



***In vivo* evaluation of *Clostridioides difficile* enoyl-ACP
reductase II (FabK) Inhibition by phenylimidazole
unveils a promising narrow-spectrum antimicrobial
strategy**

Chetna Dureja

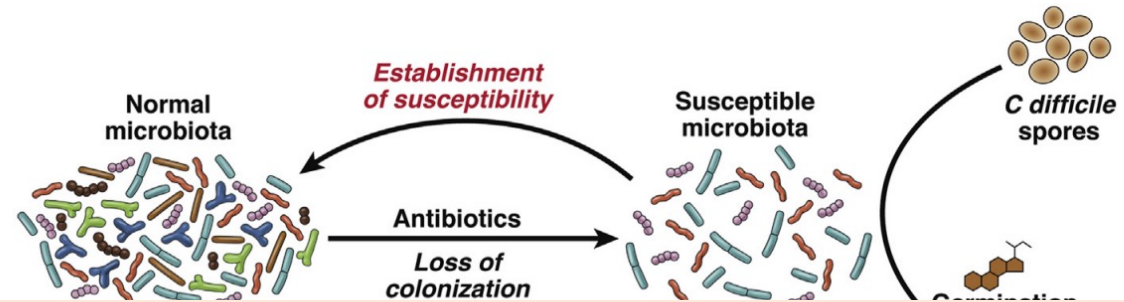
Introduction to *C. difficile*



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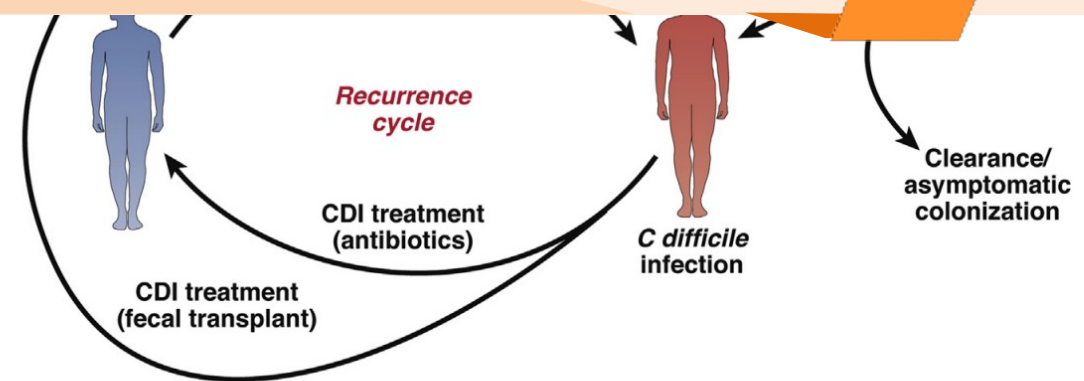
- C. difficile* is a leading cause of health-care associated infections

Estimated annual burden in the U.S of over 500,000 cases and 14,000 deaths



Which cellular processes or targets should be pursued to selectively inhibit *C. difficile*, without adversely damaging the microbiome?

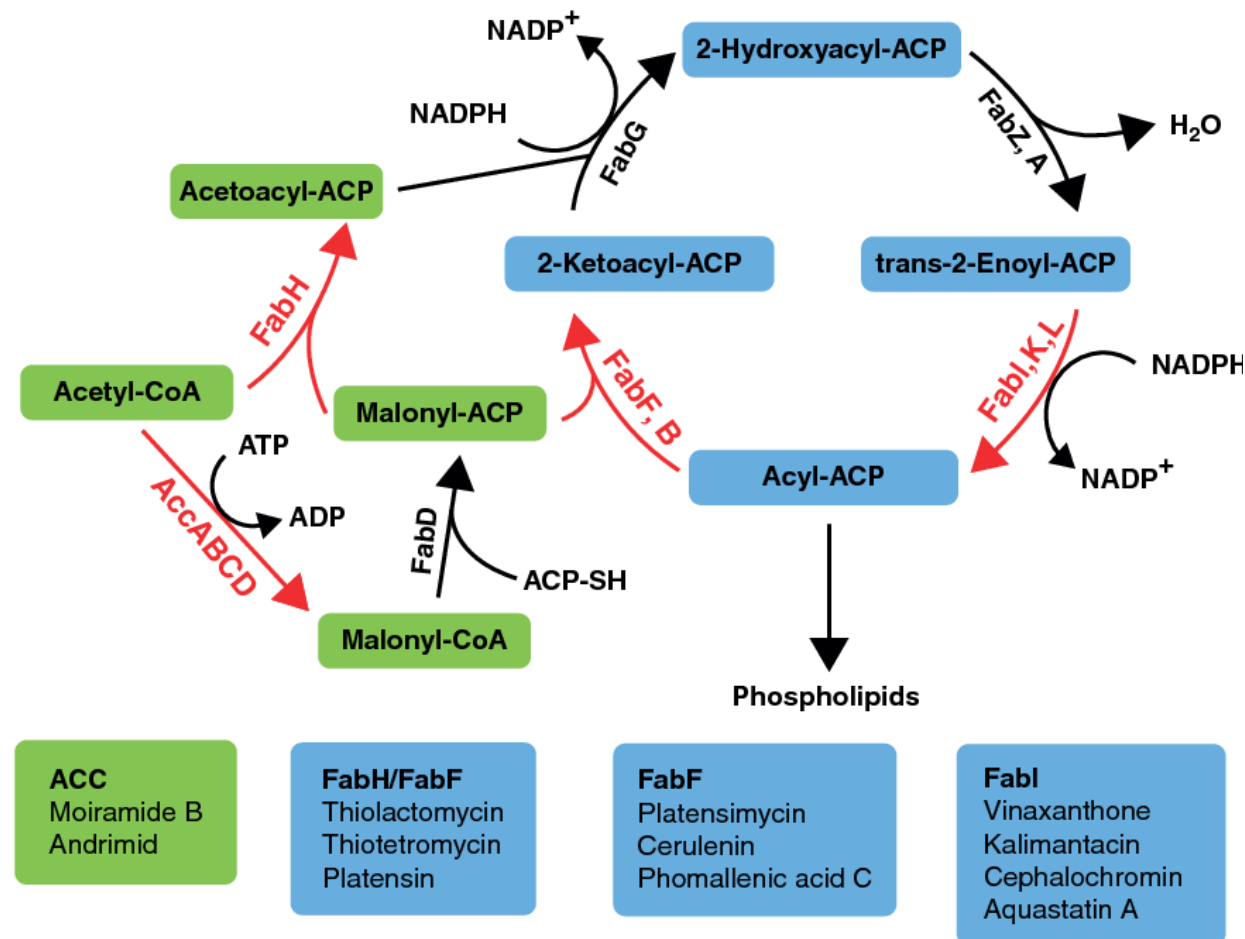
- Broad-spectrum antibiotics used to treat CDI cause microbial dysbiosis, which eases *C. difficile* colonization
- Recurrent infection ($\approx 25\%$ of cases) due to sporulation and dysbiosis
- Incidents of resistance to vancomycin, metronidazole and fidaxomicin, especially with recurrent CDI



Fatty Acid Synthesis Pathway



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Why FabK?



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It is essential

In contrast to several significant microbiota species, *C. difficile* exclusively utilizes FabK as its primary enoyl-ACP reductase.



FabK has no structural similarity other ENR (FabI, FabL and FabV)

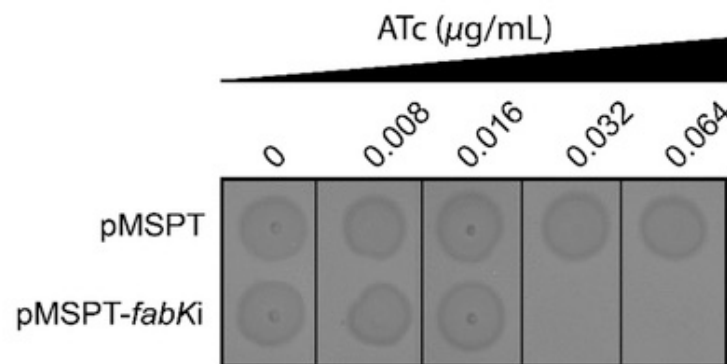
Is *fabK* essential?



