Microbiome Results from the phase 2, randomized, double-blind study of ibezapolstat compared with vancomycin for the treatment of *Clostridioides difficile* infection.

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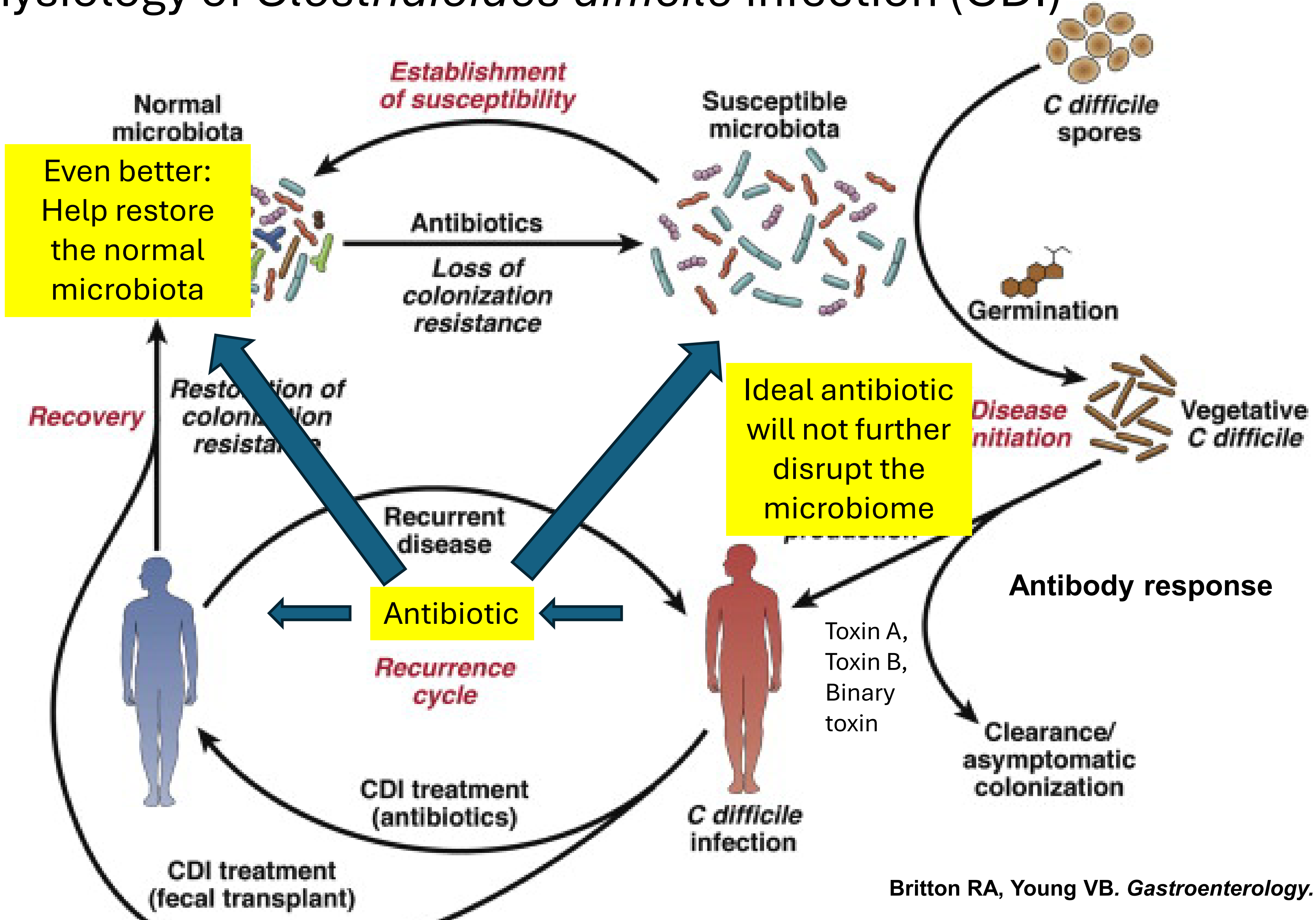
UNIVERSITY of HOUSTON | COLLEGE OF PHARMACY

Pathophysiology of *Clostridioides difficile* infection (CDI)



