A1. Supplemental Methods

A1.1 Administration of broad-spectrum antibiotics

Our first attempt administering the antibiotic cocktail was unsuccessful as the first two animals treated had lethal reactions to the antibiotics upon receiving the second gavage treatment on day 1. The antibiotic depletion protocol initially used was adopted from (Reikvam et al., 2011). In contrast to their findings, we found this mixture too harmful to continue treatment. In the second (and successful) attempt, we removed the antifungal Amphotericin B from the cocktail due to extensive reporting of acute toxicity (Jensen et al., 1999), decreased the concentrations of antibiotics in the cocktail by 1/4th, and reduced the frequency of antibiotic administration from twice daily to one treatment per day. No adverse effects occurred after making these changes.

A1.2 Construction and sequencing of v3-v4 16S metagenomic libraries

Just prior to terminal sacrifice, freshly voided fecal matter was aseptically collected into sterile 2 ml capped microtubes, snap-frozen in liquid nitrogen, and stored at -80 ºC. The samples were shipped overnight to the University of Wisconsin- Madison Biotechnology Center DNA Sequencing Facility, and DNA was isolated from 250 µL of fecal slurry using the OMNIgene adapted MO BIO PowerFecal DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, California, USA) at the University of Wisconsin-Biotechnology Center. DNA concentration was verified using the Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, California, USA). Samples were prepared as described in the 16S Metagenomic Sequencing Library Preparation Protocol, Part # 15044223 Rev. B (Illumina Inc., San Diego, California, USA) with the following modifications: The 16S rRNA gene V3/V4 variable region was amplified with nested primers (forward primer: 5-ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTAC-GGGNGGCWGCAG-3', reverse primer: 5'-GTGACTGGAGTTCAGACGTGTGCTCTT-CCGATCTGACTACHVGGGTATCTAATCC-3'), Region-specific primers were previously described in Klindworth et al., (2013) and were modified to add Illumina adapter overhang nucleotide sequences to the gene-specific sequences. Following initial amplification, library size was verified on an Agilent DNA1000 chip, and cleaned using a 1x volume of AxyPrep Mag PCR clean-up beads (Axygen Biosciences, Union

City, CA). Illumina dual indexes and Sequencing adapters were added using the following primers (Forward primer: 5' AATGATACGGCGACCACCGAGATCTACAC-[55555555]ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3', Reverse Primer: 5'-CAAGCAGAAGACGGCATACGAGAT[77777777]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3', where bracketed sequences are equivalent to the Illumina Dual Index adapters D501-D508 and D701-D712). Following PCR, cleaned using a 1x volume of AxyPrep Mag PCR clean-up beads (Axygen Biosciences). Quality and quantity of the finished libraries were assessed using an Agilent DNA1000 chip and Qubit dsDNA HS Assay Kit, respectively. Libraries were standardized to 2uM and pooled prior to sequencing. Paired end, 300 bp sequencing was performed using the Illumina MiSeq Sequencer and a MiSeq 600 bp (v3) sequence cartridge. Images were analyzed using the standard Illumina Pipeline, version 1.8.2. OTU assignments, and diversity plots were created using QIIME analysis pipeline (Caporaso et al., 2011).

Bioinformatics was performed by the UW Biotechnology Center. Briefly, sequencing reads were adapter and quality trimmed using the Skewer (Jiang et al., 2014) trimming program. Flash (Magoč & Salzberg, 2011) was used to merge paired end reads into amplicon sequences. Merged amplicons were then quality filtered. QIIME (Caporaso et al., 2011) analysis used an open-reference OTU picking process: reads are clustered against a reference sequence collection using 97% similarity, and any reads which do not hit the reference sequence collection are subsequently clustered de novo. Alignments were filtered to remove variable regions prior to creating the phylogenetic tree. Singleton OTUs and OTUs that could not be aligned using PyNAST were removed (DeSantis et al., 2006). Alpha diversity was calculated with a rarefaction upper limit of (median depth/sample count). Beta diversity was leveled according to the lowest sample depth (Hughes et al., 2016).

A2. Supplemental References

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Hughes, J., Hellmann, J., Ricketts, T., Bohannan, B., Sinclair, L., Osman, O., & Michaelakis, A. (2016). Counting the Uncountable : Statistical Approaches to Estimating Microbial Diversity MINIREVIEW Counting the Uncountable : Statistical Approaches to Estimating Microbial Diversity. App. Environ. Microbiol. 10, 4399–4406. https://doi.org/10.1128/AEM.67.10.4399

Jensen, G., Skenes, C., Bunch, T., Weissman, C., Amirghahari, N., Satorius, A., & Eley, C. (1999). Determination of the Relative Toxicity of Amphotericin B Formulations: A Red Blood Cell Potassium Release Assay. Drug. Deliv. 6, 81–88. https://doi.org/10.1080/107175499266995

