

## Introduction

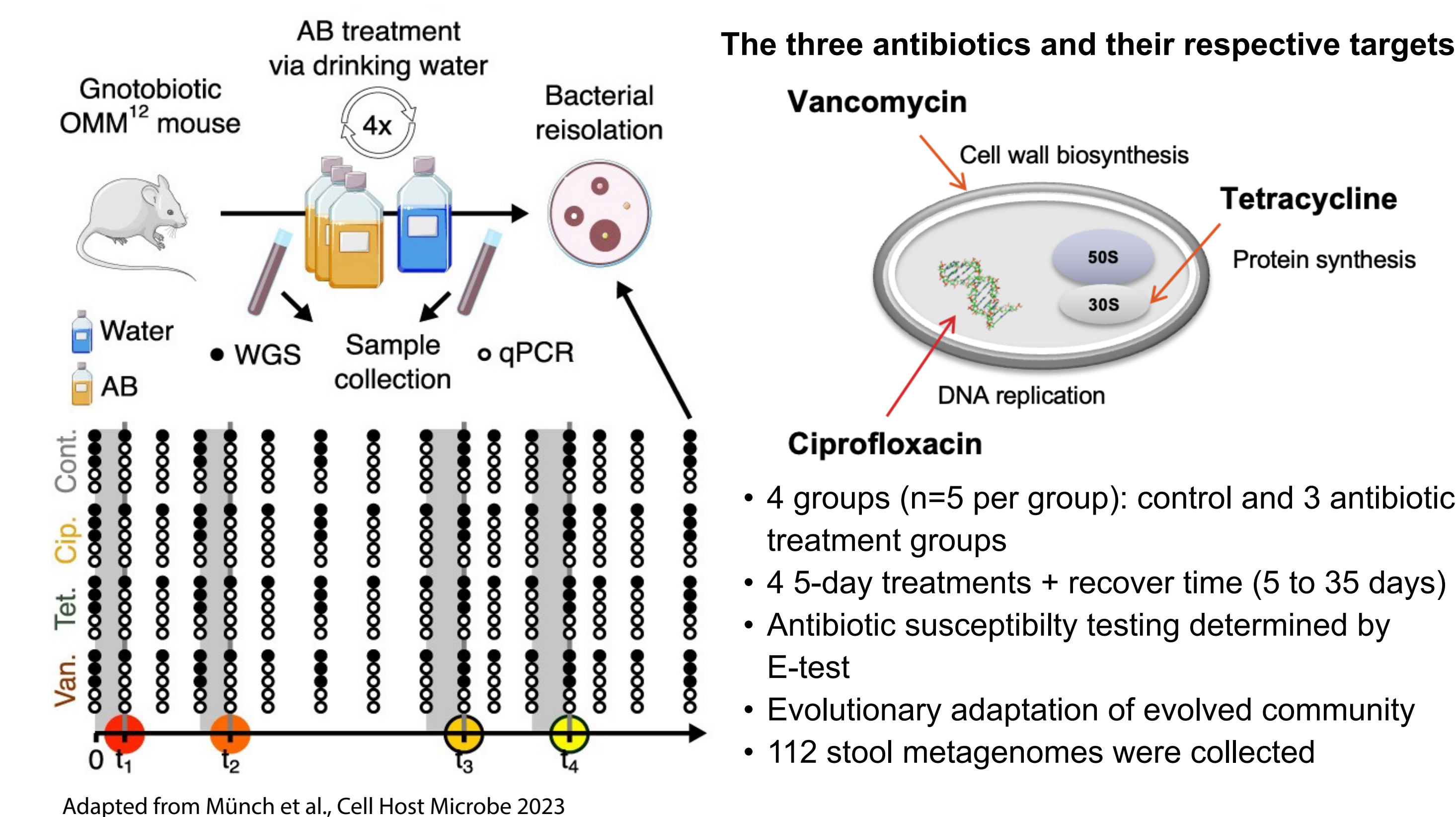
Since their discovery, antibiotics have revolutionized medicine. However, there are emerging concerns regarding overuse or misuse of antibiotics.

- Antibiotics are the largest drivers of gut microbiome compositional changes. Prolonged usage of antibiotics can lead to opportunistic pathogen infections such as *C. difficile* infection.
- Exposure to antibiotics is the driving cause of antibiotic resistance emergence and spread.
- Antimicrobial resistance makes it harder to treat infections and has become a serious global public health problem.

