

Disentangling microbiota-dependent and -independent impacts of antibiotics on host physiology

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Supplementary results

Route of antibiotic administration impacts its effect on the gut microbiota

Of the separately administered antibiotics, we found that ampicillin treatment led to the most pronounced reduction in the effective number of genera (using the Shannon diversity of the distribution, Fig. 1b). All three administration routes of ampicillin led to similar changes to microbiota composition (Fig. 1d-f and Extended Data Fig. 1e). These effects of ampicillin on gut microbial composition were also similar to those observed in mice administered the 4-antibiotic mix (Fig. 1d-f).

Of the antibiotics tested here, metronidazole had the least significant effect on bacterial composition, with no reduction in effective number genera (Fig. 1b, c, Extended Data Fig. 1f, and SI Table S1). From a compositional standpoint, metronidazole treatment was statistically indistinguishable from control (Fig. 1c-f). We also found that systemic neomycin treatment had minimal effect on bacterial composition, while oral treatment had a more pronounced effect (Fig. 1d-f and Extended Data Fig. 1g). This likely reflects the poor ability of neomycin to cross mucosal barriers¹.

Administration of all the antibiotics in combination resulted in a stark reduction in the effective number of genera and species and a change in bacterial composition in all administration routes (Fig. 1b-f and Extended Data Fig. 1h). Additionally, systemic administration of the 4-antibiotic mix led to higher bacterial community variation between different mice in the same group, as opposed to oral administration (Extended Data Fig. 1h). This difference might be due to the different luminal availability of different antibiotics when administered systemically, in contrast to oral treatment, which delivers all of the antibiotics to the gut lumen in equal amounts. Thus, altering administration routes of antibiotics leads to different effects on the gut microbiota.

Changes to gut microbiota composition do not necessarily predict alterations in gut gene expression

Our analysis showed that ampicillin treatment had a significant effect on rhythmic processes (Fig. 1i), similar to previous findings². In addition, we found induction of immune response pathways by oral and systemic administration of neomycin (Fig. 1i). Specifically, we found significant overlap in host response to both IP and IV administration, both eliciting expression of immunomodulatory and protein homeostasis pathways (Fig. 1i). It is worth

noting that these effects of systemic neomycin treatment on host gene expression were not mirrored by significant shifts to microbiota composition (Extended Data Fig. 1g). Indeed, neomycin was previously shown to induce antiviral responses in a microbiota-independent manner³.

Non-additive combination drug response and transcriptional noise

We assessed non-additive effects by modeling gene expression with a factorial design ($\sim$ DrugA + DrugB + Interaction) in pydeseq2⁴. These isolated non-additive genes where the combination's effect significantly deviated from the sum of the single drugs, categorizing them as either "synergistic" or "antagonistic". We now report that, as in the 4-antibiotic mix, combination of metronidazole with vancomycin led to antagonism of the gene induction seen in individual administration (Extended Data Fig. 3a). Furthermore, the combination of neomycin and metronidazole mainly suppressed ribosomal genes, even when compared to the control group (Extended Data Fig. 3b). Thus, combining different antibiotics, even in clinically-relevant pairs, can result in non-additive and counter-intuitive effects on the host (Extended Data Fig. 3c-d).

Next, we quantified global transcriptional noise by calculating the Euclidean distance of individual replicates to their group centroid in PCA space. This metric served as a proxy for system-wide instability, determining if the combination treatment resulted in higher inter-replicate heterogeneity than the single therapies. We found that indeed in the case of vancomycin and metronidazole the combination treatment was noisier, but for neomycin, the effect is unclear (Extended Data Fig. 3e-f).

The summary of the results presented for the additivity and interference calculations can be found in SI Table S8. The taxonomic profiles inferred from the metagenomics using MetaPhlan can be found in SI Table S10 and the 16S data in SI Table S11. The host transcriptomic feature table can be found in SI Table S12.

Supplementary references

1. Jana, S. & Deb, J. K. Molecular understanding of aminoglycoside action and resistance. *Appl Microbiol Biotechnol* **70**, 140–150 (2006).

