Supplemental Information

Cooperating commensals restore resistance to vancomycin-resistant *Enterococcus faecium*

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Figure S1

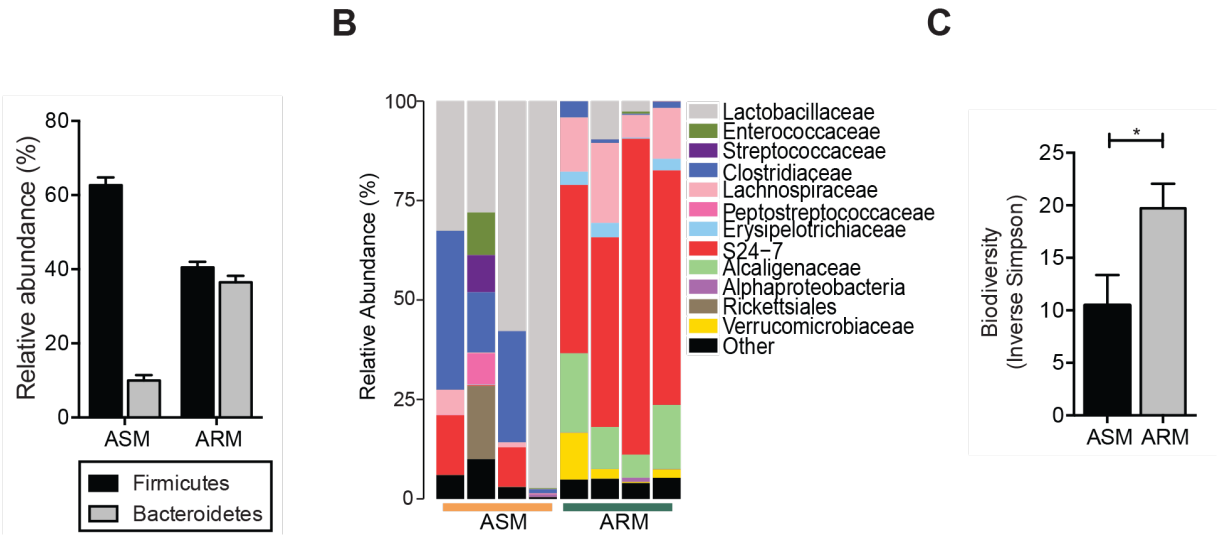
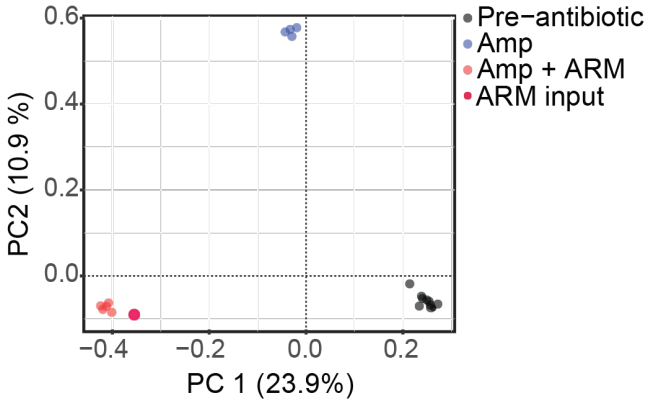

A
B
C
Relative abundance (%)
80
60
40
20
0
ASM
ARM
Firmicutes
Bacteroidetes
Relative Abundance (%)
100
75
50
25
0
ASM
ARM
Lactobacillaceae
Enterococcaceae
Streptococcaceae
Clostridiaceae
Lachnospiraceae
Peptostreptococcaceae
Erysipelotrichiaceae
S24−7
Alcaligenaceae
Alphaproteobacteria
Rickettsiales
Verrucomicrobiaceae
Other
Biodiversity
(Inverse Simpson)
25
20
15
10
5
0
*
ASM
ARM