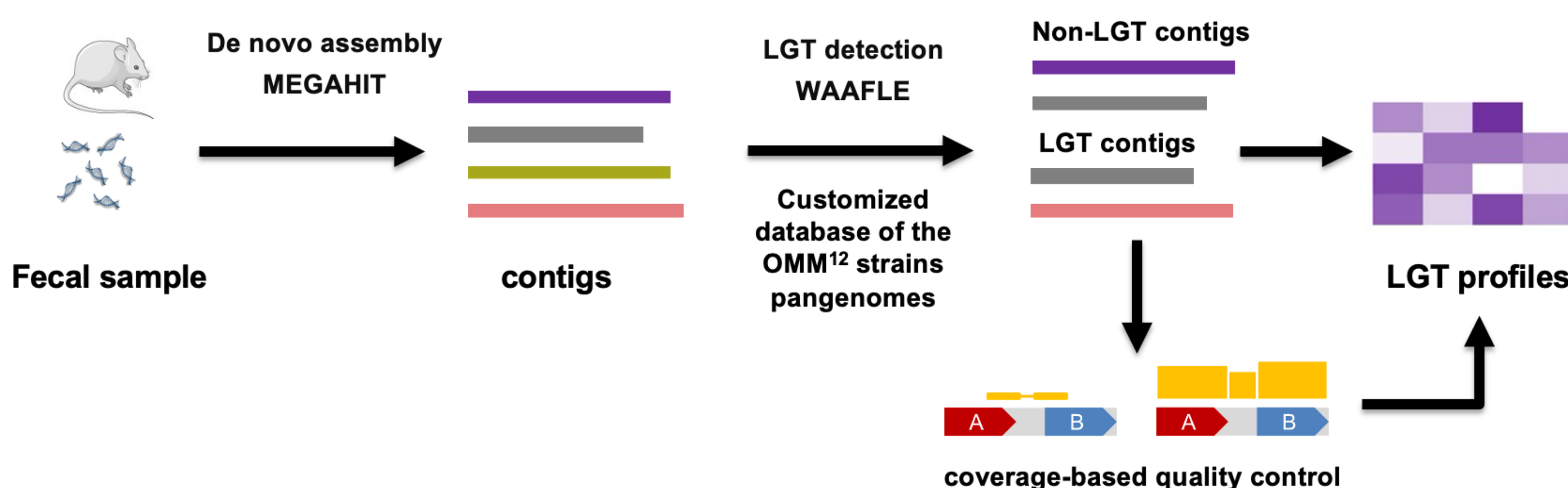
Lateral gene transfer also known as horizontal gene transfer, is an important factor in the genomic diversification of microbial communities through which recipients may acquire advantageous traits to adapt and thrive in a given ecological niche. While host/selective pressures (e.g., exposure to antibiotics) are thought to create favorable conditions for LGT, including the exchange of cassettes related to antibiotic resistance, the degree to which this occurs and whether it differs by antibiotic regimen is not well understood.

We assessed this phenomenon using gnotobiotic mice colonized with a consortium of 12 bacterial strains (oligo-mouse-microbiota, OMM<sup>12</sup>), and treated with antibiotics (Münch et al., Cell Host Microbe 2023) and used WAAFL (Hsu & Nzabarushimana et al., Nature Microbiology 2025) to profile LGT events.

