Appendix 3: Gut microbiota community composition in mice with human-derived gut microbiota

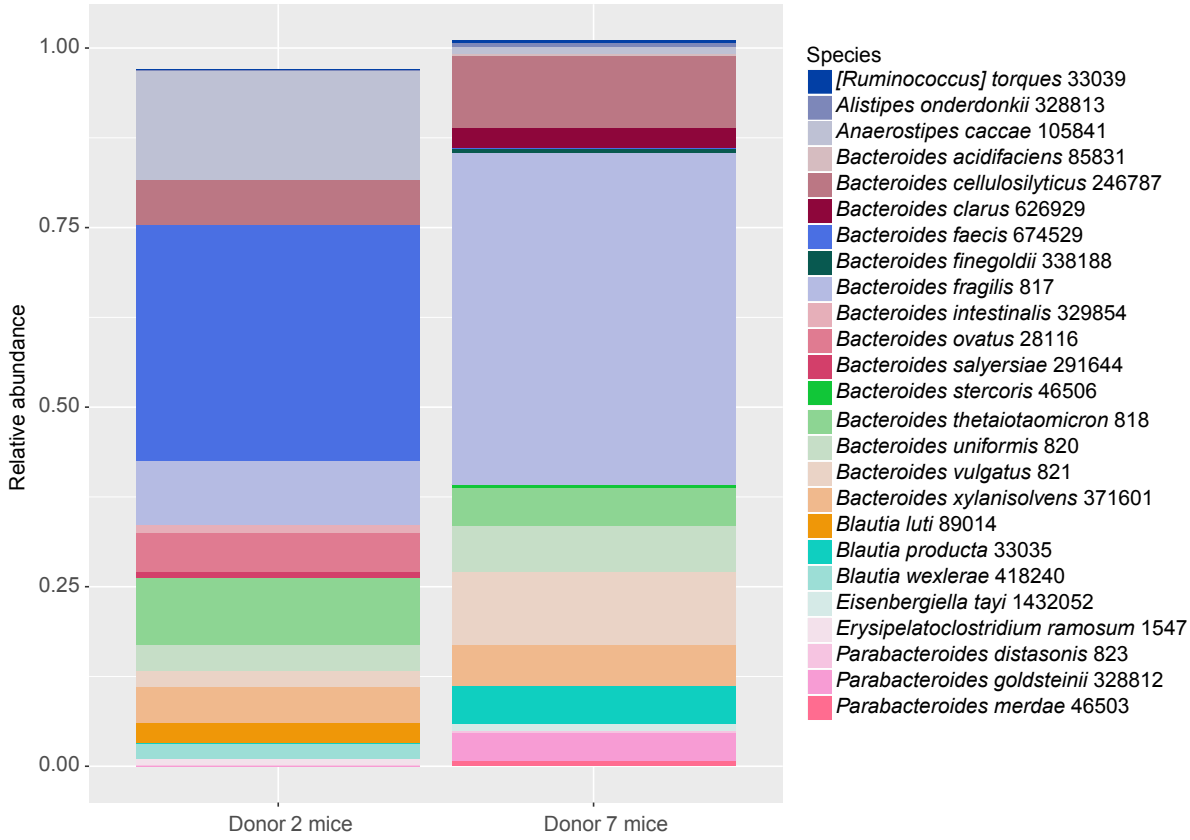

Figure A3.1. The relative abundance of species cultured from stool of Donor 2- and Donor 7-derived mice under anaerobic vegetative conditions. One faecal pellet was collected from each of five mice housed together in a single cage, of both Donor 2 and Donor 7 mouse lines. The stools were weighed and homogenised 100mg/ml, then pooled per mouse cage in equal volumes. The pooled samples were diluted and plated on YCFA agar and cultured at 37°C for 48 hours. The total growth was collected from each plate and total DNA extracted for metagenomic sequencing. Sequences reads were taxonomically classified using Kraken and a database of all publicly available gut bacterial genomes (as of December 2017) plus the HBC reference genomes. Classified read counts per species were normalised against the total number of classified reads per sample. The relative abundance of the top 25 most abundant species is shown.