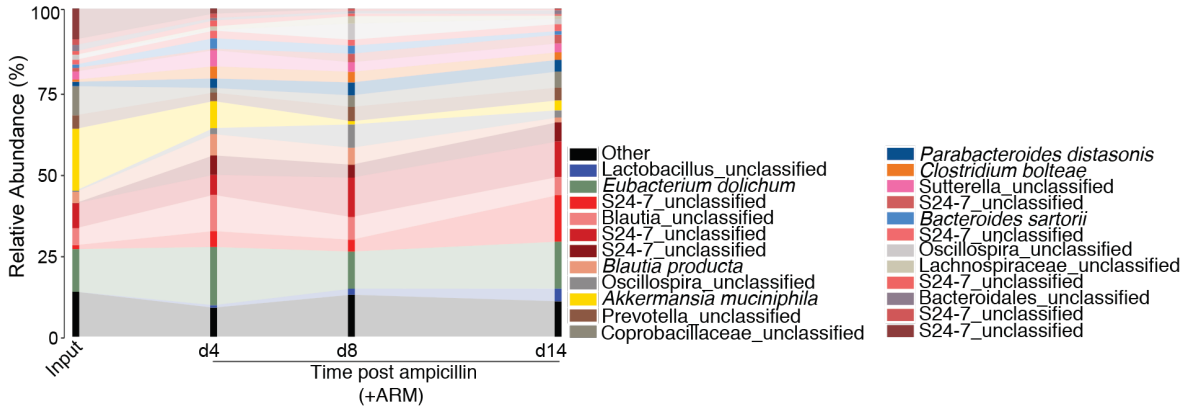
Figure S1. Related to Figure 1. Bacterial abundance and composition of ampicillin-resistant and antibiotic-sensitive microbiota. (A) Relative abundance of Firmicutes and Bacteroidetes in fecal ARM and ASM. **(B)** Family-level classification of OTUs present at >0.01% relative abundance in the ileal compartment. Each bar corresponds to an individual mouse. **(C)** Biodiversity of the ileal microbiota (**P* = 0.0137, Student's *t* test; n = 4 per group). ARM, antibiotic-resistant microbiota; ASM, antibiotic-sensitive microbiota. Data are means ± SEM.

Figure S2

A



B



C

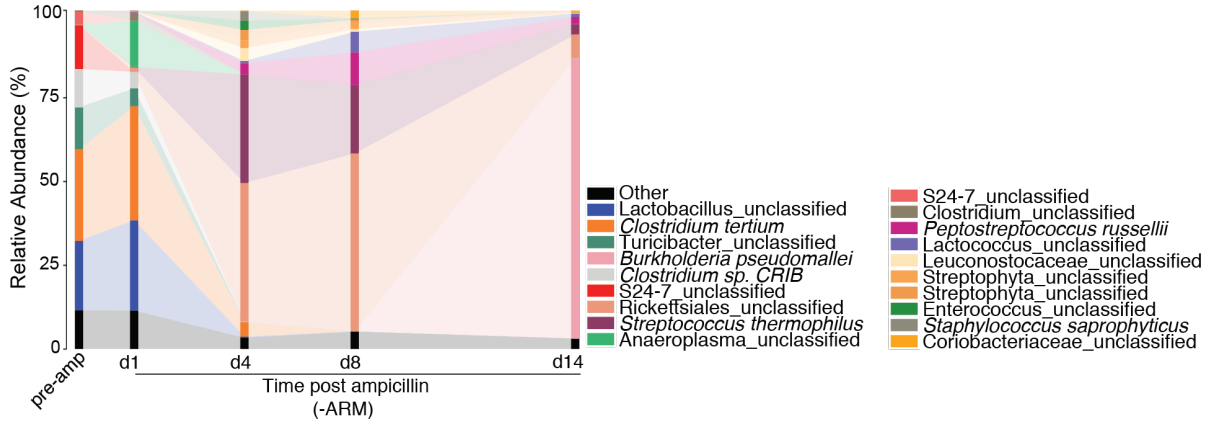
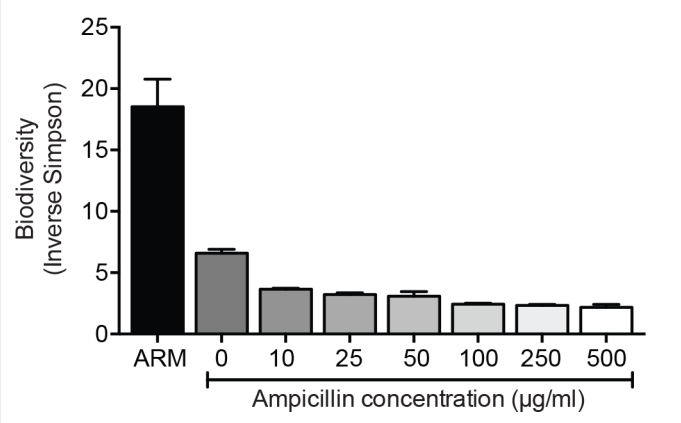