Even better:
Help restore
the normal
microbiota

Ideal antibiotic
will not further
disrupt the
microbiome

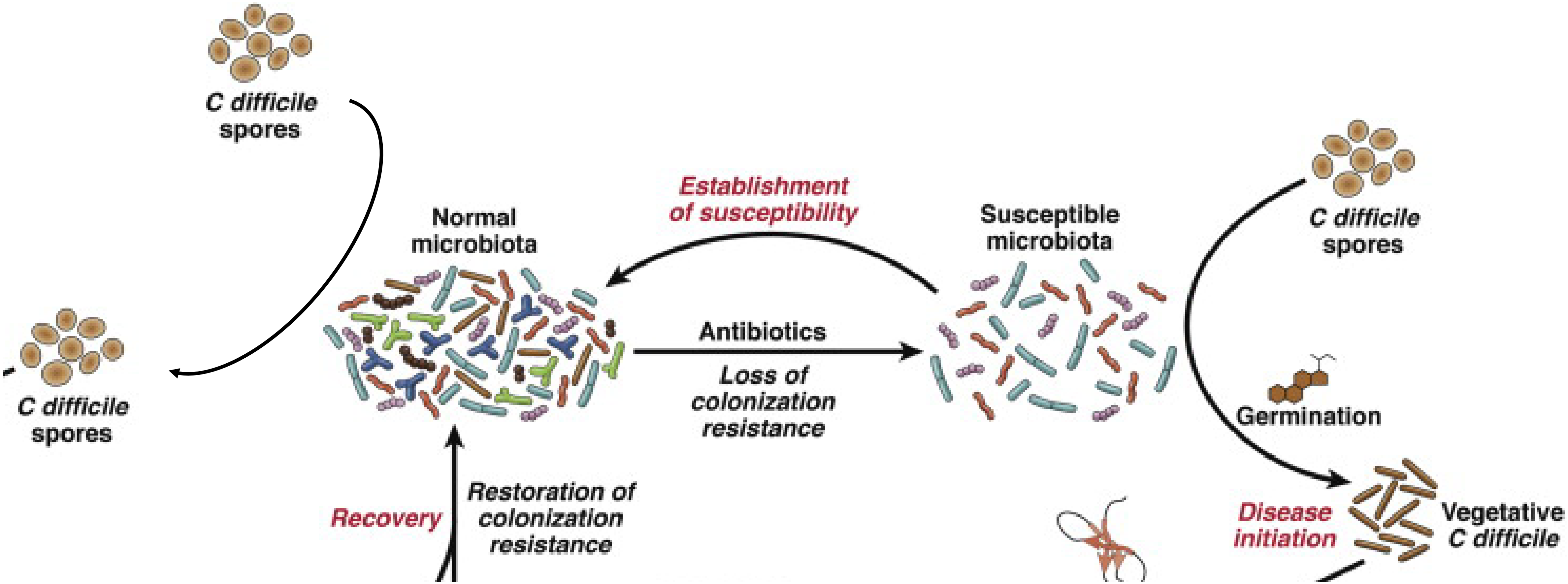
Antibiotic

Antibody response

Toxin A,
Toxin B,
Binary
toxin

Britton RA, Young VB. *Gastroenterology*. 2014;146:1547-53.

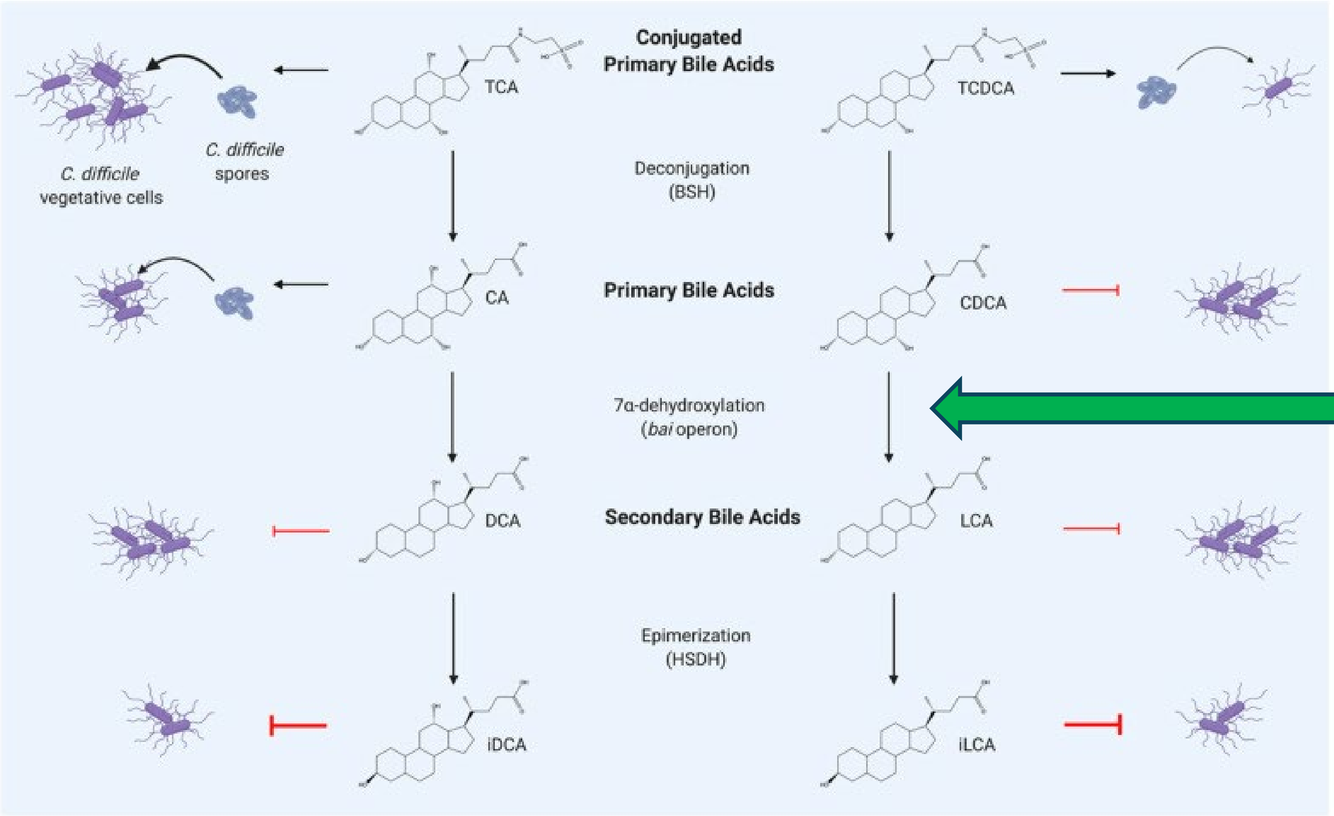
What is it about a normal microbiota that restores colonization resistance?