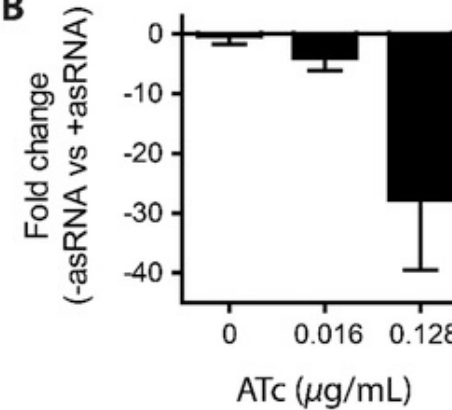
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Impact of antisense *fabK* RNA on *C. difficile* CD630 growth and gene expression

A



B

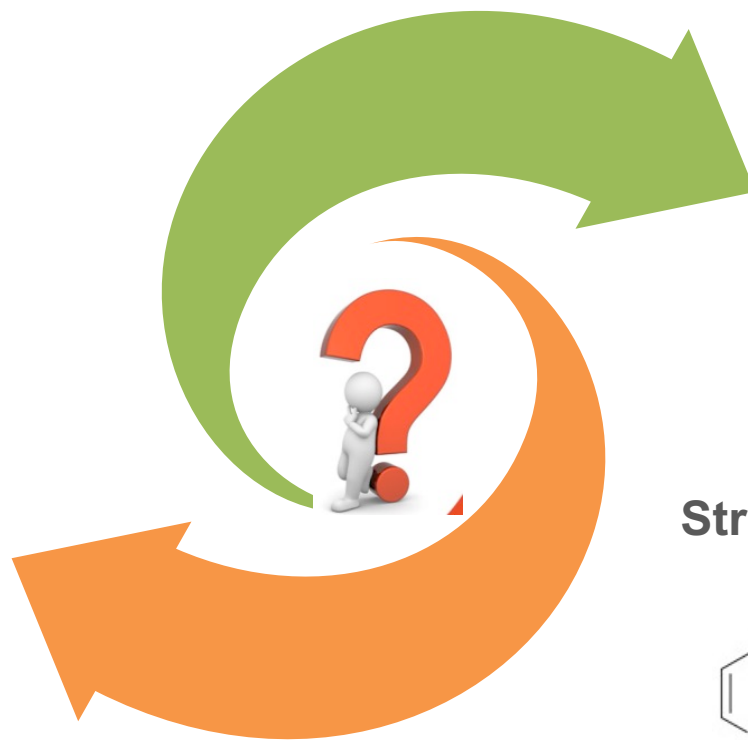


Next Questions



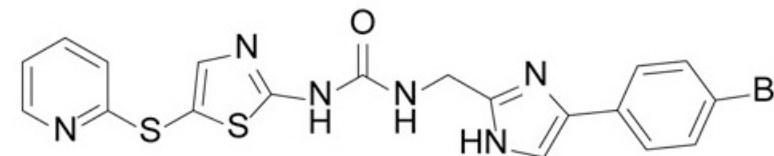
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Is FabK a narrow
spectrum target?



Is FabK a target in
various *C. difficile*
ribotypes?

Structure of the Phenylimidazole 296



296 Combats CDI ribotypes



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<i>C. difficile</i> ribotypes (n= number of strains)	MIC (µg/ml)	
	296	Vancomycin
R20291(027) control	0.5	0.5
001-072 (n=2)	0.25-0.5	0.5
002 (n=3)	1-2	0.5-1
014 (n=3)	0.5	0.25-0.5
017 (n=2)	0.125-0.25	0.25
018 (n=1)	0.5	0.5
019 (n=3)	1-2	0.5-1
020 (n=3)	0.062-0.5	0.25-0.5
024 (n=1)	2	0.5
027 (n=3)	0.5-1	0.25-0.5
047 (n=1)	0.125	0.25
054 (n=3)	0.062-0.5	0.25-0.5
078 (n=2)	1	1
106 (n=3)	0.5-1	1-2
Range	0.062-2	0.25-2
MIC ₅₀	0.5	0.5
MIC ₉₀	2	1

296 Selective for *C. difficile*



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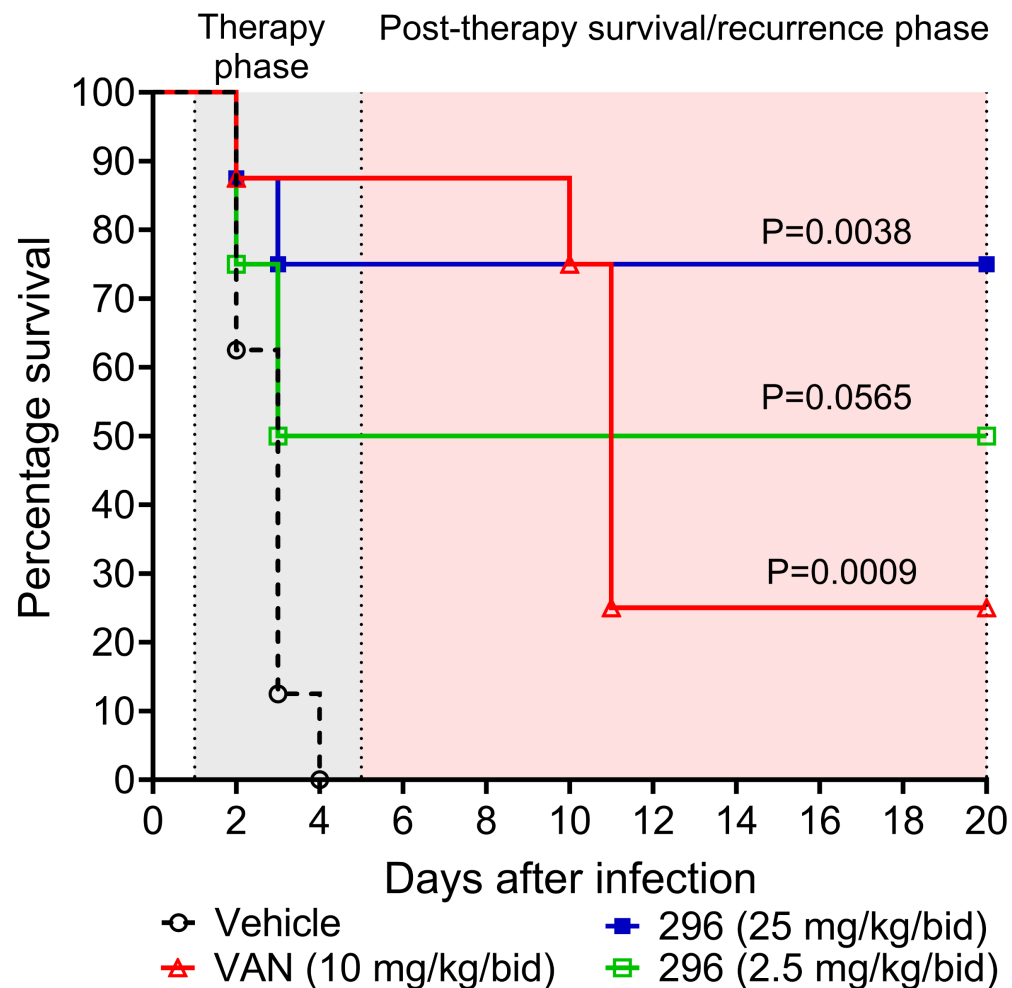
Specie/Strain	Accession No.		MIC (µg/ml)		
			296	Vancomycin	Fidaxomicin
<i>C. difficile</i> R20291 control	FN545816.1	FabK	0.5	0.5	≤0.0625
<i>Bacteroides</i> sp. (HM18)	SAMN02463734		≥64	16	≥64
<i>Bacteroides</i> sp. (HM19)	SAMN02463818		≥64	≥64	≥64
<i>Bacteroides</i> sp. (HM23)	SAMN02463690	FabK and FabI	≥64	16	64
<i>Bacteroides</i> sp. (HM28)	SAMN02463697		≥64	16	≥64
<i>Bacteroides fragilis</i> (HM20)	SAMN02463689		≥64	8	≥64
<i>Bacteroides eggerthii</i> (HM210)	SAMN02463790		≥64	16	≥64
<i>Bacteroides ovatus</i> (HM222)	SAMN02463791		≥64	16	≥64
<i>Clostridium sporogenes</i> (ATCC3584)	SAMN03742637		64	4	≤0.0625
<i>Clostridium sporogenes</i> (ATCC11437)	NA		≥64	4	≤0.0625
<i>Clostridium septicum</i> (ATCC12464)	SAMN07710202		2	2	≤0.0625
<i>Clostridium histolyticum</i> (ATCC19401)	SAMD00013616	FabK	0.5	1	≤0.0625
<i>Paraclostridium bifermentans</i> (ATCC 638)	SAMN01978893		2	0.5	≤0.0625
<i>Paeniclostridium sordellii</i> (ATCC 9714)	SAMEA1572090		0.5	1	≤0.0625
<i>Lactobacillus crispatus</i> (HM421)	SAMN00829396	FabK and FabI	≥64	4	64
<i>Bifidobacterium bifidum</i> (ATCC 11863)	NA		≥64	4	≤0.0625
<i>Bifidobacterium breve</i> (ATCC15700)	SAMN00008778	Type 1 FAS	≥64	1	≤0.0625
Activity against isogenic enterococci					
<i>E. faecalis</i> FA 2-2 (WT)	SAMN22569088	FabK and FabI	≥64	4	1
<i>E. faecalis</i> FA 2-2ΔfabK	NA	FabI	≥64	4	1
<i>E. faecalis</i> FA 2-2ΔfabI	NA	FabK	≤0.0625	4	1