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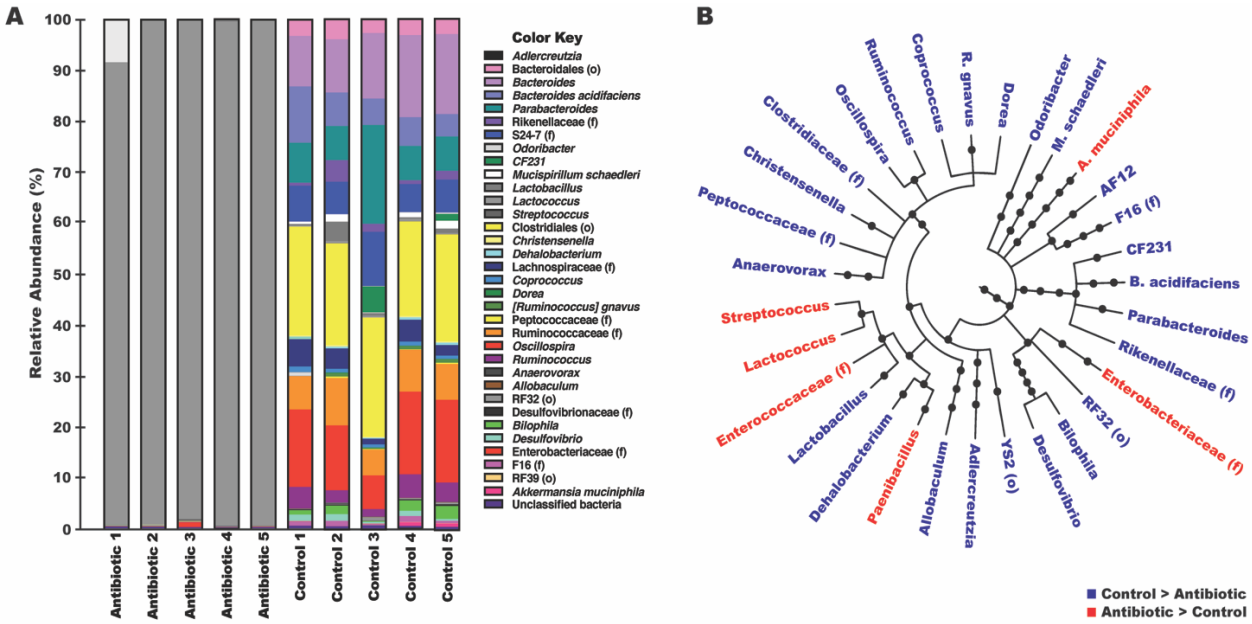
A3. Supplemental Figures



A3.1 Antibiotic treatment eradicates or reduces the vast majority of bacterial taxa. Gut microbiome profile at genus-level phylogenetic classification was determined from 16S rDNA gene sequences using the QIIME pipeline.

A4. Supplementary Tables

Table 1. Percentages of the most abundant bacterial groups.