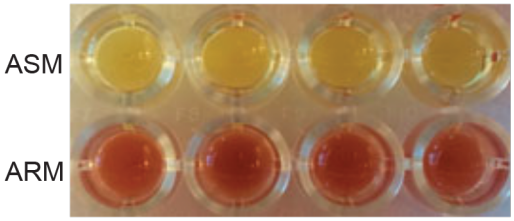
Figure S2. Related to Figure 2. ARM administration fully reconstitutes the microbiota of ampicillin-treated mice. (A) Principal component analysis of fecal microbial communities from mice prior to antibiotic treatment and two weeks following ampicillin treatment with or without ARM transplantation. Input indicates microbial composition of ARM transplant administered to mice. **(B-C)** Microbiota composition of ampicillin-treated mice transplanted with **(B)** ARM or **(C)** PBS at different time points during ampicillin (amp) treatment. Each bar represents the average of five mice per group.

Figure S3

A



B



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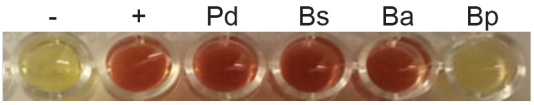
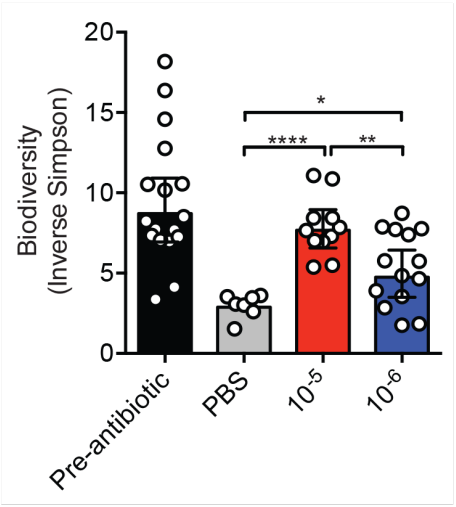


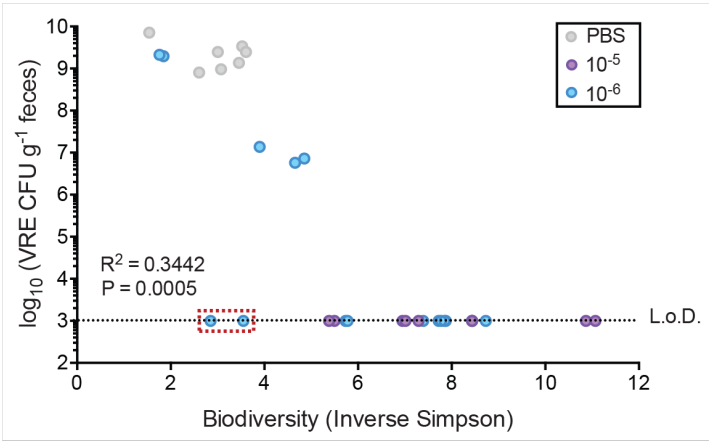
Figure S3. Related to Figure 4. A subset of bacterial strains within ARM resists ampicillin by producing β-lactamase. (A) Biodiversity of cultures from 10^{-5}-fold diluted ARM grown on plates containing 0, 10, 50, 100 and 500 μg/ml of ampicillin (n = 4 mice per group). Data are means ± SEM. **(B-C)** β-lactamase activity in **(B)** ARM and ASM fecal samples and (**C**) bacterial isolates derived from ARM. Pd, *Parabacteroides distasonis*; Bs, *Bacteroides sartorii*; Ba, *Bacteroides acidifaciens*; Bp, *Blautia producta*; -, PBS (negative control); +, Amp^R *E. coli* DH5α (positive control).