Efficacy in CDI Colitis Model



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Kaplan-Meier Survival Analysis

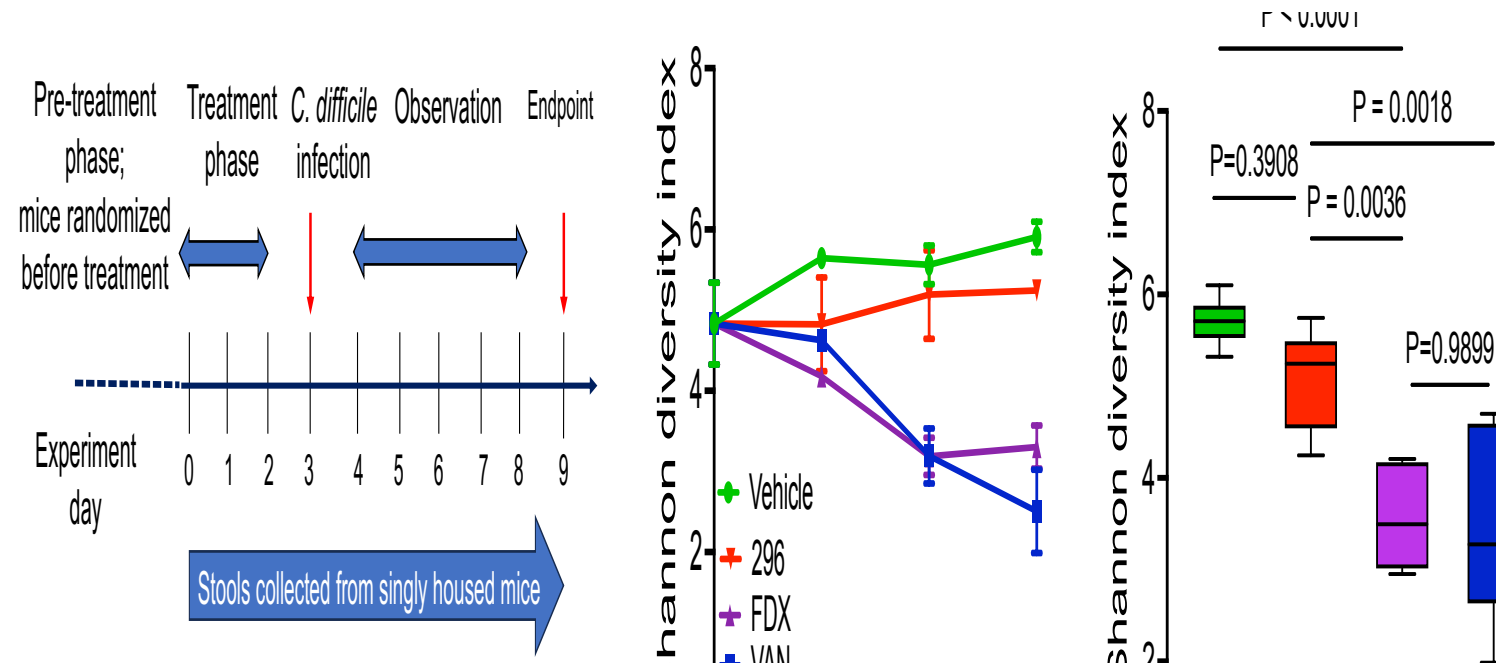


296 Effects on Mouse Microbiome



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Schematic of the Experimental Plan of Studies

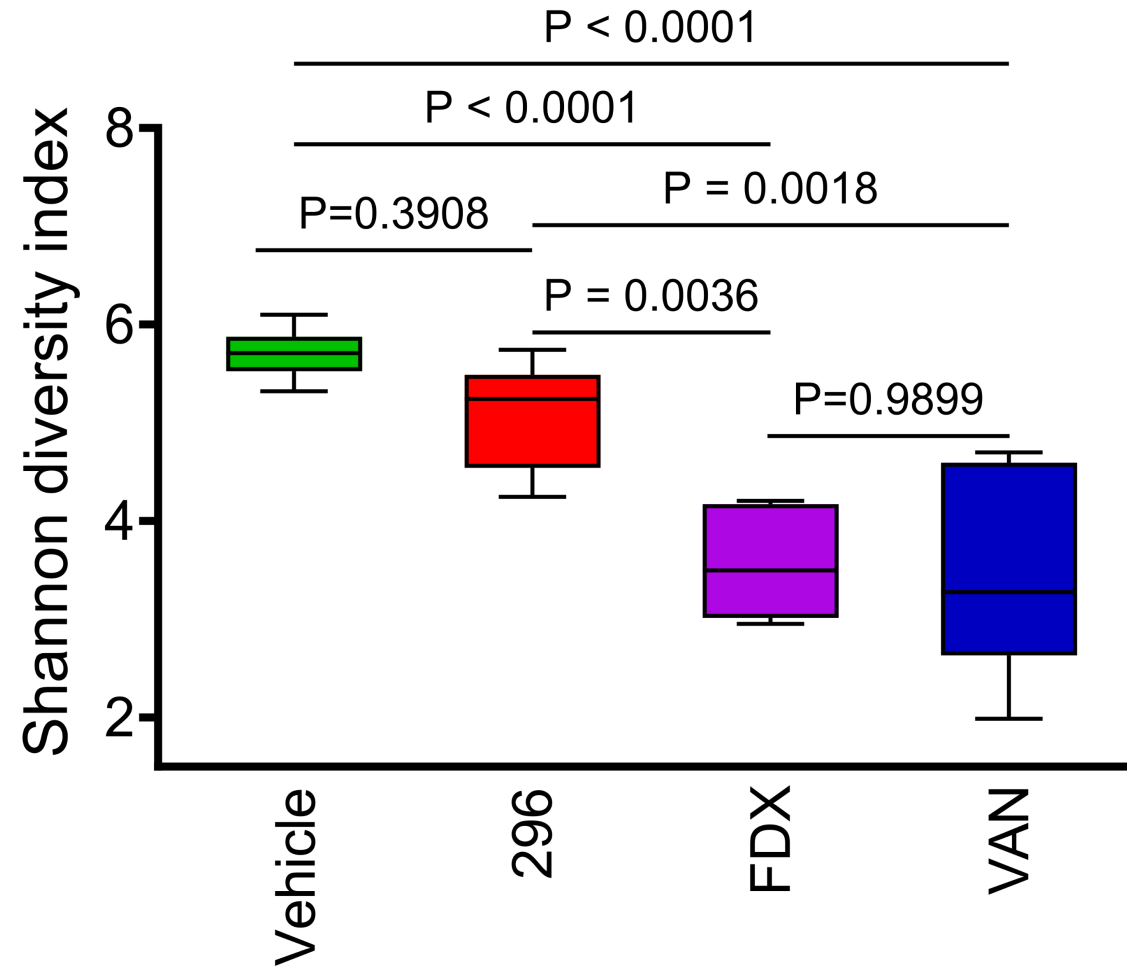


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Statistical Analysis of the Alpha Diversity

