Supplementary Information:
Human-associated microbiota suppress invading bacteria even under disruption by antibiotics

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Materials and Methods

Focal strain

We used an *Escherichia coli* K-12 MG1655 strain with a yfp::ampicillin resitance cassette and nucleotide changes at nine positions relative to the ancestral strain, including mutations in *rpoB* and *basS*, conferring observed resistance to polymyxin [1] and rifampicin [2]. One day prior to the microcosm experiment, we inoculated *E.coli* in LB and incubated it overnight at 37°C in a shaking incubator.

Microcosm experiment

We used stool samples from consenting human donors that we collected on the 15th of May 2018. The sampling protocol, approved by the ETH Zürich Ethics Commission (EK 2015-N-55) is described in [3]. In brief, we collected samples from three anonymous, healthy human donors and mixed the samples with 200 ml of anaerobic peptone wash (1 g/l peptone, 0.5 g/l L-Cysteine, 0.5 g/l bile salts and 0.001 g/l Resazurin; Sigma-Aldrich) to make a 10% (w/v) anaerobic faecal slurry. We divided the faecal slurry in two equal volumes and autoclaved one of the aliquots to create sterilized (community-free) slurry and stored it at -80°C. We added to the other half of the aliquot glycerol as a cryo-protective agent and stored it at -80°C until the day of the experiment. There is a risk that freeze-thawing could lead to the loss of some taxa from the sampled microbiota [4], but previous studies indicate this risk is small, with frozen slurry containing rich, viable communities [5–7]. Note also that we do not exclude loss of some taxa, but for this experiment the aim was to capture abundant and rich communities sampled from humans (not to recover 100% of all species present in the GI tract).

Prior to the microcosm experiment, we filled 54 Hungate tubes with 7.2 ml basal medium (2 g/l Peptone, 2 g/l Tryptone, 2 g/l Yeast extract, 5 g/l NaCl, 0.04 g K2HPO4, 0.04 g/l KH_2PO_4, 0.01 g/l $MgSO_4$x7H_2O, 0.01 g/l $CaCl_2$x6H_2O, 0.005 g/l Hemin, 0.5 g/l L-Cysteine, 2g/l Starch, 1.5 g/l casein, 0.001g/l Resazurin, pH adjusted to 7; Sigma-Aldrich) flushed the headspace with nitrogen to remove oxygen, sealed and autoclaved the tubes. We thawed sterile and live slurries from each human donor on the morning before the experiment. For each human donor background, we inoculated 2x10^6 of focal strain with the resident microbial community (n=9), containing 350 μl of live faecal plus 500 μl of the sterilized slurry, and in the absence of the community, containing 850 μl of sterilized slurry (community-free treatment, n=9). To each of these tubes we added either rifampicin (128 μg/ml), polymyxin (4 μg/ml) or no antibiotics as a control treatment. Before and after incubation at 37°C in a shaking incubator for 24h, we took samples for plating, flow cytometry and sequencing. For plating, we diluted samples in PBS (Phos-

phate Buffered Solution) and plated them on chromatic MH agar (Liofilchem) supplemented with rifampicin to count abundance of our focal strain.

We quantified total bacterial densities by flow cytometry (Novocyte 2000R, ACEA Biosciences Inc.). We diluted each sample containing live community with PBS, stained them with Sybrgreen (final concentration of 10^{-4} of the stock solution, Life Technologies, Zug, Switzerland) and detected bacteria based on their signature in a cytogram of green fluorescence versus forward scattered light. For sequencing, we took samples from all microbiome treatments at 0 and 24h, supplemented them with glycerol and stored them at -80°C until further processing.

Amplicon sequencing

We used the powerlyzer power soil kit (Qiagen) to extract DNA for amplicon sequencing according to the manufacturers protocol with the following modification. We concentrated each sample before extraction by adding 1.5 ml of each sample in an extraction tube provided by the kit, centrifuged for 10 min at 10000 rpm, discarded the supernatant and repeated this step. DNA quality and quantity was assessed by Nanodrop and Qubit.

We sequenced the variable regions V3-V4 of the 16S rRNA gene with previously described primers [8]. Following the Illumina 16S Metagenomic Sequencing Library preparation guide for the MiSeq Illumina sequencing platform we amplified target regions with a limited 17 cycle PCR, followed by a PCR clean-up and adapter attachment of the Nextera XT Index Kit v2 in a second PCR step. We assessed quality and quantity of amplicon library by TapeStation and qPCR using the KAPA library quantification kit. After normalization and pooling we sequenced the library on the MiSeq sequencing platform using 2x300 V3 kit. We used Trimmomatic and FLASH to demultiplex and quality-filter the raw paired-end sequencing reads. We used Usearch software to cluster ZOTUs with a 99% similarity threshold. For taxonomic assignment prediction against the Silva 16S rRNA database we used SINTAX.