Figure S4

A



B



C

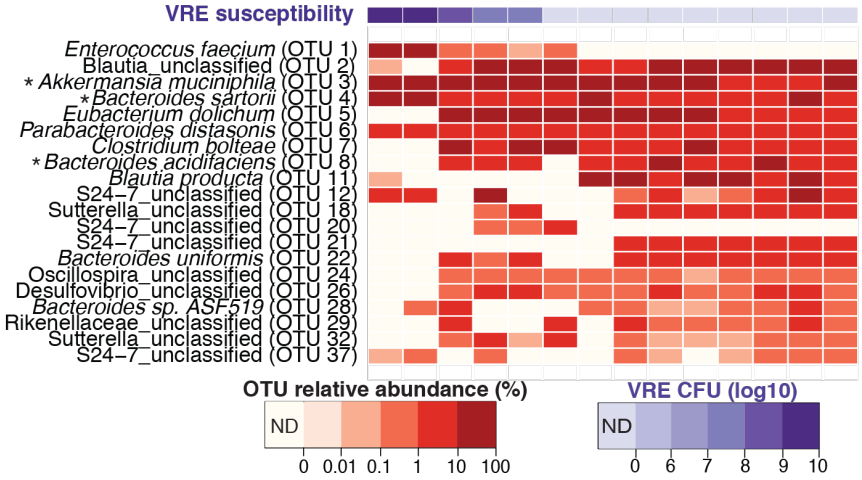
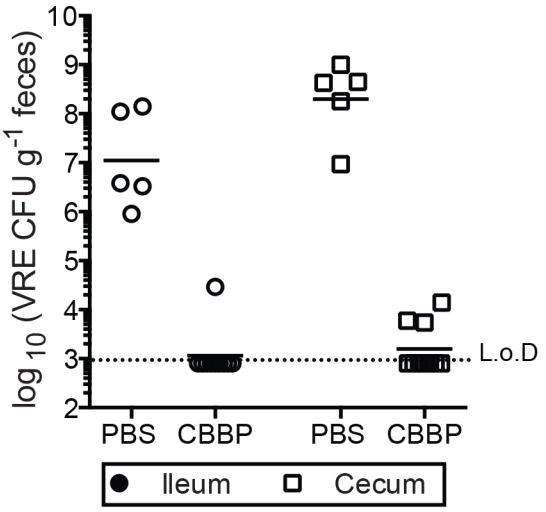


Figure S4. Related to Figure 4. Low biodiversity microbiota can provide VRE resistance. (A) Biodiversity of fecal microbiota of antibiotic-naïve mice and ampicillin-treated mice inoculated with PBS, 10^{-5} or 10^{-6} plate cultures from ARM (*P = 0.0193, **P = 0.0071, ****P < 0.0001, Student's t test; n = 8-16 mice per group). Data are means ± SEM. **(B)** Pearson correlation between VRE levels and biodiversity. Red box indicates mice that received 10^{-6} plate cultures and became VRE-resistant despite exhibiting low biodiversity. L.o.D., limit of detection. **(C)** Sequences from fecal samples from ampicillin-treated mice inoculated with PBS or 10^{-6} plate cultures were binned into OTUs. Mice were stratified based on their levels of VRE colonization. The relative abundance of the top 20 OTUs (horizontal bars) in each individual mouse (vertical bars) and corresponding VRE density 3 days post challenge are shown. *, high abundance OTUs not significantly-associated with protection but present in all mice. L.o.D., limit of detection.

Figure S5

A


log 10 (VRE CFU g-1 feces)
10
9
8
7
6
5
4
3
2
L.o.D
PBS
CBBP
PBS
CBBP
Ileum
Cecum