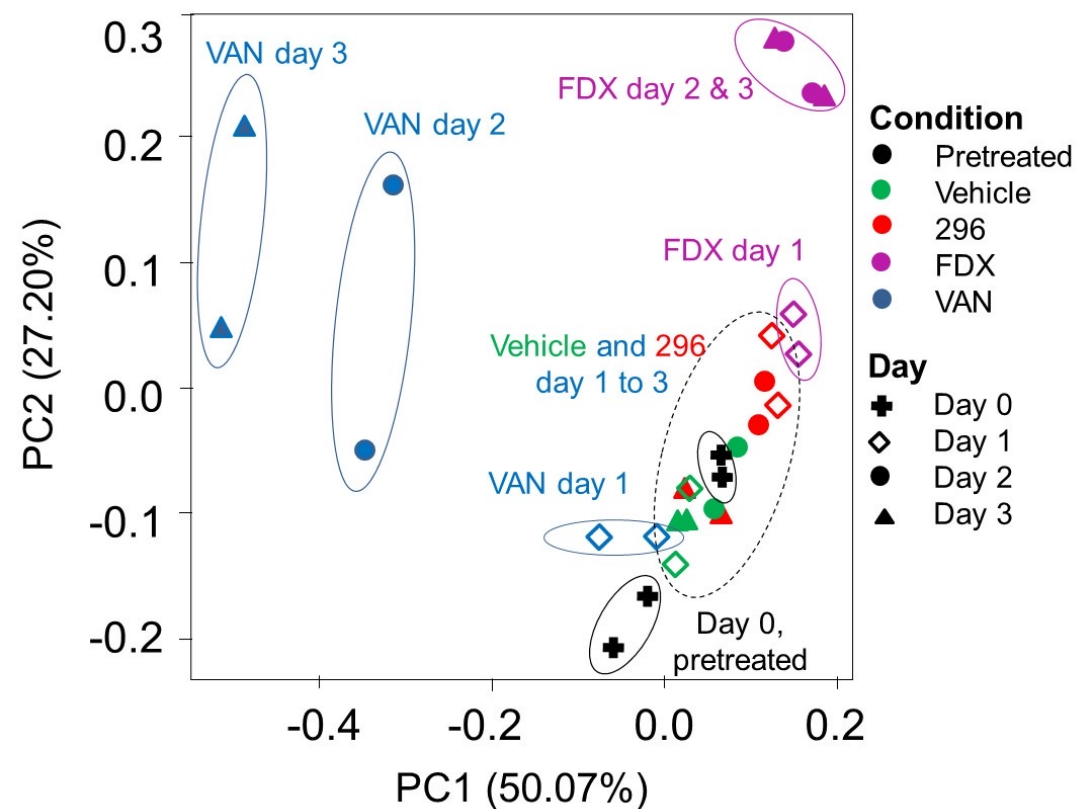


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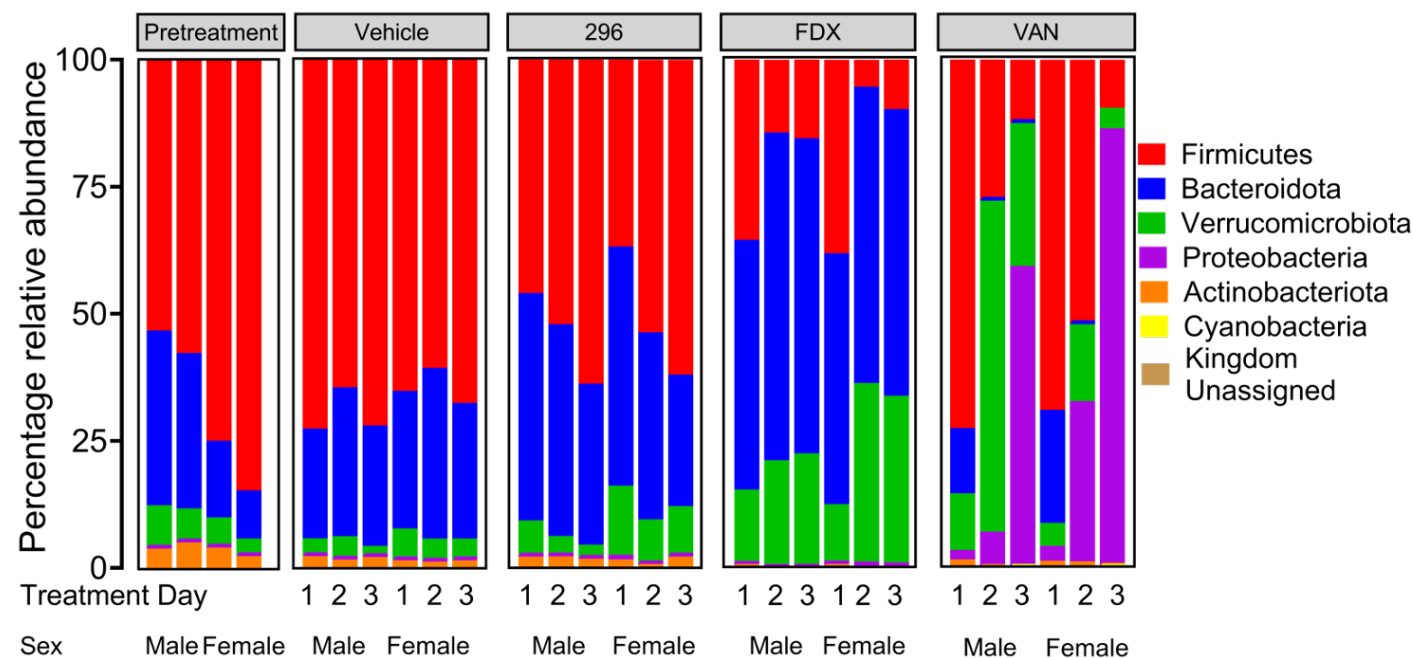


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A Principal-Coordinate Analysis (PCoA)



Relative Abundance of Each Phylum During the Treatment Period

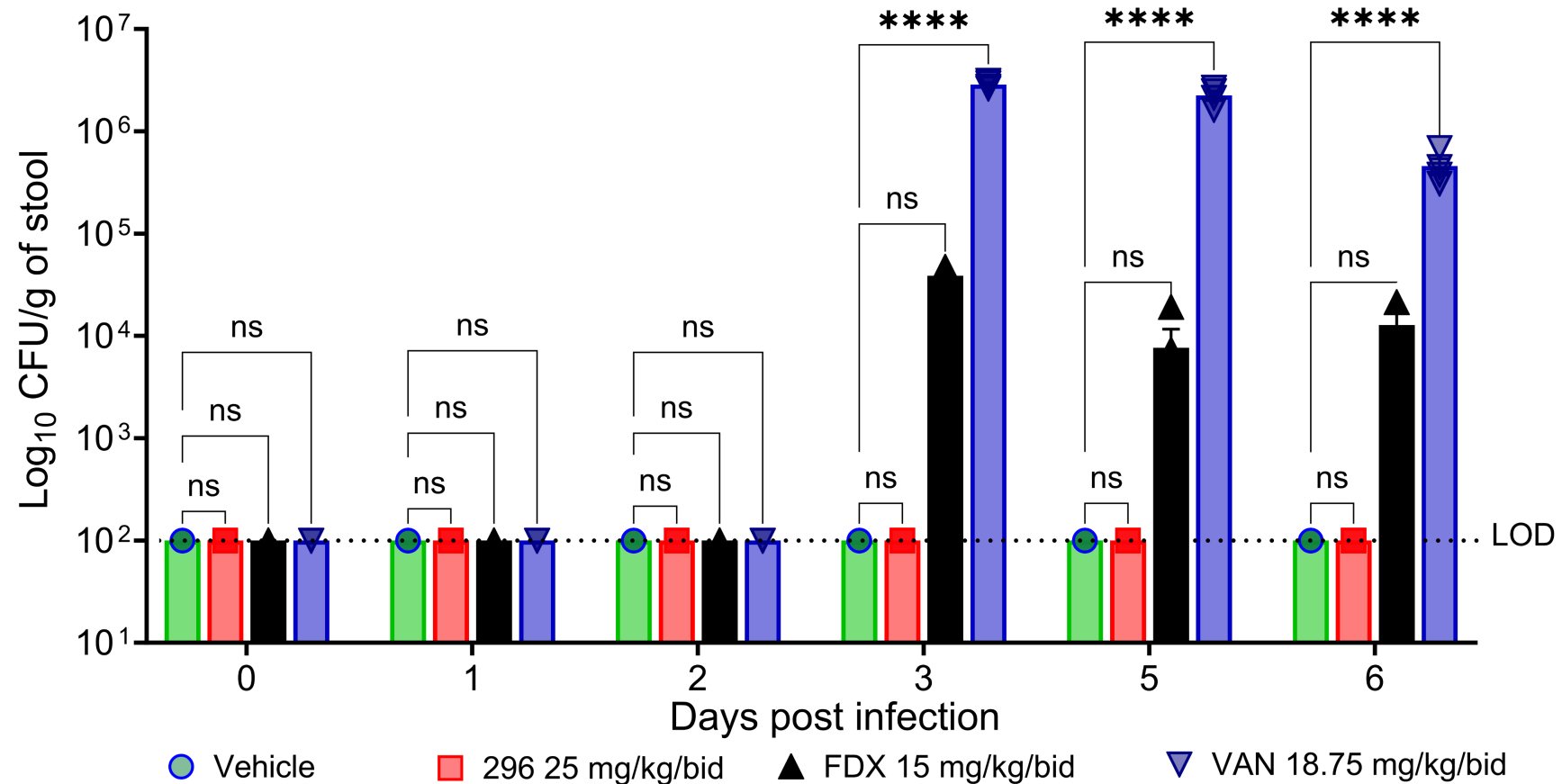


Colonization Resistance



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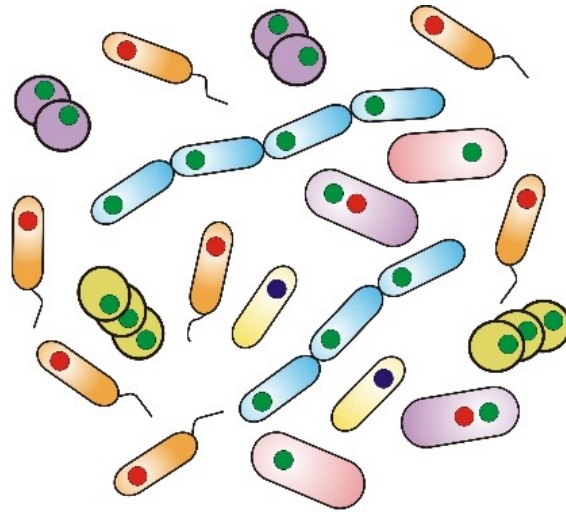
Assessment of Colonization Resistance Following Treatment