Statistical analysis

Generalised linear models for both focal strain abundance and total biomass with negative binomial errors and a log link were fit in a Bayesian framework using the R package *brms* v2.12.0 [9]. Predictors in both models included community (presence/absence), treatment (antibiotic free, rifampicin and polymyxin) and donor community/slurry (three levels) and the interaction between community presence and donor, between community presence and treatment, and between community presence, donor and treatment. We used default (uninformative) priors, and ran

74 four chain for 2,000 iterations with a warm-up period of 1,000 iterations each, for
75 a combined total of 4,000 samples. We checked model convergence by visualising
76 traceplots and checking diagnostics (Rhat = ∼1).

77 16S RNA compositional data was analysed using the R package *Phyloseq* v1.30.0
78 [10]. We first excluded any OTUs with a total read abundance across all samples
79 of less than five. To visualise compositional similarity across samples (Fig 2b), we
80 performed non-metric multidimensional scaling based on the Bray-Curtis distance
81 between samples.

82 Data availability

83 16S rRNA Amplicon sequencing data is deposited in the European Nucleotide
84 Archive under the Project accession number PRJEB38496.

85 Supplementary Figure S1-S3

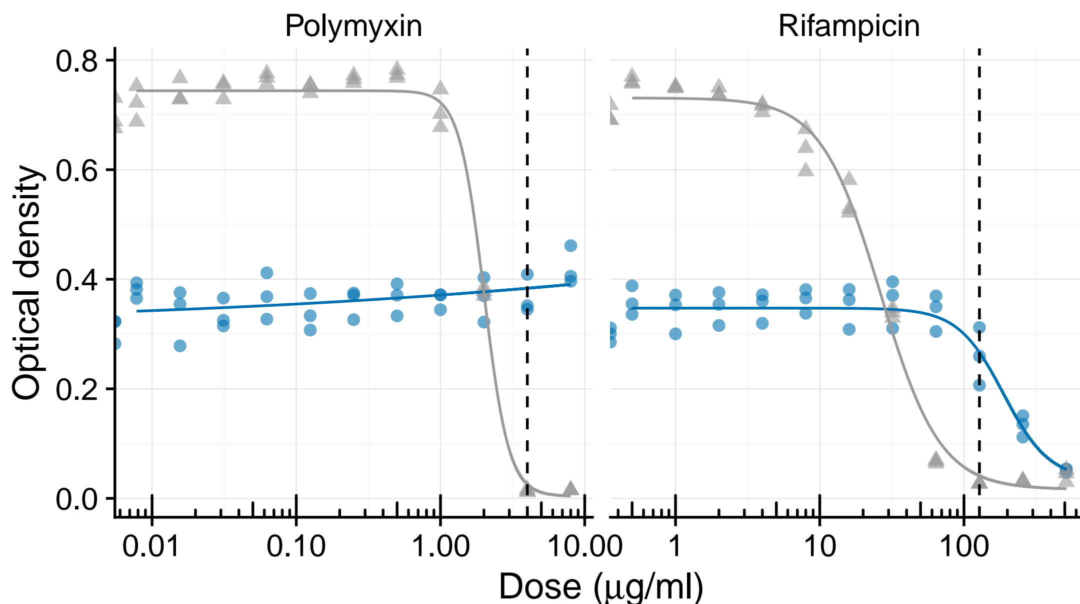

Figure S1: Optical density of the focal strain (blue) and its drug sensitive ancestor (grey) after growing for 24 hours in LB supplemented with different concentrations of polymyxin or rifampicin. Vertical dashed lines indicate antibiotic concentrations used in main experiment. Experimental concentrations chosen such that the focal strain can be expected to have an advantage over other sensitive strains in the microbiota.