B

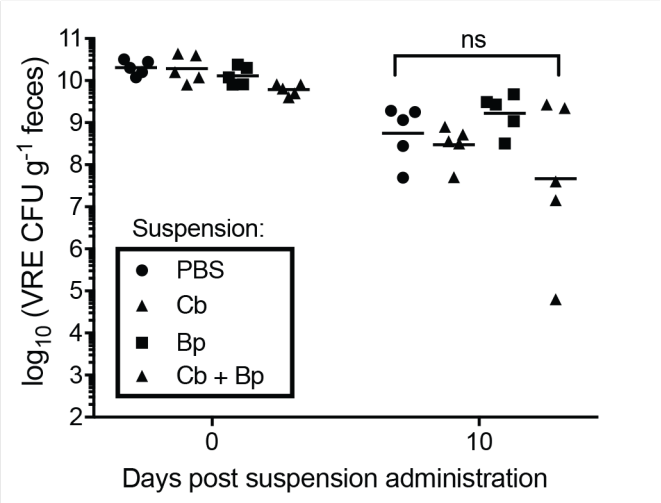
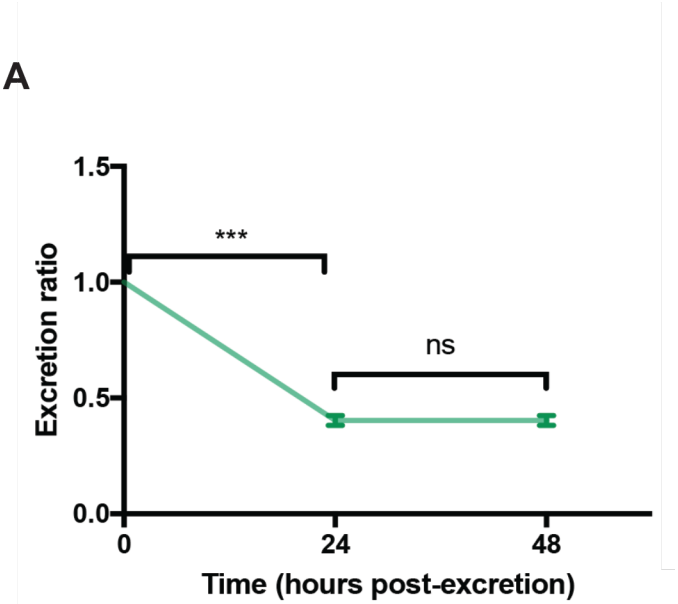

log10 (VRE CFU g-1 feces)
11
10
9
8
7
6
5
4
3
2
ns
Suspension:
PBS
Cb
Bp
Cb + Bp
0
10
Days post suspension administration

Figure S5. Related to Figure 6. All members of CBBP are required for VRE clearance. (A) Ampicillin-treated, VRE-colonized mice received either PBS or the CBBP consortium for 3 consecutive days following ampicillin cessation. Mice were sacrificed 12 days post PBS/CBBP treatment and VRE burden was quantified in content from the ileal and cecal compartments. L.o.D., limit of detection. **(B)** Ampicillin-treated mice were challenged with VRE and following discontinuation of antibiotics received the first of three doses of PBS, *C. bolteae* (Cb), *B. producta* (Bp) or a combination of both (Cb + Bp) by oral gavage starting on the third day post VRE challenge. VRE levels were quantified prior to treatment (day 0) and 10 days after the first gavage (ns = non-significant, Mann-Whitney test; n=5 mice per group).

Figure S6

A


1.5
1.0
0.5
0.0
Excretion ratio
***
ns
0
24
48
Time (hours post-excretion)

B

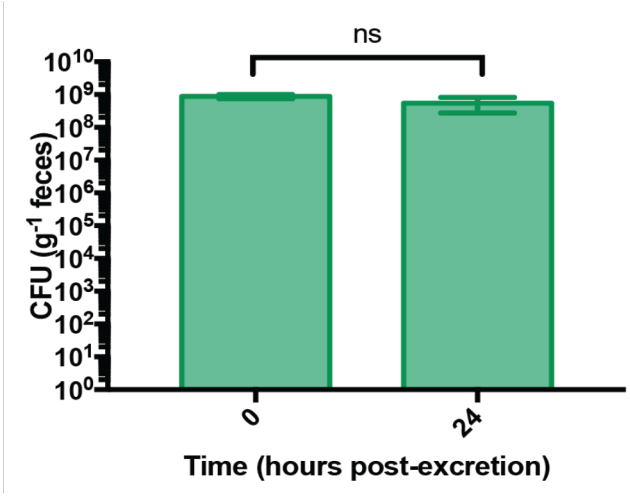

ns
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
CFU (g^{-1} feces)
0
24
Time (hours post-excretion)

Figure S6. Related to Figure 7. Fecal weight kinetics and VRE viability. Ampicillin-treated mice were inoculated with VRE on the fifth day of ampicillin treatment. **(A)** All fecal pellets were freshly collected and weighed following a room temperature incubation of 0, 24, and 48 hours. The excretion ratio was calculated by dividing the fecal weight at time = 24 or 48 by the fecal weight at time = 0. A 50% reduction in the excretion ratio between fresh pellets and pellets dried for 24 hours is the result of evaporation (***$P < 0.0002$ by the Mann-Whitney test, ns = non-significant; n = 7 mice). **(B)** VRE quantification in fresh fecal pellets (0) and fecal pellets incubated at room temperature for 24 hours to assess the effects of evaporation on VRE viability.