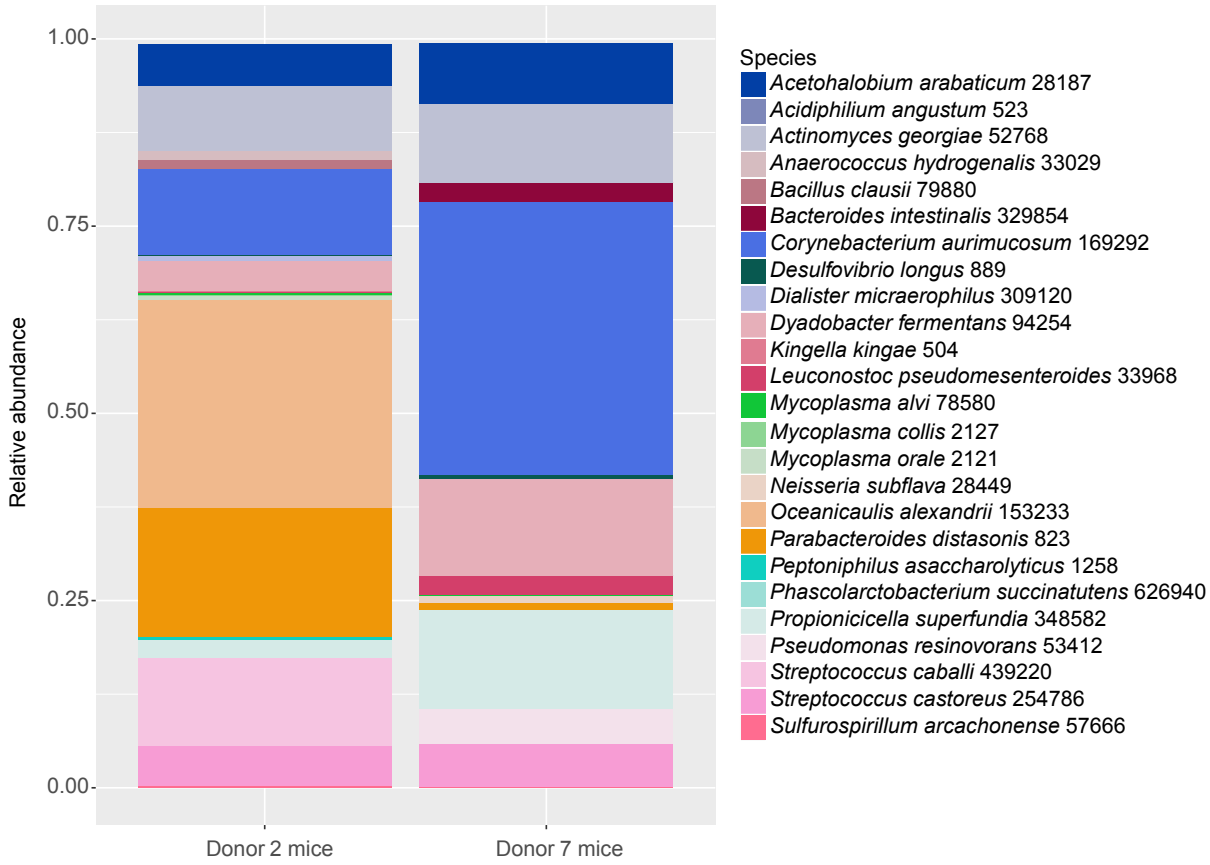

Figure A3.2. The relative abundance of amoxicillin-resistant species cultured from stool of Donor 2- and Donor 7-derived mice under anaerobic vegetative conditions. One stool was collected from each of five mice housed together in a single cage, of both Donor 2 and Donor 7 mouse lines. The stools were weighed and homogenised 100mg/ml, then pooled per cage in equal volumes. The pooled samples were diluted and plated on YCFA agar containing 8 mg/L amoxicillin (a level considered 'clinically resistant' by CLSI and EUCAST) and cultured at 37C for 48 hours. The complete growth was collected from each plate and total DNA extracted for metagenomic sequencing. Sequences reads were taxonomically classified using Kraken and a database of all publicly available gut bacterial genomes plus the HBC reference genomes. Classified read counts per species were normalised against the total number of classified reads per sample. The relative abundance of the top 25 most abundant species is shown.

