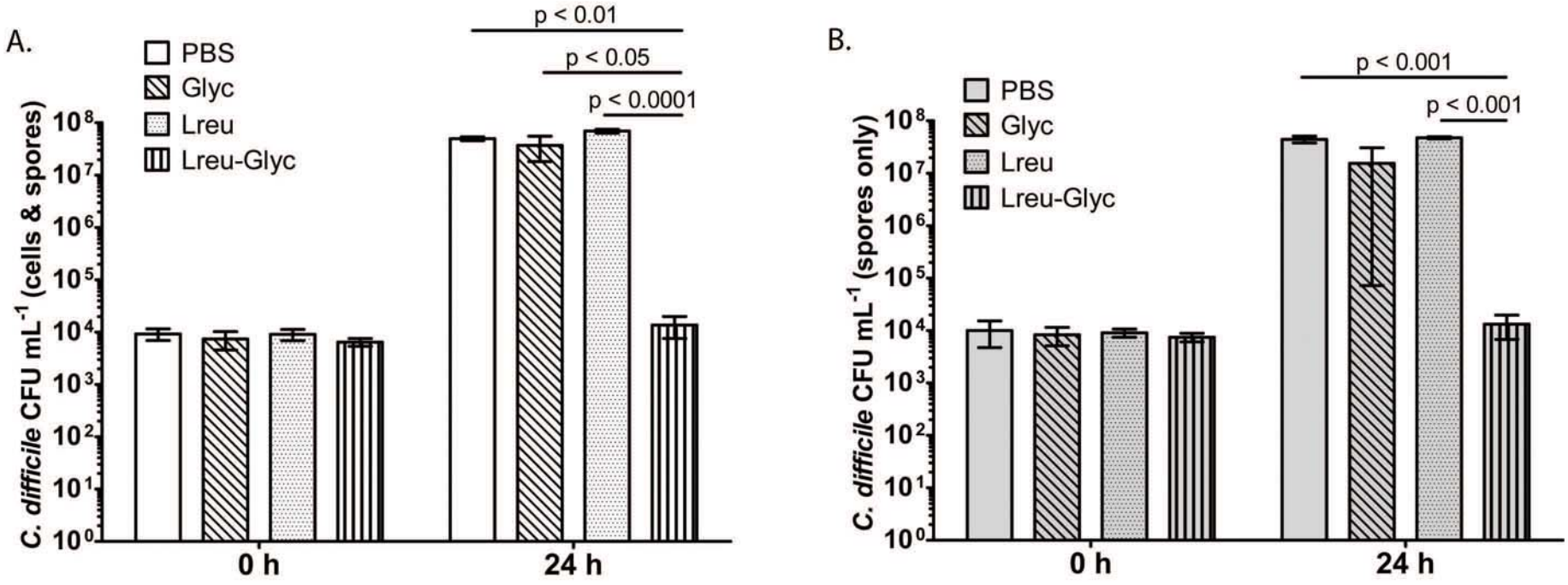

Figure S2. *Ex vivo* analysis of glycerol-dependent *L. reuteri*-mediated growth inhibition of *C. difficile*. *Ex vivo* germination and outgrowth of *C. difficile* VPI 10463 spores were measured in cecal contents from germ-free mice (n = 3 mice per group). Results for (A) spores and vegetative cells and (B) spores alone that outgrew after incubation for 24 h. Data represent the mean +/- S.E.M. Significance between groups was determined by using two-way ANOVA with Bonferroni multiple-comparison correction.

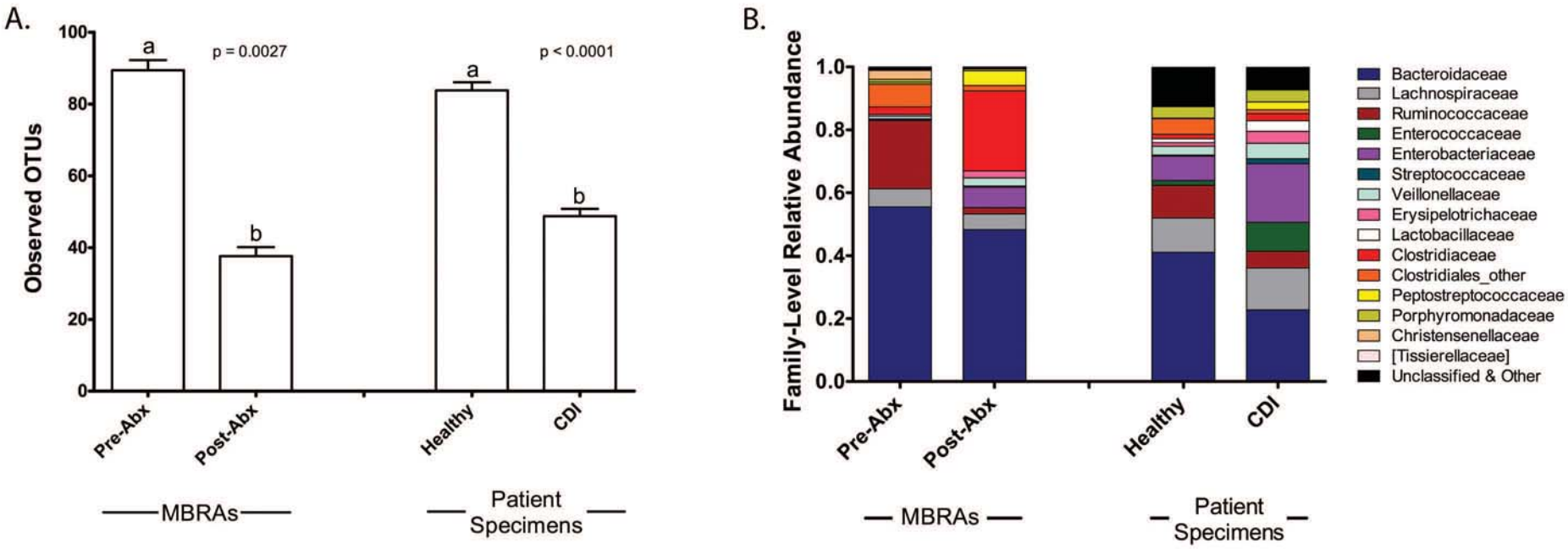

Figure S3. Antibiotic treatment of human fecal MBRAs results in community dynamics mimicking CDI. (A) Antibiotic treatment of MBRAs results in a significant loss in observed species similar to that seen when comparing differences in feces of healthy adults to patients with CDI. Significance was determined by the Mann-Whitney U test. (B) Taxonomic summary illustrating similar family-level relative abundance changes in microbiomes before and after antibiotic treatment (MBRAs) and in feces from healthy subjects (Healthy) or patients with CDI (CDI).