Conclusions

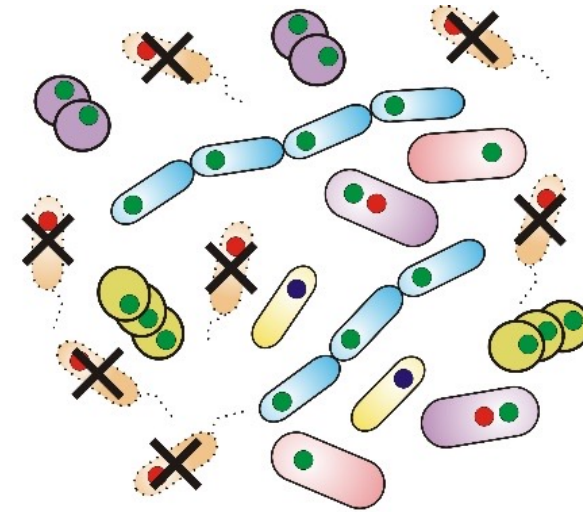


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C. difficile infected gut
microbial composition

FabK inhibitors
→



Specific eradication of *C. difficile*

- Enoyl-ACP reductase (FabK)
- Other enoyl-ACP reductase (e.g. FabI/FabL)
- *C. difficile*

Acknowledgement



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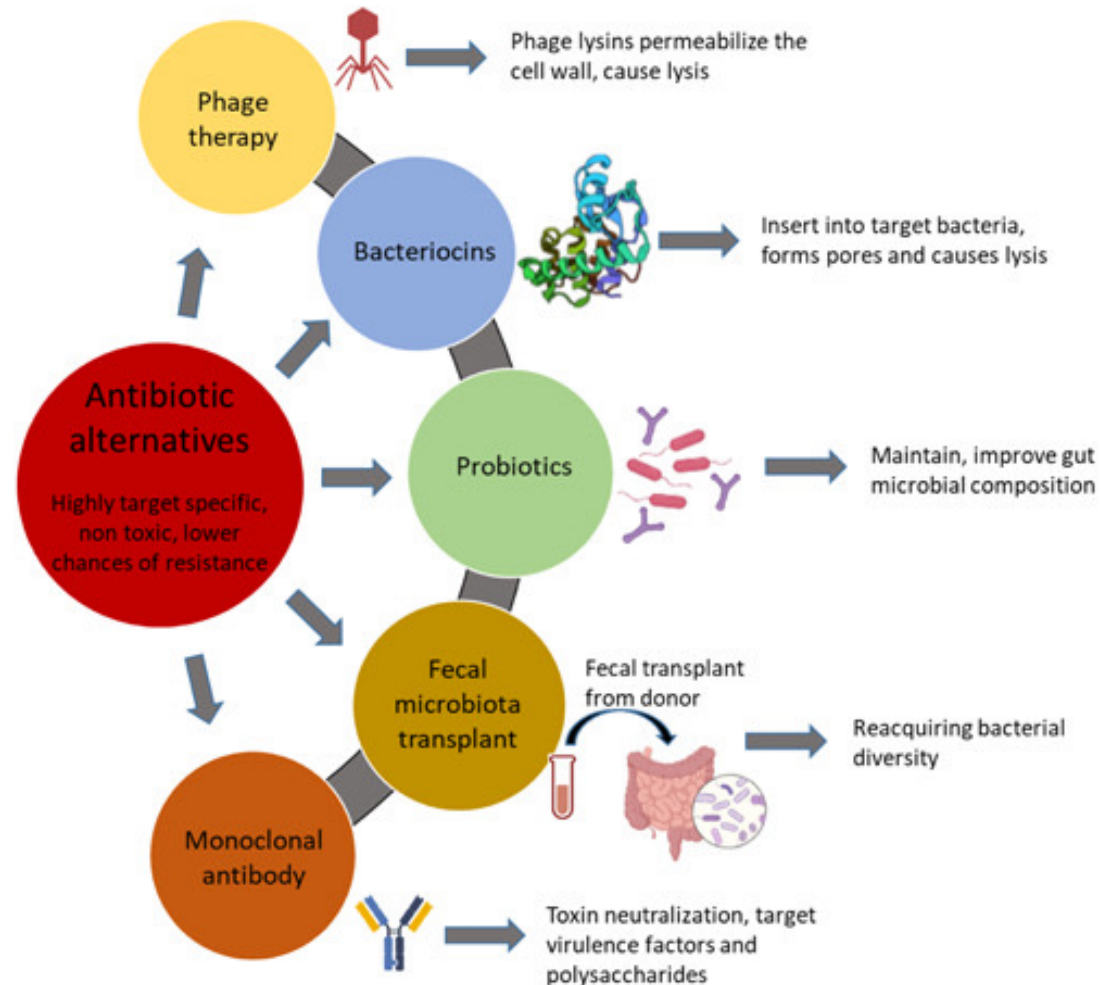
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Alternative Therapeutics



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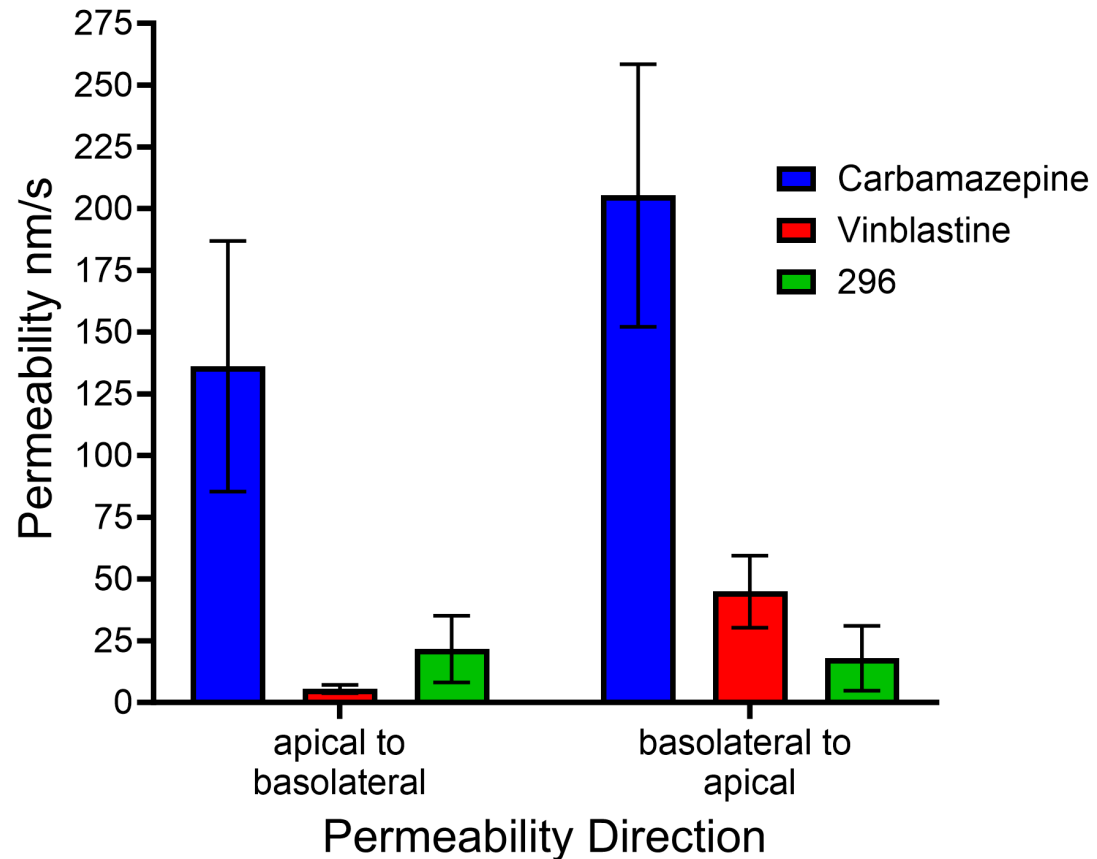