## Pulsed antibiotic treatment study design

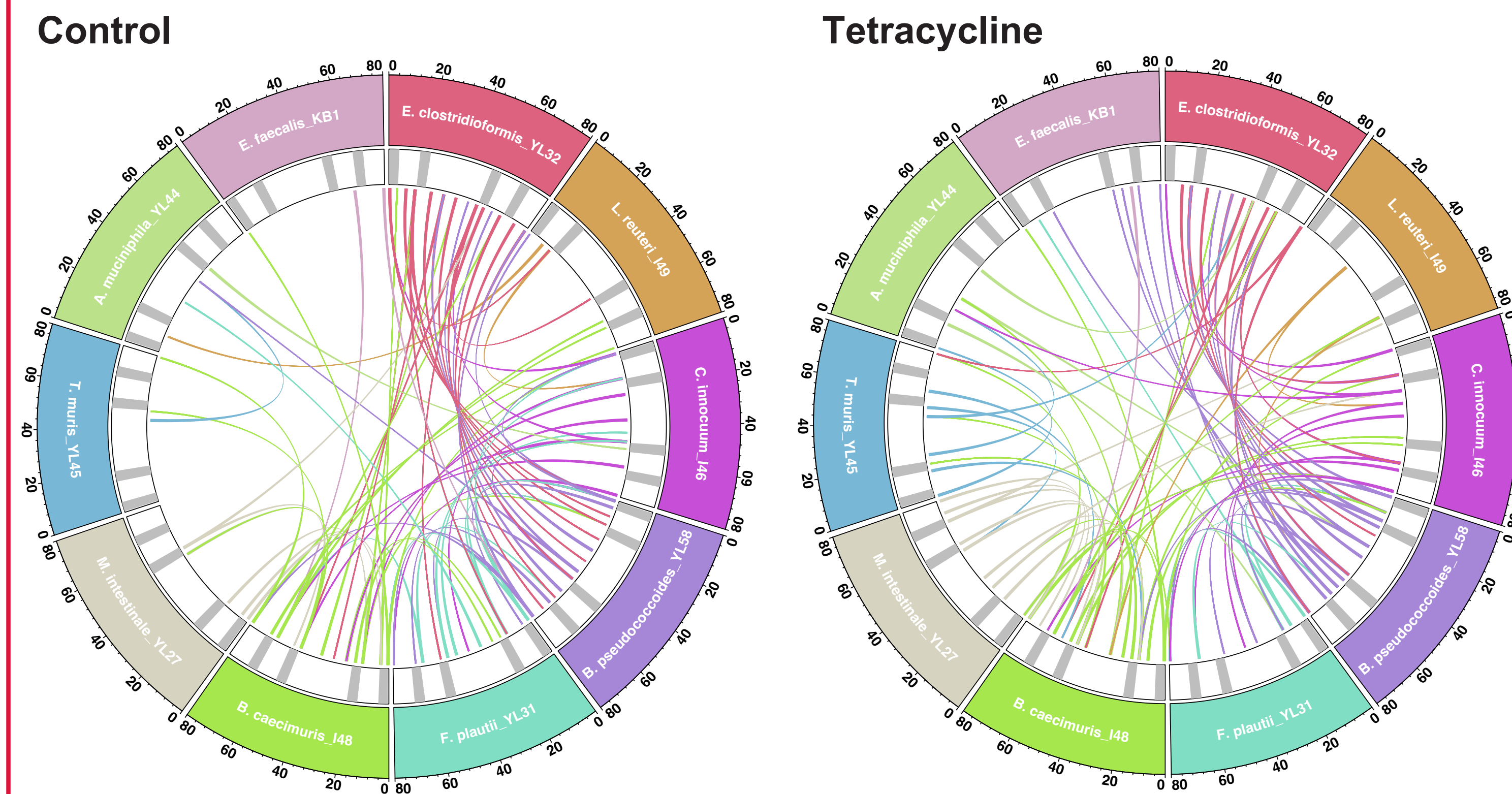


## Detection of LGT events in OMM<sup>12</sup> colonized mice



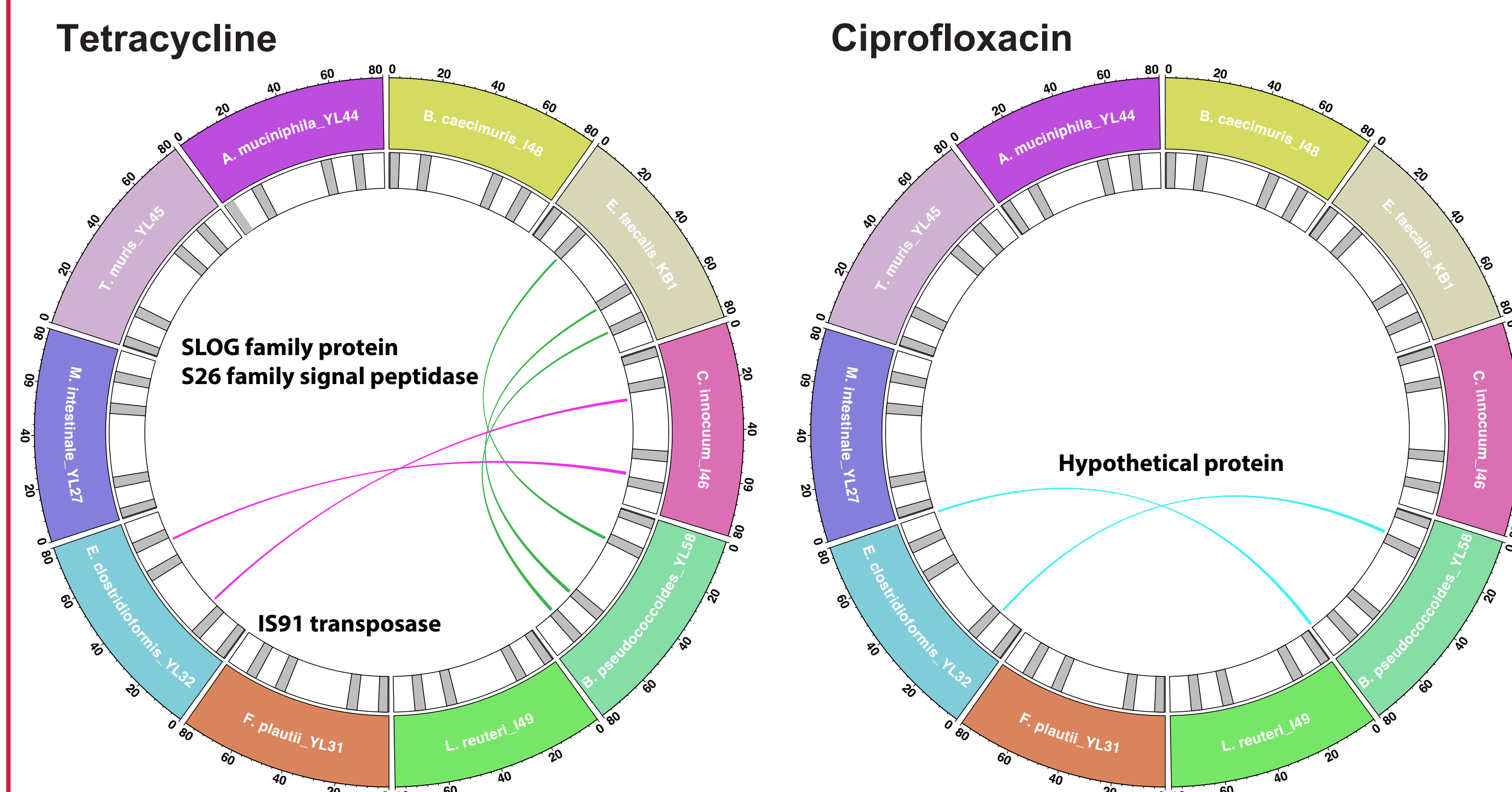
To detect LGT in these metagenomes, we generated *de novo* assemblies and used WAAFL to detect LGT events. We used a customized database using pangenomes of the 12 strains of the OMM<sup>12</sup> community. WAAFL LGT candidates were further filtered and retained if they either had paired-end read covering the entire LGT junction or if the junction gap was too wide, the coverage of the function was at least half of the mean coverage of the flanking regions. In total we detected 317 high-quality LGT events across the treatment groups.

