A Metagenomic Analysis for Combination Therapy of Multiple Classes of Antibiotics on the Prevention of the Spread of Antibiotic Resistant Genes

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Introduction

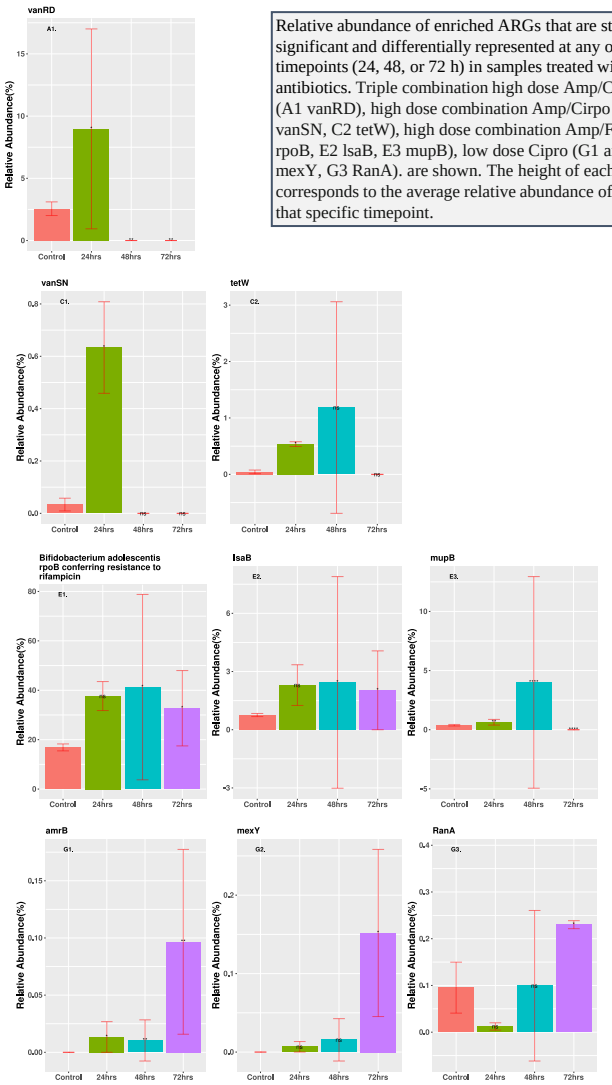
Antibiotic resistance is a global problem exacerbated by the use and overuse of antibiotics. Overuse has caused the rapid spread of antibiotic resistant genes (ARGs) and the emergence of multidrug resistant bacteria. Metagenomic analysis of the gut microbiome has shown to be viable method to study the spread of ARGs, mobile genetic elements (MGEs), and bacterial compositional profiles.

Methods

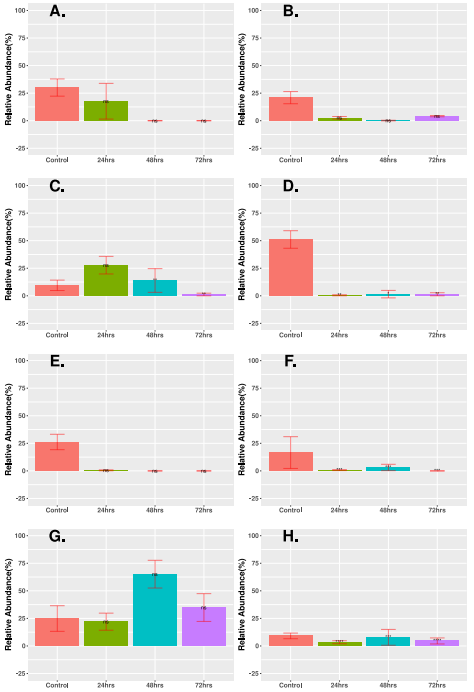
This project used next-generation sequencing, a custom-built metagenomics pipeline, and differential abundance analysis to study the effect of antibiotic (ampicillin, ciprofloxacin, and Fosfomycin) combination and monotherapy at high and low doses on resistome and taxonomic composition in the gut of Balb/c mice to investigate the evolution and emergence of antibiotic resistance.

Results

Triple combination, high-dose treatment killed nearly all bacteria and no ARGs were detectable after treatment. Dual combination treatments caused the emergence of clinically relevant multidrug resistant bacteria including *Acinetobacter radioresistens*, *Delftia acidovorans*, *Enterococcus faecalis*, and *Stenotrophomonas maltophilia*, despite a decrease in microbiota diversity. Only a maximum of 2 ARGs showed enrichment after treatment in any of the dual combination cohorts. Low-dose monotherapy treatments showed little change in microbiome composition but saw enrichment of over 30 ARGs. Relative abundances of MGEs either decreased or remained unchanged for combination therapy with increases in low-dose monotherapy.



Relative abundance of enriched ARGs that are statistically significant and differentially represented at any of the three timepoints (24, 48, or 72 h) in samples treated with antibiotics. Triple combination high dose Amp/Cirpo/Fosfo; (A1 vanRD), high dose combination Amp/Cirpo (C1 vanSN, C2 tetW), high dose combination Amp/Fosfo (E1 rpoB, E2 lsaB, E3 mupB), low dose Cipro (G1 amrB, G2 mexY, G3 RanA). are shown. The height of each bar corresponds to the average relative abundance of ARG for that specific timepoint.



Statistically significant change in relative abundance of MGE integrase between control and treatment groups. Triple combination high dose Amp/Cipro/Fosfo (a), triple combination low dose Amp/Cirpo/Fosfo (b), high dose combination Amp/Cirpo (c), low dose combination Amp/Cirpo (d), high dose combination Amp/Fosfo (e), high dose combination Cipro/Fosfo (f), low dose Cipro (g), low dose Fosfo (h) treatments. The height of each bar corresponds to the average relative abundance of integrase for that specific timepoint.

Conclusions

Combination therapy appears to be a feasible option to prevent the spread of ARGs, but caution needs to be taken to prevent possible superinfection. Low-dose monotherapy treatment did not greatly change bacteria diversity but did cause an increase in ARGs that could cause cross-resistance of antibiotics.