Oral Bioavailability

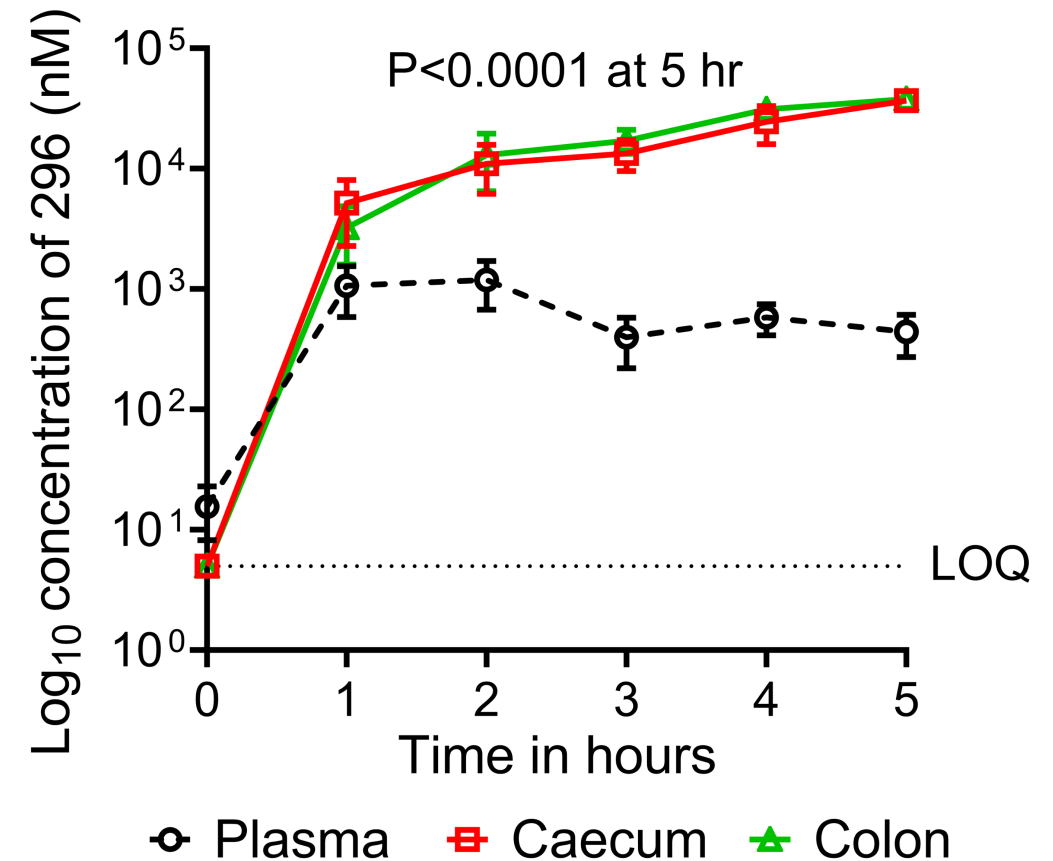


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Caco2 Permeability Assay



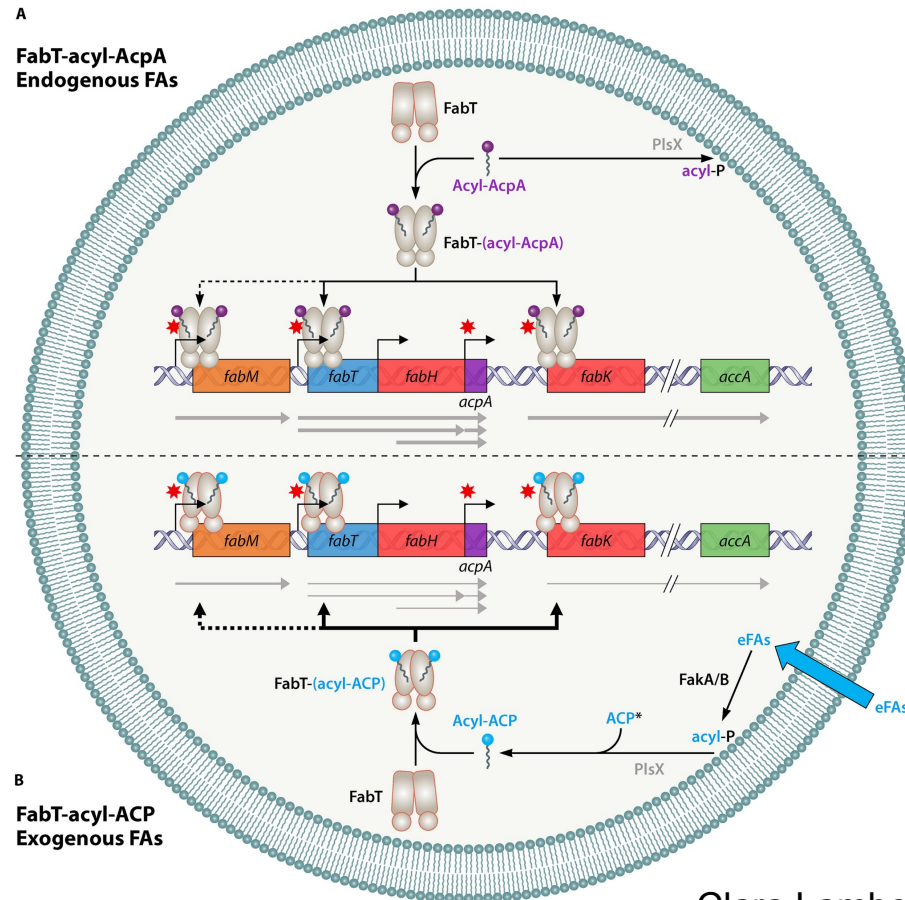
in vivo Pharmacokinetics



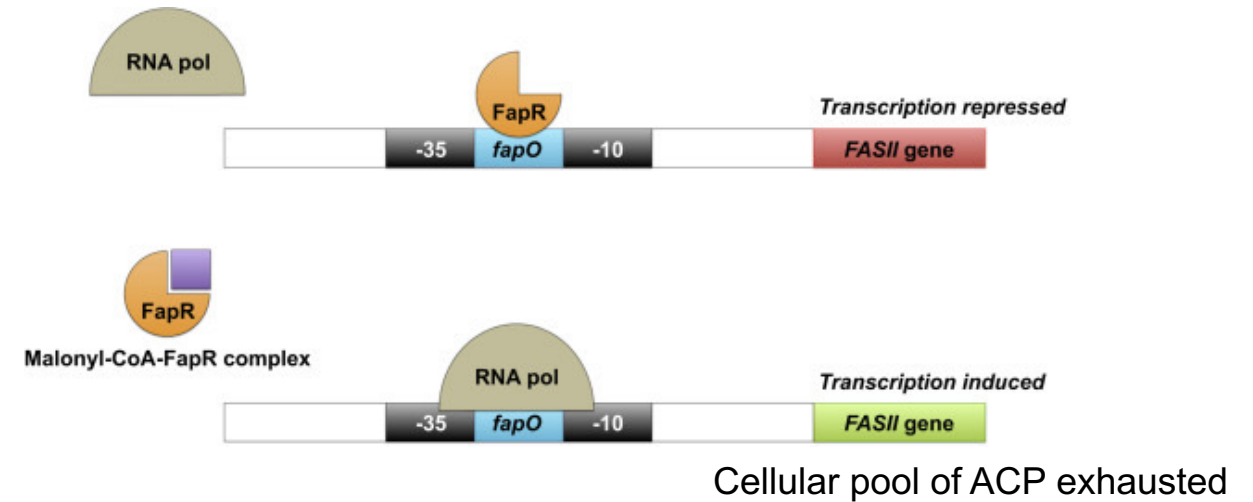
Host lipids Compensation?