Britton RA, Young VB. *Gastroenterology.* 2014;146:1547-53.

Why does C. *difficile* require dysbiosis to cause infection?

Answer: These organisms maintain gut health. For example: Bile acids and CDI



Primary bile acids: BAD
(promote C diff germination)

Healthy colon concentration: usually very low

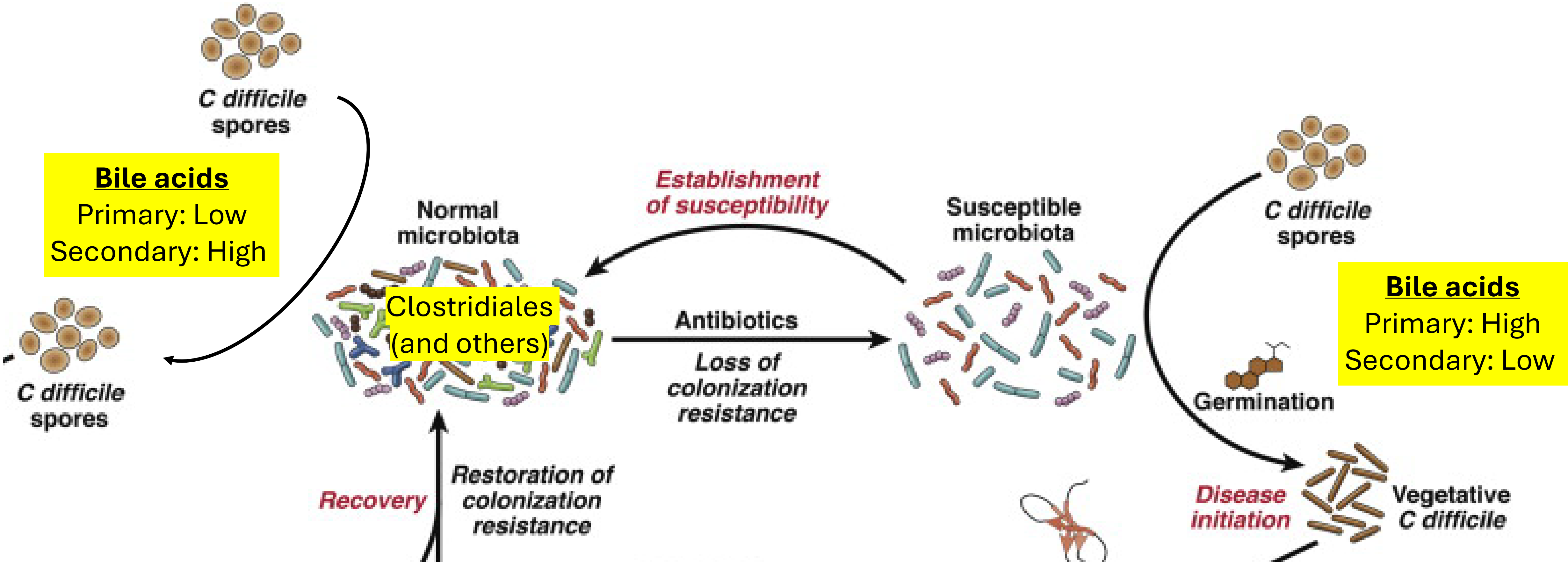
Bile acids are converted by specific gut microbiota

Secondary bile acids: Good (inhibit C diff growth)

Healthy colon concentrations: usually high

Most important taxa responsible for converting primary to secondary bile acids: Clostridiales

What is it about a normal microbiota that restores colonization resistance?