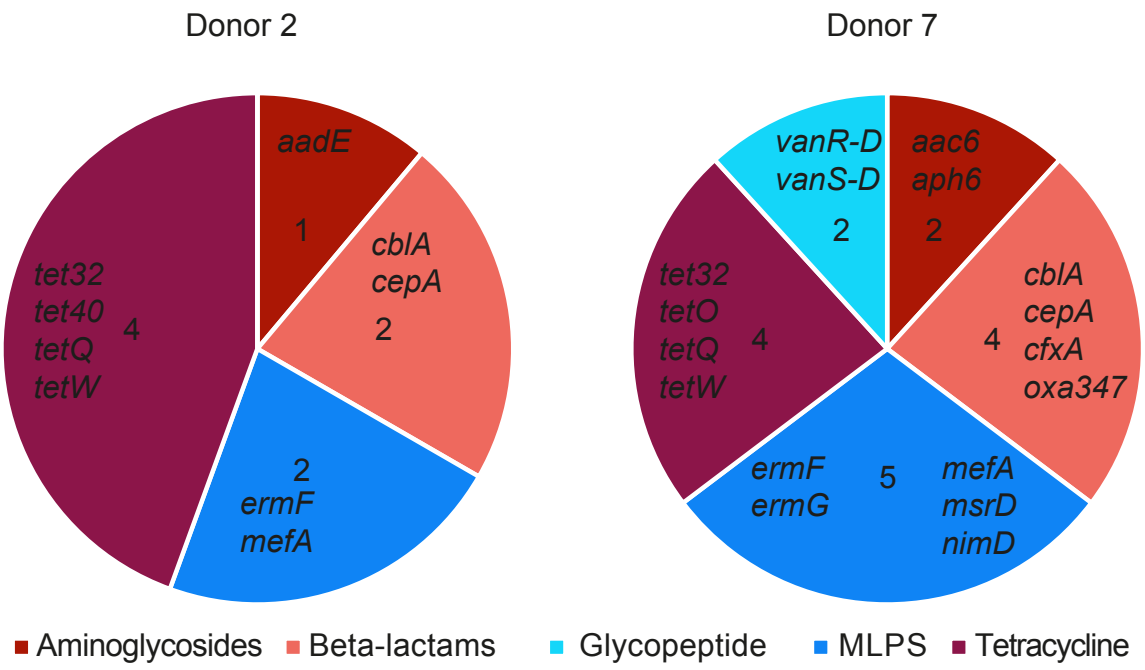

Figure A3.3. Antibiotic resistance genes (ARGs) identified in Donor 2- and Donor 7-derived mice. One stool was collected from each of five mice housed together in a single cage, of both Donor 2 and Donor 7 mouse lines. The stools were weighed and homogenised 100mg/ml, then pooled per cage in equal volumes. The pooled samples were diluted and plated on YCFA agar and cultured at 37C for 48 hours. The complete growth was collected from each plate and total DNA extracted for metagenomic sequencing. The presence of ARGs in the CARD, ResFinder and MegaRes databases were predicted from the metagenomic sequence reads using ARIBA. Results using each database were combined to count the different ARGs predicted to be present in the gut community of each mouse line. 19 different ARGs were predicted in total, with Donor 7 mice predicted to harbour more antibiotic resistance genes than Donor 2 (17 vs. 9); this includes the presence of a Class D beta-lactamase (Oxa-347) in the Donor 7 mice.