Taxa	Control (%)	Antibiotic (%)	T-test	P-Value
Adlercreutzia	0.1 ± 0.0	0.0 ± 0.0	**	**0.0008**
Unclassified Bacteroidales (o)	3.1 ± 0.2	0.0 ± 0.0	**	**< 0.0001**
Bacteroides	13.0 ± 1.3	0.0 ± 0.0	**	**< 0.0001**
Bacteroides acidifaciens	6.6 ± 1.2	0.0 ± 0.0	**	**0.0005**
Parabacteroides	9.5 ± 2.5	0.0 ± 0.0	**	**0.0052**
Unclassified Rikenellaceae (f)	1.8 ± 0.7	0.0 ± 0.0	*	**0.0269**
Unclassified S24-7 (f)	7.2 ± 0.9	0.0 ± 0.0	**	**< 0.0001**
Odoribacter	0.1 ± 0.1	0.0 ± 0.0	ns	0.0773
CF231	1.3 ± 1.0	0.0 ± 0.0	ns	0.2315
Mucispirillum schaedleri	0.9 ± 0.3	0.0 ± 0.0	*	**0.0127**
Paenibacillus	0.0 ± 0.0	0.1 ± 0.0	**	**0.0075**
Unclassified Enterococcaceae (f)	0.0 ± 0.0	1.7 ± 1.6	ns	0.3328
Lactobacillus	1.1 ± 0.7	0.1 ± 0.0	ns	0.1462
Lactococcus	0.3 ± 0.1	97.1 ± 1.6	**	**< 0.0001**
Streptococcus	0.0 ± 0.0	0.1 ± 0.1	ns	0.2061
Unclassified Clostridiales (o)	20.8 ± 0.8	0.0 ± 0.0	**	**< 0.0001**
Christensenella	0.2 ± 0.0	0.0 ± 0.0	**	**< 0.0001**
Dehalobacterium	0.4 ± 0.1	0.0 ± 0.0	**	**0.0002**
Unclassified Lachnospiraceae (f)	3.3 ± 0.8	0.0 ± 0.0	**	**0.0026**
Coprococcus	0.8 ± 0.1	0.1 ± 0.0	**	**< 0.0001**
Dorea	0.1 ± 0.0	0.0 ± 0.0	**	**0.0011**
[Ruminococcus] gnavus	0.6 ± 0.1	0.0 ± 0.0	**	**0.0002**
Unclassified Peptococcaceae (f)	0.2 ± 0.1	0.0 ± 0.0	*	**0.0105**
Unclassified Ruminococcaceae (f)	7.2 ± 0.7	0.1 ± 0.0	**	**< 0.0001**
Oscillospira	13.4 ± 1.8	0.0 ± 0.0	**	**< 0.0001**
Ruminococcus	3.3 ± 0.6	0.0 ± 0.0	**	**0.0005**
Allobaculum	0.1 ± 0.0	0.0 ± 0.0	*	**0.0380**
Unclassified RF32 (o)	0.3 ± 0.1	0.0 ± 0.0	*	**0.0387**
Unclassified Desulfovibrionaceae (f)	0.2 ± 0.1	0.0 ± 0.0	**	**0.0060**
Bilophila	1.5 ± 0.4	0.0 ± 0.0	**	**0.0080**
Desulfovibrio	0.9 ± 0.2	0.0 ± 0.0	**	**0.0045**
Unclassified Enterobacteriaceae (f)	0.0 ± 0.0	0.2 ± 0.2	ns	0.3831
Unclassified F16 (f)	0.8 ± 0.2	0.0 ± 0.0	**	**0.0027**
Akkermansia muciniphila	0.3 ± 0.2	0.0 ± 0.0	ns	0.1416
Other bacteria (< 0.1%)	0.6 ± 0.1	0.5 ± 0.1	**	**0.0090**

Values are mean ± SEM; t-test, *p < 0.05, **p < 0.01.

Unclassified denotes unclassified within the respective taxonomic group: (o) order, (f) family.

Table 2. Effects of gut microbiota depletion on sleep and locomotor behaviors.

Sleep Parameters	Control	Antibiotic	T-test	P-Value
24 h % sleep	63.9 ± 3.1	57.1 ± 4.8	ns	0.2435
Light phase % sleep	72.8 ± 2.6	72.1 ± 2.5	ns	0.8058
Dark phase % sleep	55.1 ± 3.7	42.2 ± 0.9	**	**0.0098**
Diurnal sleep ratio	1.3 ± 0.1	1.7 ± 0.1	*	**0.0198**
# of light phase S/W transitions	18.1 ± 1.6	17.4 ± 1.4	ns	0.7399
# of dark phase S/W transitions	20.2 ± 1.3	19.1 ± 1.5	ns	0.5837
Light phase sleep bout length (min)	8.3 ± 0.5	8.3 ± 0.7	ns	0.8214
Dark phase sleep bout length (min)	6.3 ± 0.6	5.1 ± 0.2	ns	0.0562
Light phase wake bout length (min)	3.8 ± 0.3	3.5 ± 0.2	ns	0.2418
Dark phase wake bout length (min)	5.0 ± 0.5	6.7 ± 0.4	*	**0.0362**
Light phase sleep onset latency (min)	23.9 ± 5.8	16.4 ± 7.5	ns	0.0944
Dark phase sleep onset latency (min)	9.7 ± 3.0	30.4 ± 7.4	*	**0.0181**
Light phase wake after sleep onset (min)	4.9 ± 1.7	7.6 ± 4.2	ns	0.1043
Dark phase wake after sleep onset (min)	5.9 ± 2.0	13.2 ± 1.9	*	**0.0175**
Locomotor Activity Parameters				
Light phase distance traveled (m)	3.3 ± 0.7	1.6 ± 0.5	ns	0.0721
Dark phase distance traveled (m)	3.9 ± 0.9	8.3 ± 1.1	*	**0.0139**
Light phase maximum speed (mm/s)	12.2 ± 0.9	13.2 ± 0.8	ns	0.4194
Dark phase maximum speed (mm/s)	17.2 ± 1.9	24.8 ± 1.9	*	**0.0200**
# of light phase body rotations	7.3 ± 4.5	3.9 ± 0.9	ns	0.0814
# of dark phase body rotations	8.8 ± 2.2	17.4 ± 3.8	ns	0.0834

Values are mean ± SEM, n = 5 mice per group. Student's upaired t-test, *p < 0.05, **p < 0.01. Light phase sleep parameters; ZT 0-12. Dark phase sleep parameters; ZT 12-24. Light phase locomotor activity parameters; ZT 2-3, ZT 8-9. Dark phase locomotor activity parameters; ZT 16-17, ZT 22-23.