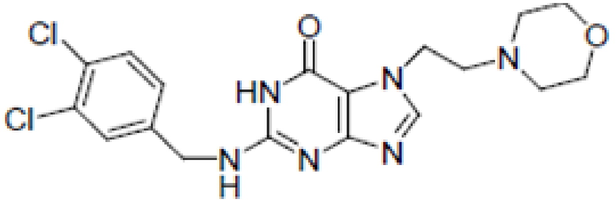
Bile acids
Primary: Low
Secondary: High

Clostridiales
(and others)

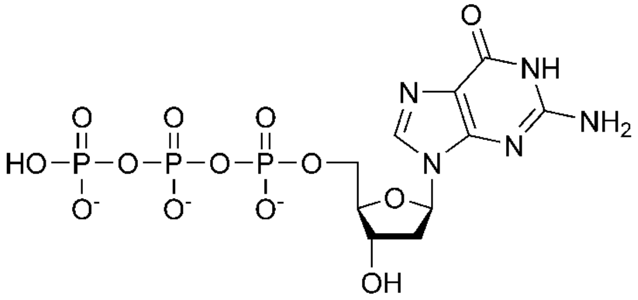
Bile acids
Primary: High
Secondary: Low

Britton RA, Young VB. *Gastroenterology.* 2014;146:1547-53.

Ibezapolstat (IBZ; ACX362E)