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Clara Lambert *et al* 2022

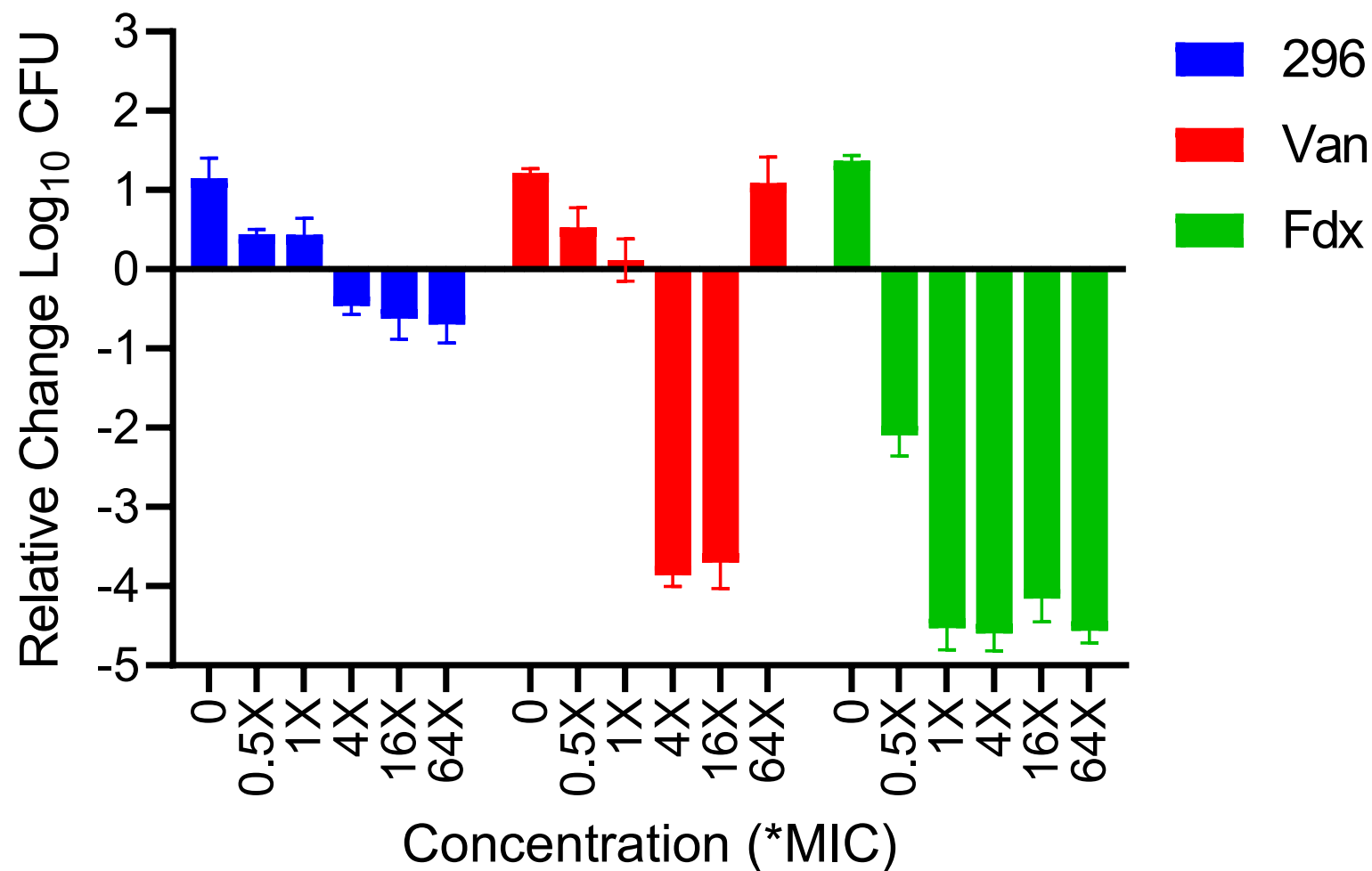


Johnson *et al* 2017

Bacteriostatic or Bactericidal?



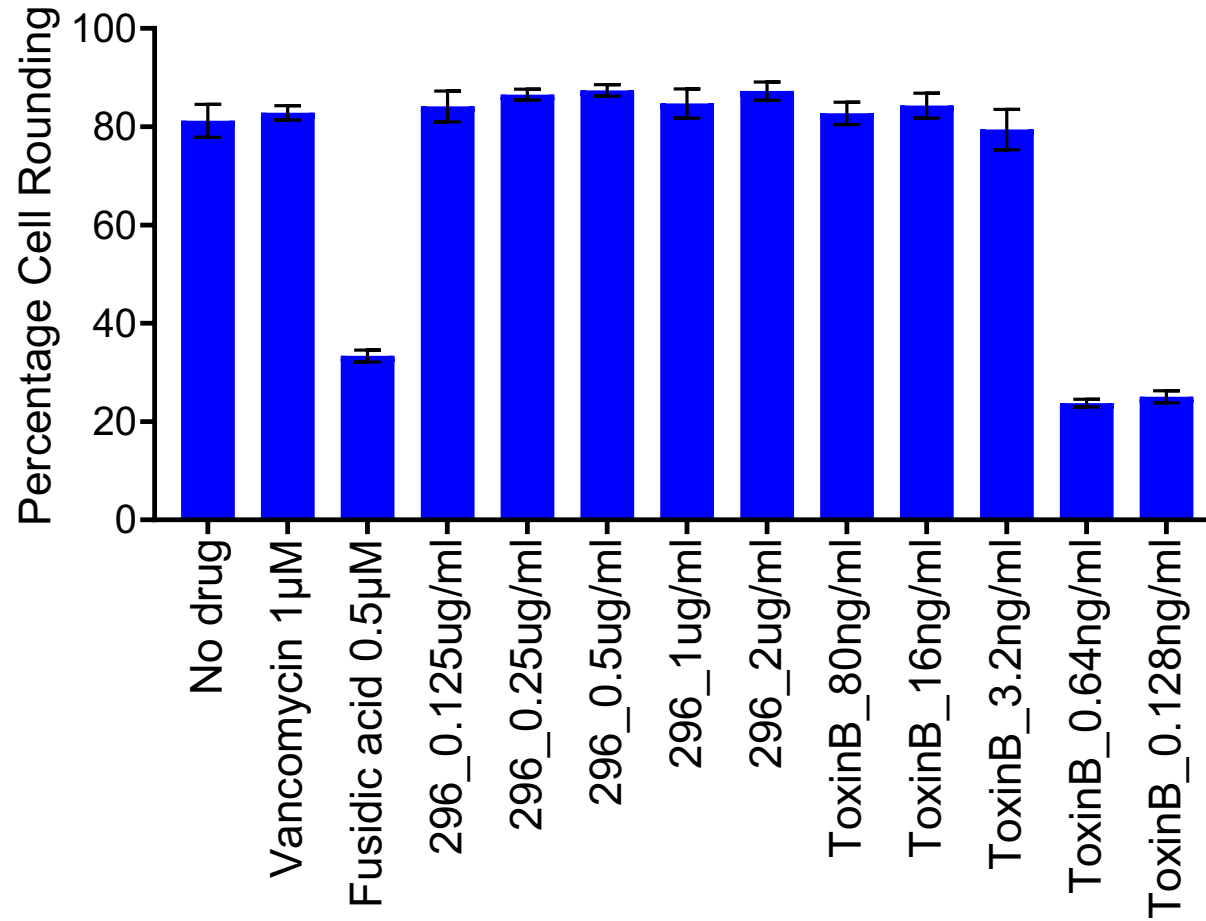
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Effects on Toxins



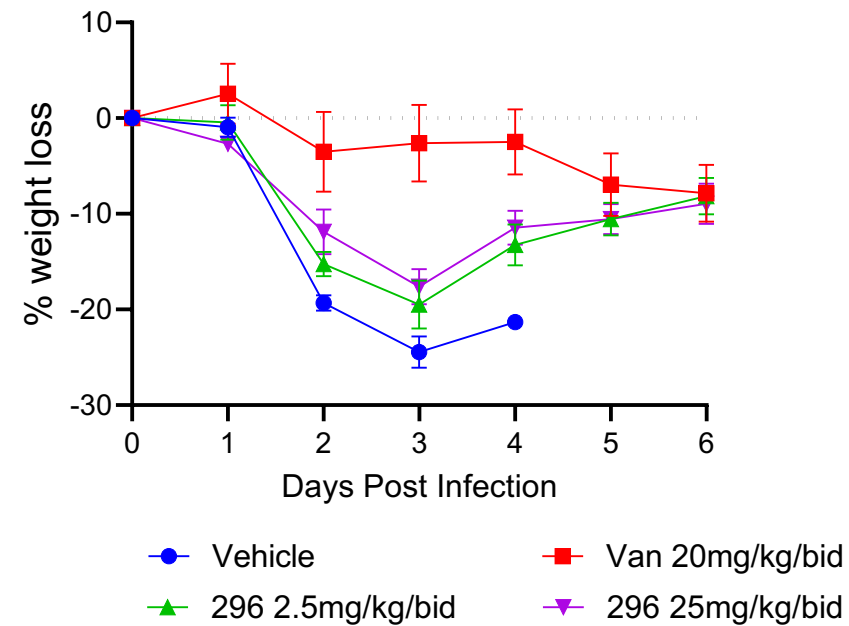
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Percentage weight change