## Lateral gene transfer is prevalent in OMM<sup>12</sup> colonized mice



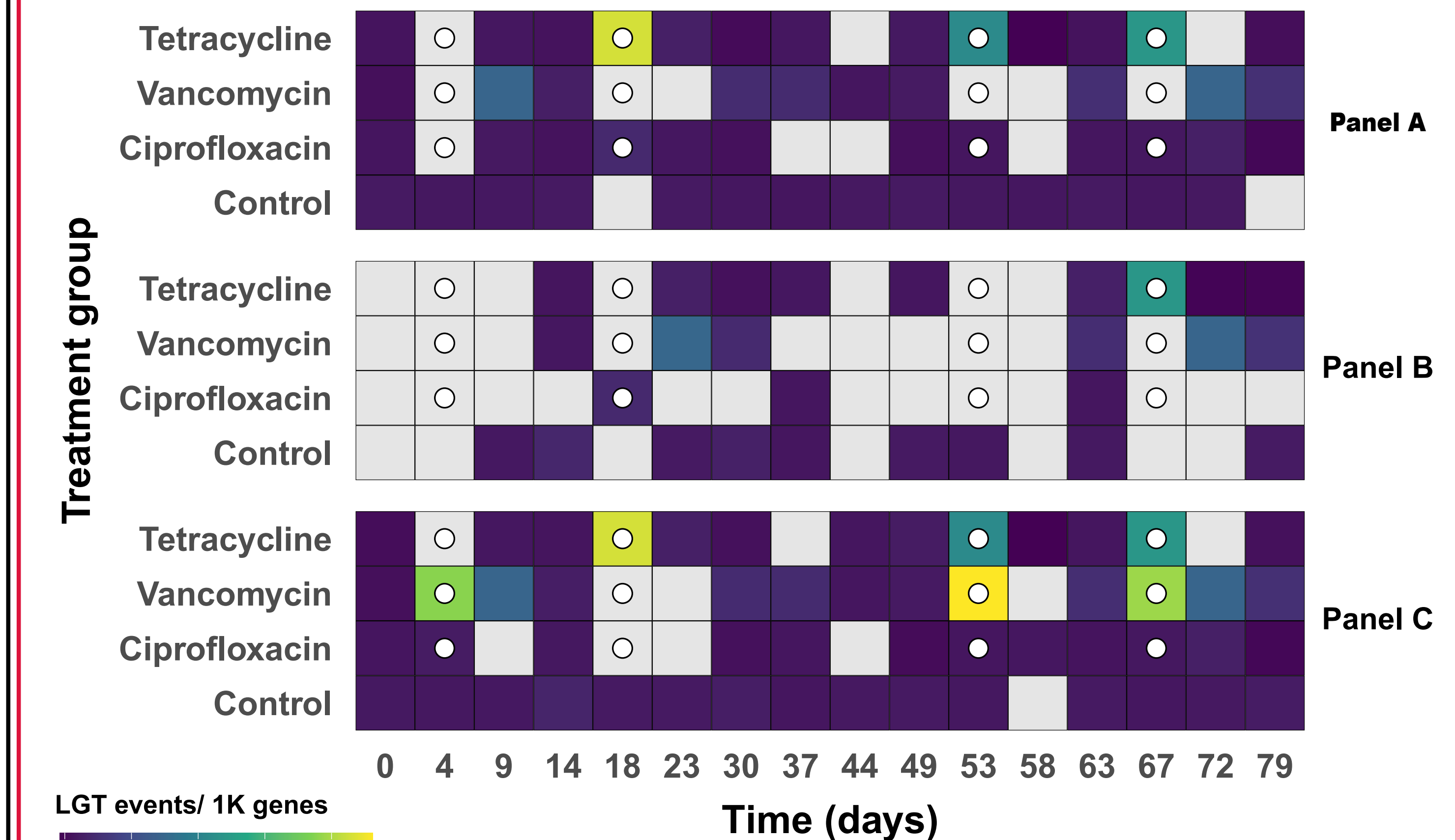
On the circo figures, each sector represents a member of the OMM<sup>12</sup> species. The x-axis shows the time in days. The links indicate two sharing partners at a given time point. The gray bar in the inner ring represents the active dates of antibiotic treatment. We profiled events between the species and traced them back to treatment events. We detected LGT events in all 4 treatment groups. LGT is prevalent and strains seem to preferentially exchange with certain partners. For each LGT event we further identify the genes involved and their respective functions. We observed bacterial strains have consistent preferred partners though tended to exchange different genes with different partners.

## Tetracycline and ciprofloxacin induced drug-specific LGT events in OMM<sup>12</sup> colonized mice



We found 2 unique exchanges attributable to tetracycline and one LGT gene attributable to ciprofloxacin which included a gene of unknown function. One tetracycline specific LGT involved a mobile genetic element of the IS91 family transposases. The LGT event contains a cassette of two genes, a SLOG superfamily protein gene and a S26 family signal peptidase, all detected during active antibiotic exposure. The SLOG gene contained a known uncharacterized SPBc2 prophage-derived protein YoqJ showing this LGT event could be phage-mediated. Previous studies have shown that SLOG protein family can play role in nucleic-acid sensing and nucleotide dependent signaling (Burroughs et al., Nucleic Acid Res, 2015) and have been shown to stimulate growth or provide resistance to oxidative stress in Mycobacterium tuberculosis through proteasome control of cytokinin (Samanovic et al., Mol Cell, 2015). Taken together this cassette of two genes provide insights into how tetracycline exposure may lead to an adaptive or resistance response.

## LGT dynamics and patterns in OMM<sup>12</sup> colonized mice



We grouped the observed patterns into three categories:

- LGT events that are detected before the experiment at time 0 and shared across the 4 groups and observed at later time points during the pulsed antibiotic experiment (Panel A). These events might be occurring just naturally and maybe playing role in sustaining the stable coexistence of the OMM<sup>12</sup> strains.
- LGT events that were only observed post-antibiotic exposure or after the experiment started and these events once observed they were shared or persisted within or across the treatment groups (Panel B). This is the most likely pool of events that can be attributed to antibiotic selection pressure.
- LGT events that are transient (Panel C). These events were only observed at one time point and never seen again.

## Conclusions

- Pulsed antibiotic treatment leads to resilience effects in the bacterial community
- We detected 371 LGT events, 172 of which occurred post-antibiotic exposure, highlighting high LGT prevalence in OMM<sup>12</sup> colonized mice
- OMM<sup>12</sup> strains preferentially exchange genes with specific patterns across treatments
  - E. clostridioformis* YL32 exchanges with *B. pseudococcoides* YL58
- Exchanging strains tend to share different genes under different antibiotic exposure
- Exposure to tetracycline led to drug-specific exchange of genes that may confer adaptive or resistance mechanism

## Funding

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<https://github.com/biobakery/waafle>

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