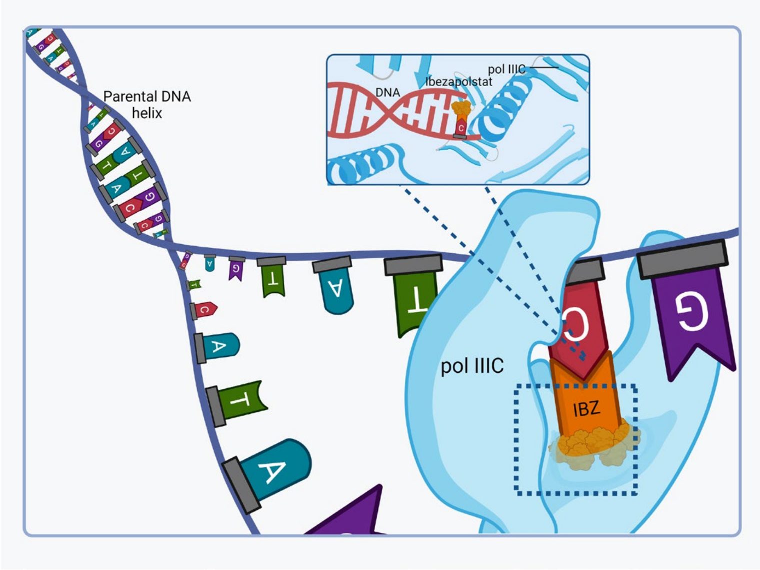


ACX-362



dGTP

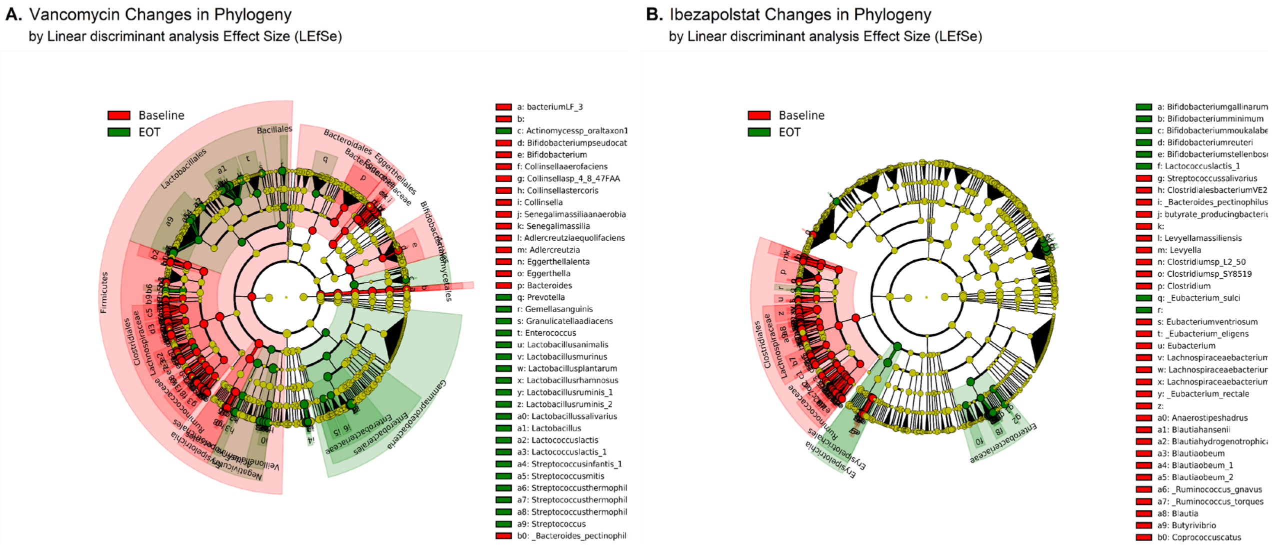
- Ibezapolstat: small-molecule inhibitor of DNA pol IIIC enzyme based upon competitive inhibition of dGTP (guanosine analog)
- DNA pol IIIC: essential for DNA replication of low G+C content Gram-positive bacteria (Bacillota / Firmicutes)
- Novel mechanism of action GPSS™ (**G**ram **P**ositive **S**elective **S**pectrum) including selective killing of certain Firmicutes but not others
  - Other DNA pol IIIC inhibitor compounds, in preclinical development for systemic treatment of resistant infections, show in vitro activity vs. the bioterrorism Category A pathogen *B. anthracis (Anthrax)*, including a ciprofloxacin-resistant strain, with MICs of 0.5-2 ug/mL. Selective microbiome effects will be tested in these compounds as well



Xu et al. Bioorg Med Chem. 2019
https://www.nature.com/articles/d43747-021-00149-0

IBZ has been shown to have favorable effects on the microbiome

IBZ Phase 1 Healthy volunteer study in comparison with VAN



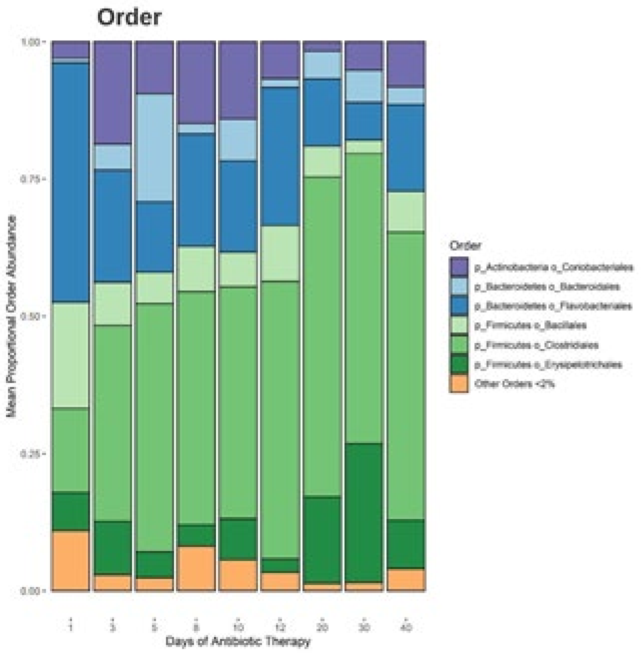
A. Vancomycin Changes in Phylogeny by Linear discriminant analysis Effect Size (LEfSe)

B. Ibezapolstat Changes in Phylogeny by Linear discriminant analysis Effect Size (LEfSe)

IBZ:
More narrow spectrum
Increased proportion of Actinobacteria

McPherson et al AAC 2022

IBZ Phase 2a. Single arm, no-comparator study of CDI patients (n=10)



IBZ:
Increased proportion of Actinobacteria
Increased proportion of Clostridiales

Garey et al CID 2022

Phase 2b Study design

Patients with mild/moderate CDI diagnosed using an EIA free toxin kit

Ibezapolstat 450 mg BID X 10 days

Vancomycin 125 mg QID X 10 days

ClinicalTrials.gov ID NCT04247542

Patients followed daily for 12 days + follow-up

Outcome Measures

Initial clinical cure (day 12 evaluation)

Sustained clinical cure (day 38)

Extended clinical cure (3 months)

Time to resolution of diarrhea (days 0-12)

Safety (day 38)

Pharmacokinetics (days 0-12)