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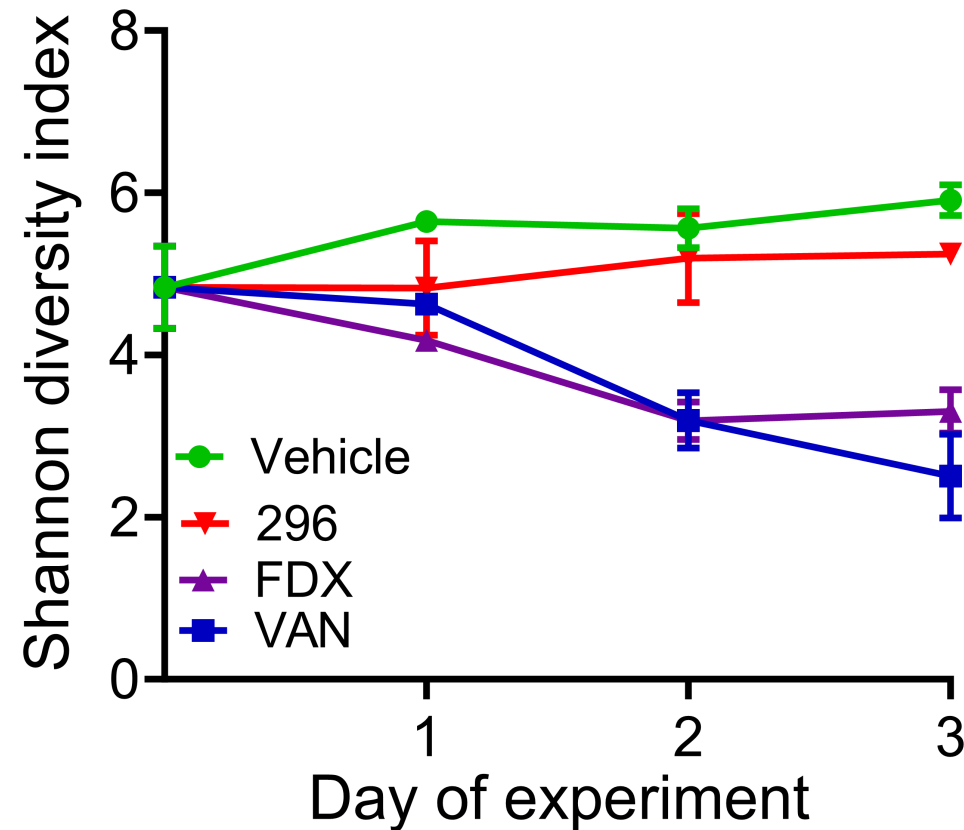


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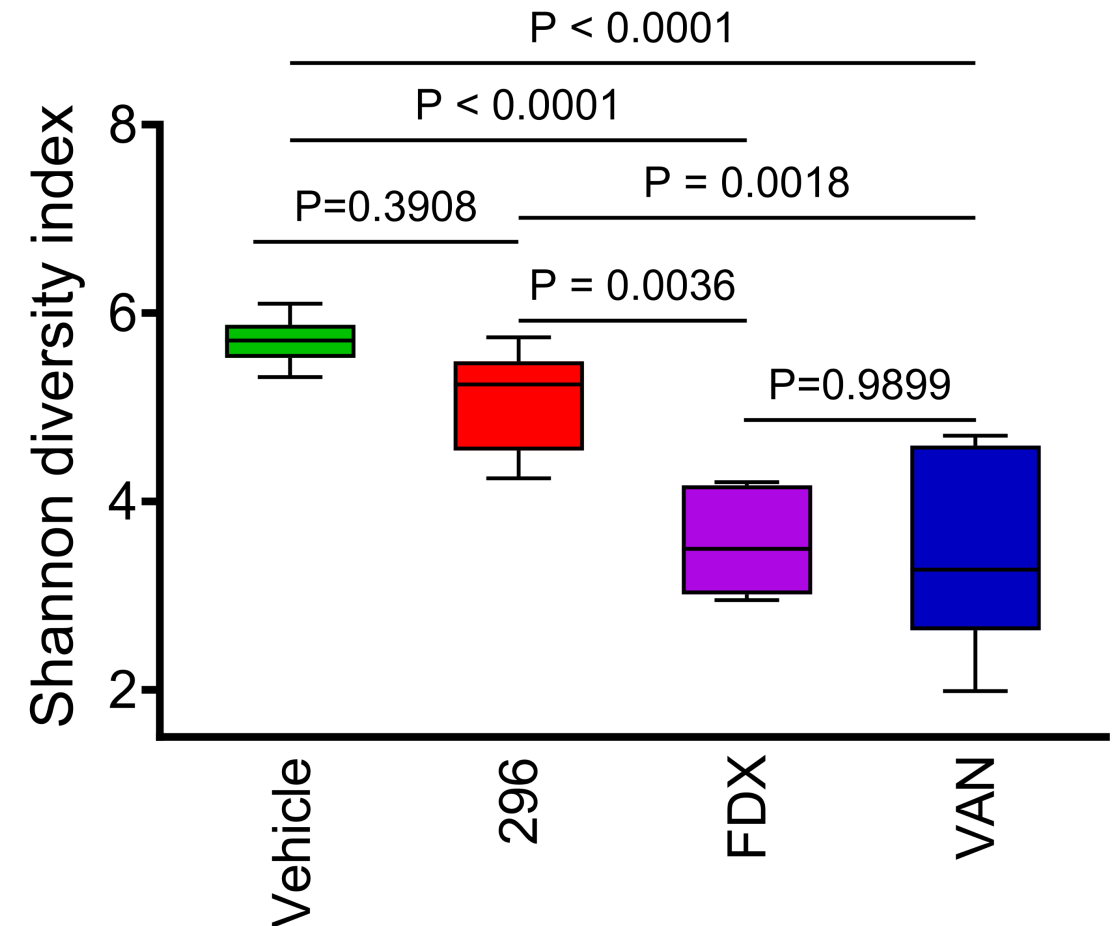


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Shannon Diversity Index



Statistical Analysis of the Alpha Diversity

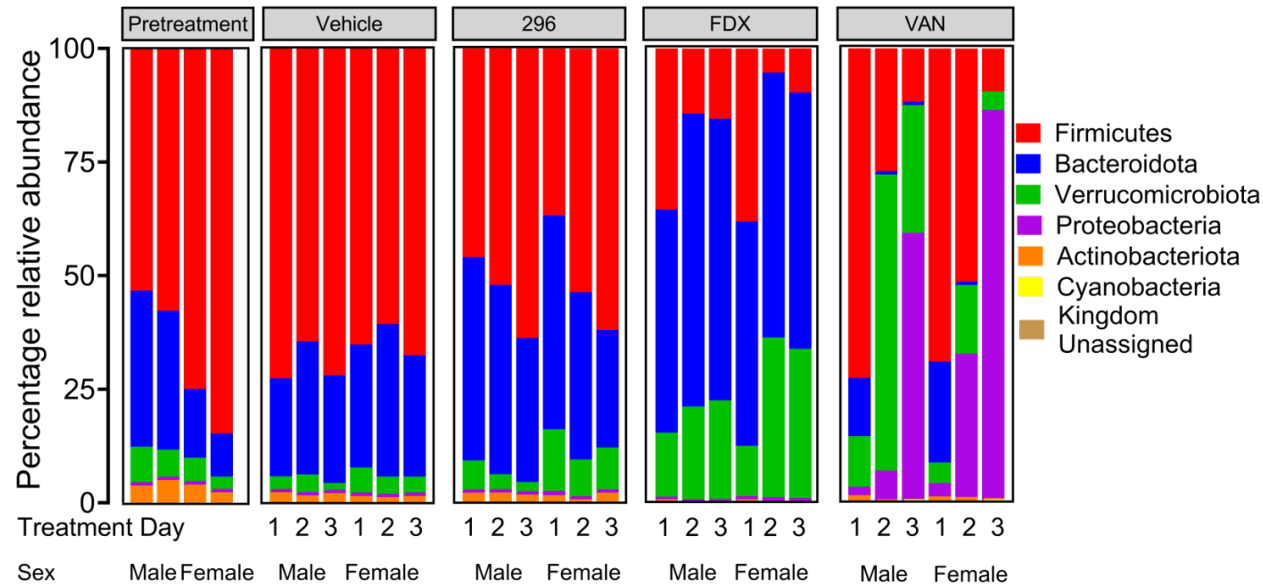


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Relative Abundance of Each Phylum During the Treatment Period



Relative Abundance of Each Class During the Treatment Period