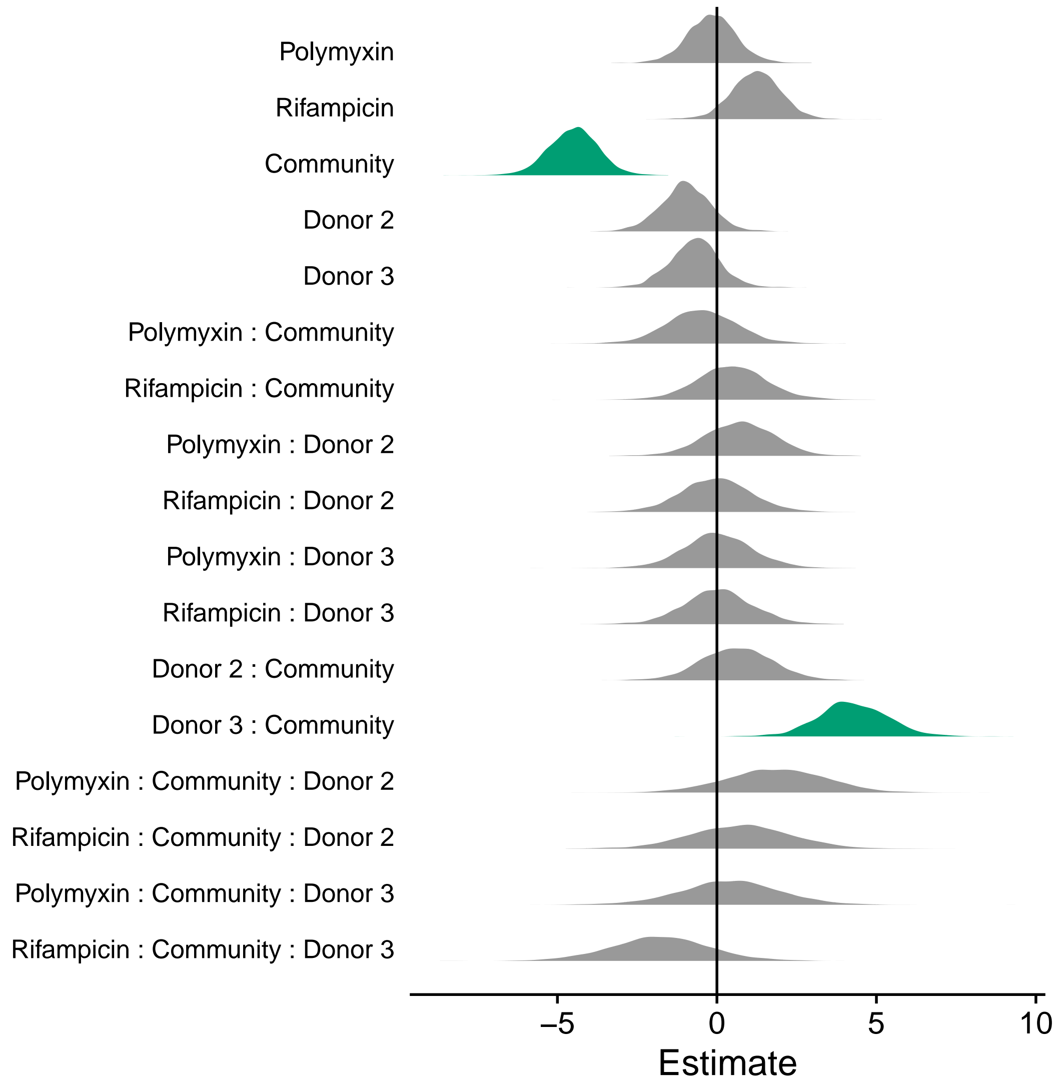

Figure S2: Model coefficients (posterior distributions) from a linear model (negative binomial errors) of focal strain abundance as a function of community, antibiotic, and donor, and all pairwise and three-way interactions. Posteriors in green have 95% credible intervals that do not overlap with 0 (i.e., there is less than 5% probability there is no effect of the variables/interactions captured by these coefficients). Intercept (not shown) = donor 1 in the no antibiotic treatment in the absence of the community (i.e., sterilized slurry).

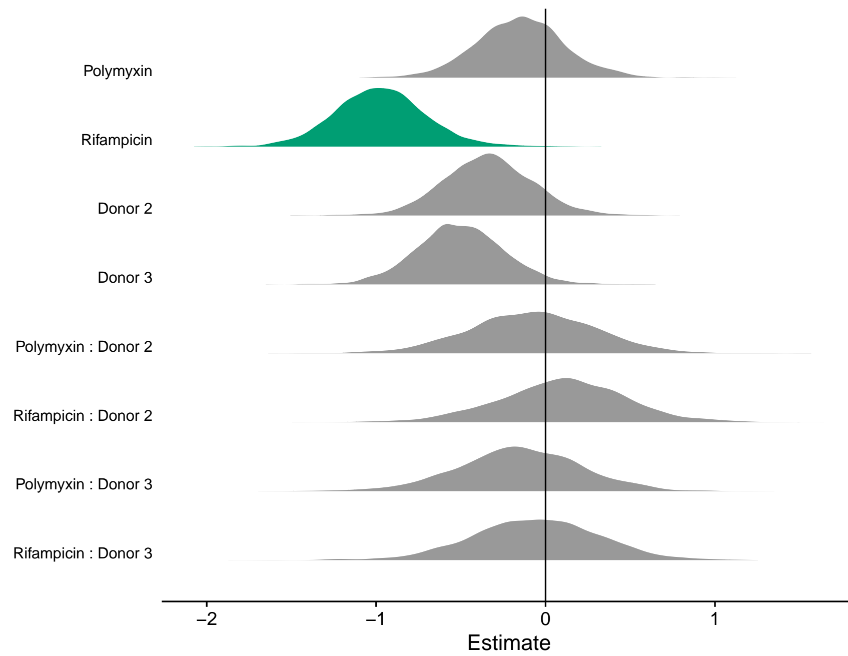

Figure S3: Model coefficients (posterior distributions) from a linear model (negative binomial errors) of total bacterial abundance as a function of antibiotic, donor, and their interaction. Posteriors in green have 95% credible intervals that do not overlap with 0 (i.e., there is less than 5% probability there is no effect of the variables/interactions captured by these coefficients). Intercept (not shown) = Donor 1 in the no antibiotic treatment.

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