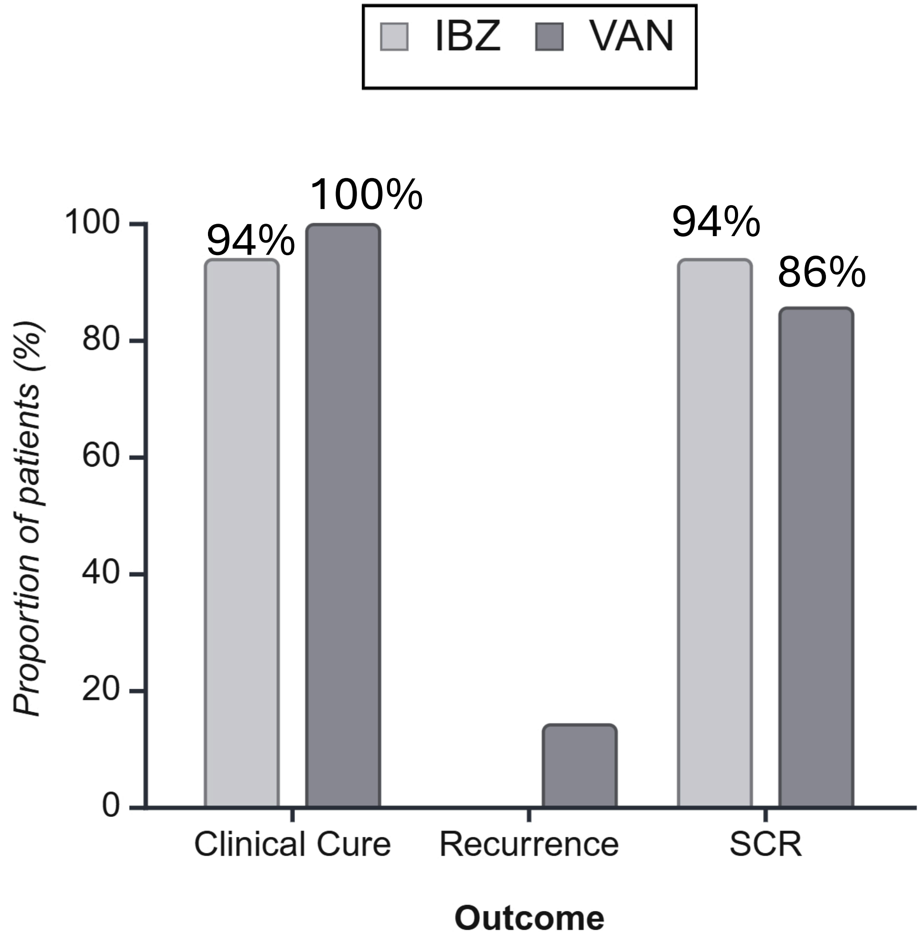
Microbiome changes (days 0-12)
qPCR and 16S rRNA

Bile acid changes (days 0-12)
LC-MS/MS

RESULTS: Demographics and Baseline Information

	IBZ (n=16)	**VAN (n=14)**	**P value**
N	16	14	
Age, years ≥75 yo	64±13 5 (31.2%)	62±10 2 (14.3%)	0.57
Female	13 (81%)	11 (79%)	0.85
White	16 (100%)	13 (93%)	0.27
Hispanic or Latino	11 (69%)	11 (79%)	0.54
Charlson Comorbidity index	2.6±1.5	2.2±1.5	0.47
Number of UBMs at baseline Median (minimum, maximum)	6 (3-15)	6 (4-13)	
Baseline *C. difficile* ribotype strains			
F014-020	0	3	
F027	1	2	
F106	3	1	
F002	1	1	
F116	0	1	
Other	6	3	
Safety	Both treatments were well-tolerated with no drug-related serious adverse events or drug-related treatment withdrawals		