Figure S7

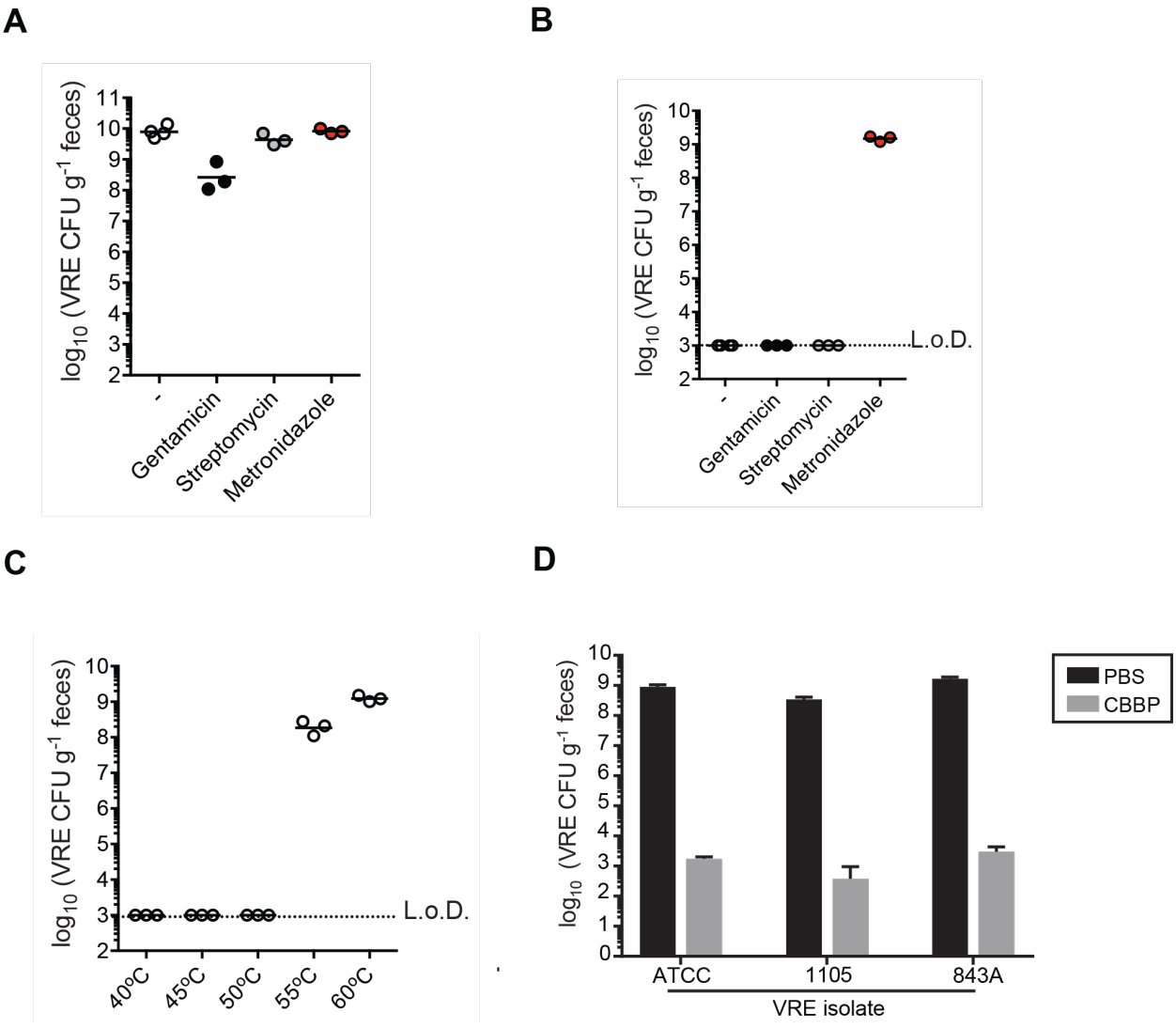

A
B
C
D
log10 (VRE CFU g-1 feces)
-
Gentamicin
Streptomycin
Metronidazole
L.o.D.
40°C
45°C
50°C
55°C
60°C
PBS
CBBP
ATCC
1105
843A
VRE isolate