2. Thaïss, C. A. *et al.* Microbiota Diurnal Rhythmicity Programs Host Transcriptome Oscillations. *Cell* **167**, 1495-1510.e12 (2016).
3. Gopinath, S. *et al.* Topical application of aminoglycoside antibiotics enhances host resistance to viral infections in a microbiota-independent manner. *Nat Microbiol* **3**, 611–621 (2018).
4. Muzellec, B., Teleńczuk, M., Cabeli, V. & Andreux, M. PyDESeq2: a python package for bulk RNA-seq differential expression analysis.
<https://dx.doi.org/10.1093/bioinformatics/btad547>.

Tables

Table S14. Summary of new resource data and analyses

Data type	Figs	Raw Data	Analysis type
Stool microbial composition data in response to antibiotics	1, Extended data 1	GSE293029	• 16S Compositional Analysis • Pairwise Adonis2 comparison between treatment and PBS. • ANCOM analysis.
Host transcriptomics in response to antibiotics (colon)	1	GSE293364	• GSEA analysis of differential expression of GO clusters.
	2, Extended data 2		• RF classification of different antibiotics
	2		• RF classification of administration routes
Microbial stool composition and host transcriptomics	3	GSE293029 GSE293364 GSE260592	• CompoRes p-value and correlation table for host gene expression with microbiota composition • CompoRes results comparing FMT and GF experiments.
Diurnal host transcriptomics in response to vancomycin (GF+SPF)	4 and 5	GSE293364	• Genes selected as changed in the diurnal dynamics for both the SPF and GF experiments.

Paired antibiotics: host transcriptomics in response to antibiotics (colon) coupled with stool metagenomics and 16S profiling	Extended Data 3,4	PRJNA1442090	• Gene expression treatment additivity • CompoRes agreement between gene expression and both 16S and metagenomic stool profiling.
Differentiated PC12 cells transcriptional response to vancomycin <i>in vitro</i>	5	GSE324684	Differentially expressed genes

Table S15. Software used for analysis

Software or Algorithm	Reference (Paper URL)	Version	Link or Download Source
Python		3.12.3	https://www.python.org/downloads/release/python-3123/
scikit-learn	https://jmlr.csail.mit.edu/papers/v12/pedregosa11a.html	1.5.0	https://scikit-learn.org/stable/
goatools		1.4.7	https://pypi.org/project/goatools/
pandas		2.2.2	https://pypi.org/project/pandas/
numpy		1.26.4	https://pypi.org/project/numpy/
matplotlib		3.9.0	https://pypi.org/project/matplotlib/
seaborn		0.13.2	https://pypi.org/project/seaborn/
scipy		1.13.1	https://pypi.org/project/scipy/

statsmodels		0.14.2	https://pypi.org/project/statsmodels/
anytree		2.12.1	https://pypi.org/project/anytree/
CompoRes		1.0.0	https://github.com/AmirErez/CompoRes
GSEA	https://www.pnas.org/doi/10.1073/pnas.0506580102	V4.3.3	https://www.gsea-msigdb.org/gsea/index.jsp
QIIME2	https://doi.org/10.1038/s41587-019-0209-9	2024.2	https://library.qiime2.org/quickstart/amplicon
DADA2	https://www.nature.com/articles/nmeth.3869	1.26.0	https://github.com/benjjneb/dada2/releases/tag/v1.26
Greengenes2	https://doi.org/10.1038/s41587-023-01845-1	2022.1	https://ftp.microbio.me/greengenes_release/2022.10-rc1/2022.10.backbone.v4.nb.qza
ANCOM-BC	https://doi.org/10.1038/s41467-020-17041-7	2.4.0	https://www.bioconductor.org/packages/release/bioc/html/ANCOMBC.html
phyloseq	https://doi.org/10.1371/journal.pone.0061217	1.46.0	https://www.bioconductor.org/packages/release/bioc/html/phyloseq.html
vegan		2.6.10	https://cran.r-project.org/web/packages/vegan/
dplyr		1.1.4	https://cran.r-project.org/web/packages/dplyr/
compositions		2.0.8	https://cran.r-project.org/web/packages/compositions/
MetaPhlAn	https://doi.org/10.1038/s41587-023-01688-w	4.1.0	https://github.com/biobakery/MetaPhlAn
DIABLO (mixOmics)	https://doi.org/10.1093/bioinformatics/bty1054	6.32	https://github.com/mixOmicsTeam/mixOmics
pydeseq2	https://doi.org/10.1093/bioinformatics/btad547	0.4.12	https://github.com/scverse/PyDESeq2