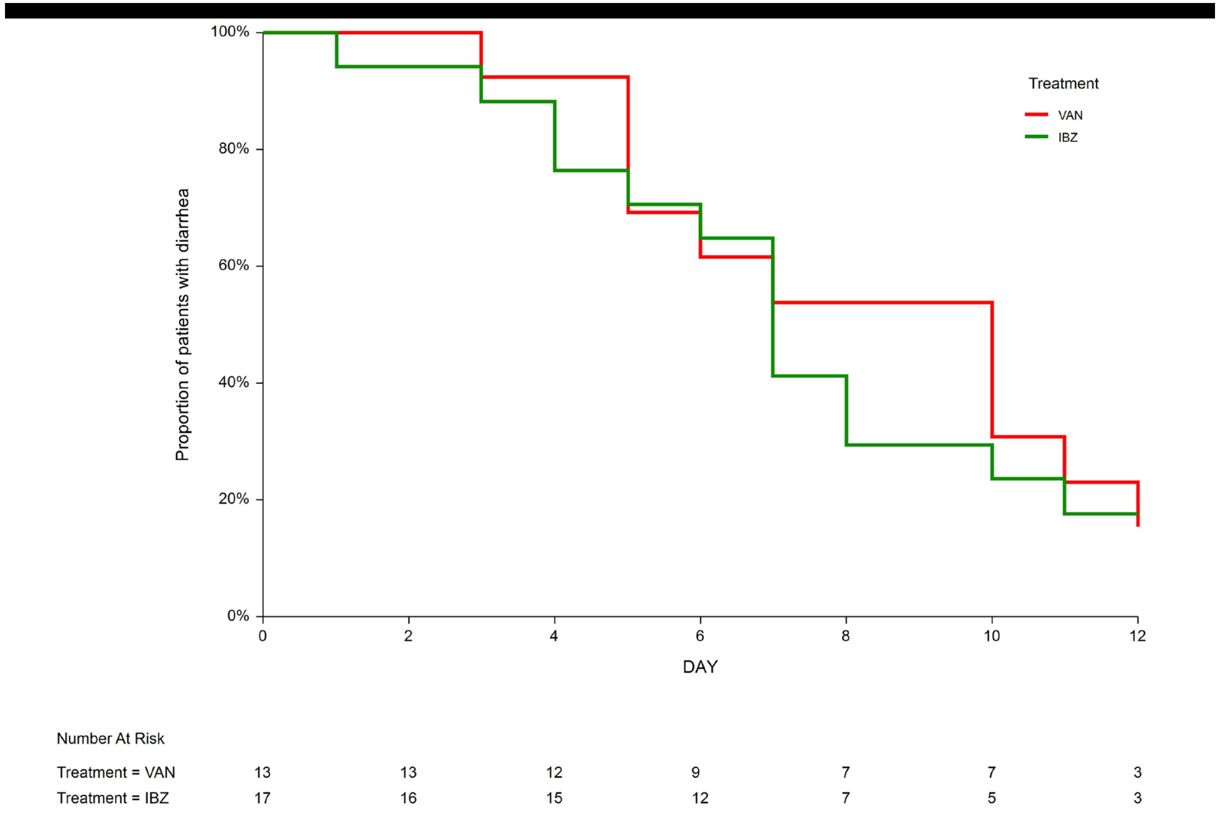
Efficacy analysis



	IBZ	VAN	IBZ	VAN	IBZ	VAN
Number of events	15	14	0	2	15	12
Number of patients	16	14	16	14	16	14