Figure S7. Related to Figure 7. Suppression of VRE expansion *ex vivo* and *in vitro*. **(A-B)** VRE growth in fecal pellets from **(A)** ampicillin-treated mice and **(B)** ampicillin-treated mice colonized with ARM cultured in the presence of antibiotics of varying anaerobic coverage (gentamicin, streptomycin, metronidazole). -, no antibiotics. **(C)** VRE expansion in fecal samples from ampicillin-treated, ARM-colonized mice following heat-treatment at the indicated temperatures for 15 min prior to VRE inoculation. **(D)** VRE growth in the presence or absence of CBBP. 1105 and 843A, clinical isolates isolated from stool of VRE-colonized patients (n = 5 per group). L.o.D., limit of detection.

Table S1.

Strain	Sequenced length (bp)	Close relatives	Similarity to close relative (%)	Match length (bp)	Isolated clones	100% OTU match
Strain_01	912	Bacteroides sartorii strain JCM 17136 Bacteroides chinchillae strain JCM 16487	99 99	909 907	9	**OTU 4**
Strain_02	886	Blautia producta strain JCM 1471 Blautia coccoides strain JCM 1395 Blautia producta strain ATCC 27340	98 98 98	867 867 865	15	**OTU 11**
Strain_03	833	Clostridium innoccuum strain B-3 Eubacterium dolichum strain JCM 10413	97 92	805 769	17	**OTU 5**
Strain_04	916	Bacteroides acidifaciens strain JCM 10556 Bacteroides acidifaciens strain A-40	98 98	896 895	5	
Strain_05	914	Parabacteroides distasonis strain ATCC 8503 Parabacteroides distasonis strain JCM 5825	98 98	892 892	56	**OTU 6**
Strain_06	911	Parabacteroides goldsteinii strain JCM 13446 Parabacteroides goldsteinii strain WAL 12034	99 98	902 877	3	
Strain_07	892	Akkermansia muciniphila strain ATCC BAA-835 Akkermansia muciniphila strain Muc2	99 99	882 881	2	**OTU 3**
Strain_08	881	Clostridium hylemonae strain TN-272 Clostridium hylemonae strain TN-271	95 96	838 789	12	
Strain_09	838	Clostridium bolteae strain JCM 12243 Clostridium bolteae strain 16351	99 98	826 825	3	**OTU 7**
Strain_10	890	Bacteroides uniformis strain JCM 5828 Bacteroides rodentium strain JCM 16496	99 99	887 884	10	
Strain_11	858	Flavonifractor plautii strain 265 Flavonifractor plautii strain Prevot S1	99 99	853 851	32	
Strain_12	873	Enterococcus gallinarum strain LMG 13129	99	865	1	
Strain_13	462	Anaerostipes caccae strain L1-92	97	447	7	
Strain_14	457	Erysipelatoclostridium ramosum strain JCM 1298	99	456	4	
Strain_15	1069	Blautia schinkii strain B Blautia glucerasea strain HFTH-1	94 94	1006 954	3	**OTU 2**
Strain_16	958	Barnesiella viscericola	82	784	10	
Strain_17	921	Barnesiella intestinihominis strain JCM 15079	88	811	4	
Strain_18	919	Alistipes senegalensis strain JC50	95	874	1	
Strain_19	1015	Olsenella umbonata strain lac31 Olsenella uli strain DSM 7084	94 94	950 935	5	

Table S1. Related to Figure 5. 16S rRNA gene analysis of isolated strains. The full length of the 16S ribosomal RNA gene was amplified by colony-PCR. Amplified 16S genes were Sanger-sequenced and classified using the 16S ribosomal RNA Sequence Database in BLAST. Percent similarities to closely related species and to resistance-associated OTUs are shown. Shaded boxes indicate isolated organisms significantly correlated with VRE suppression.