Time to resolution of diarrhea

Cumulative incidence of UBM resolution



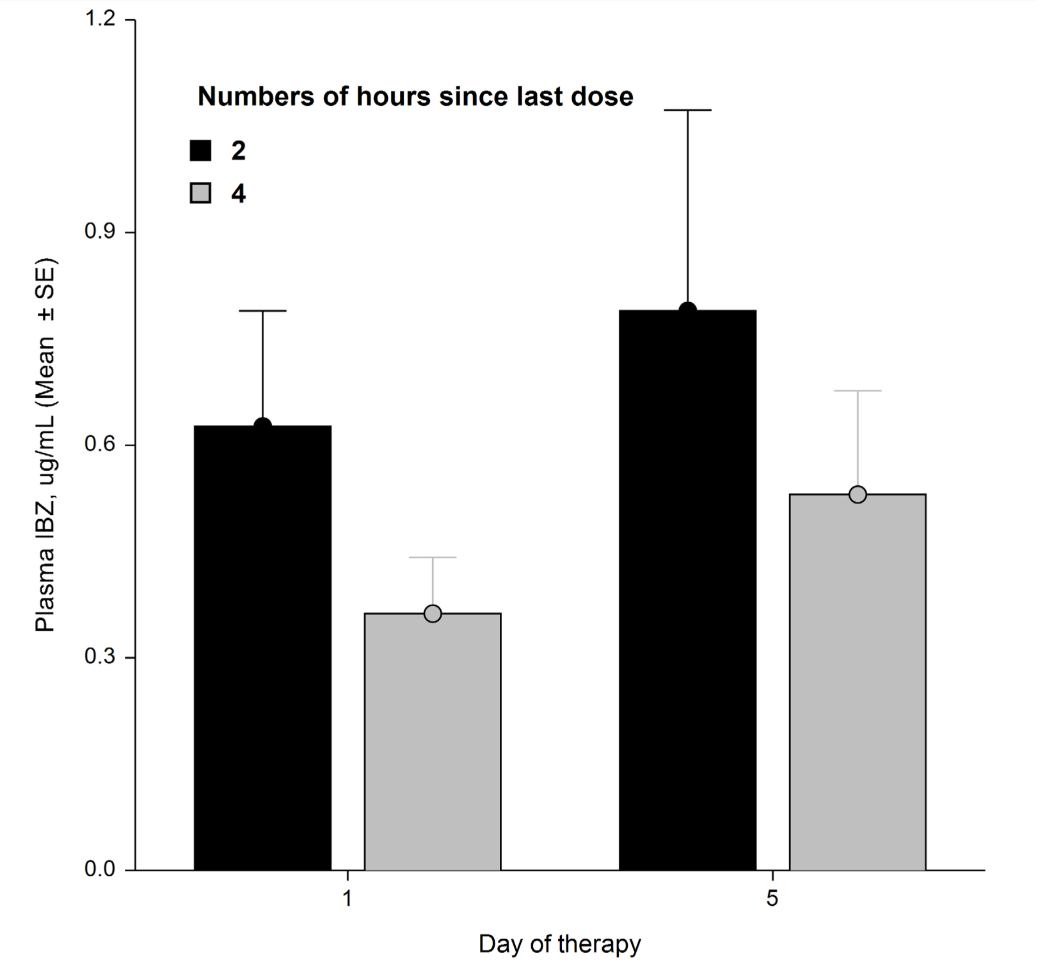
Number At Risk

	0	2	4	6	8	10	12
Treatment = VAN	13	13	12	9	7	7	3
Treatment = IBZ	17	16	15	12	7	5	3

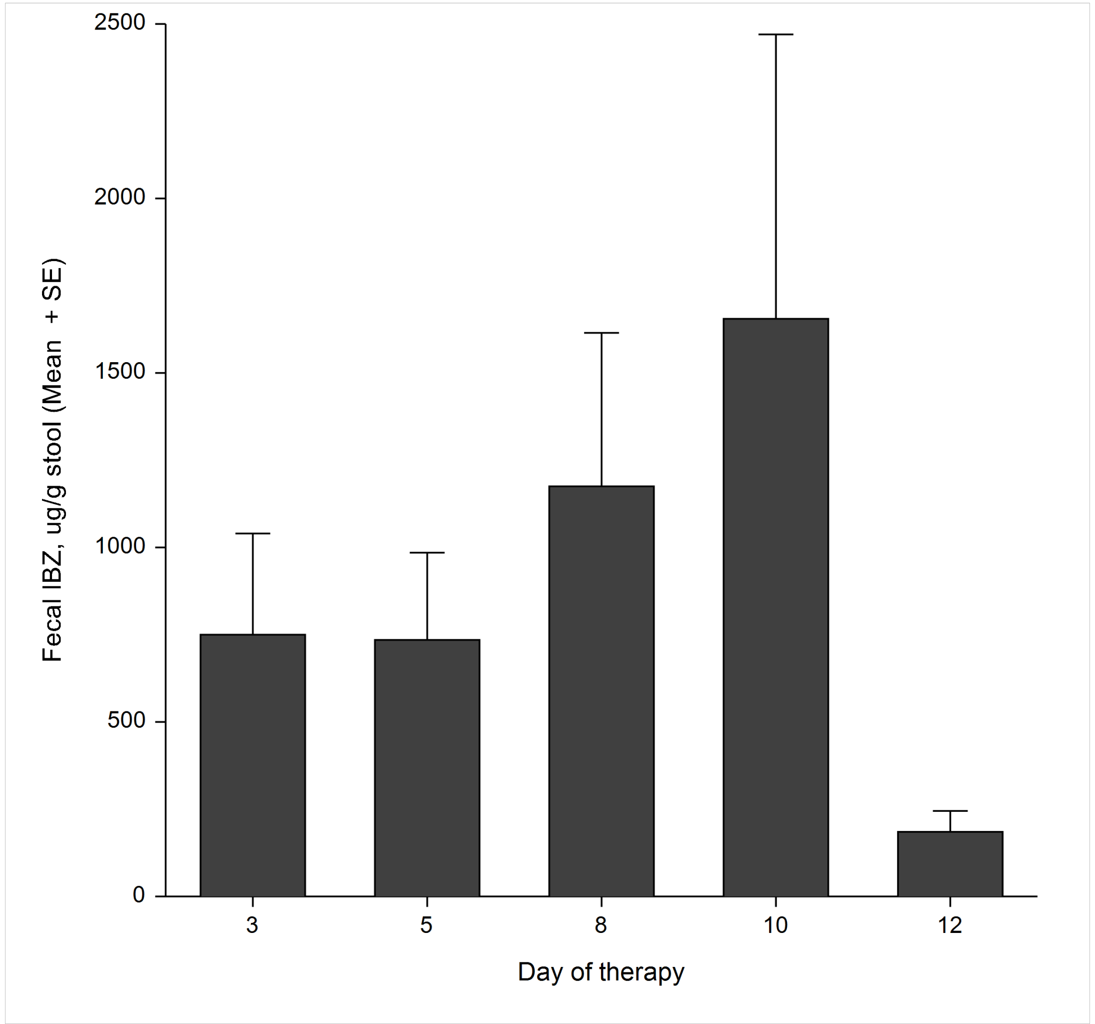
SCR: sustained clinical response; UBM: unformed bowel movement

IBZ Plasma and Fecal Concentrations

a. Plasma concentrations

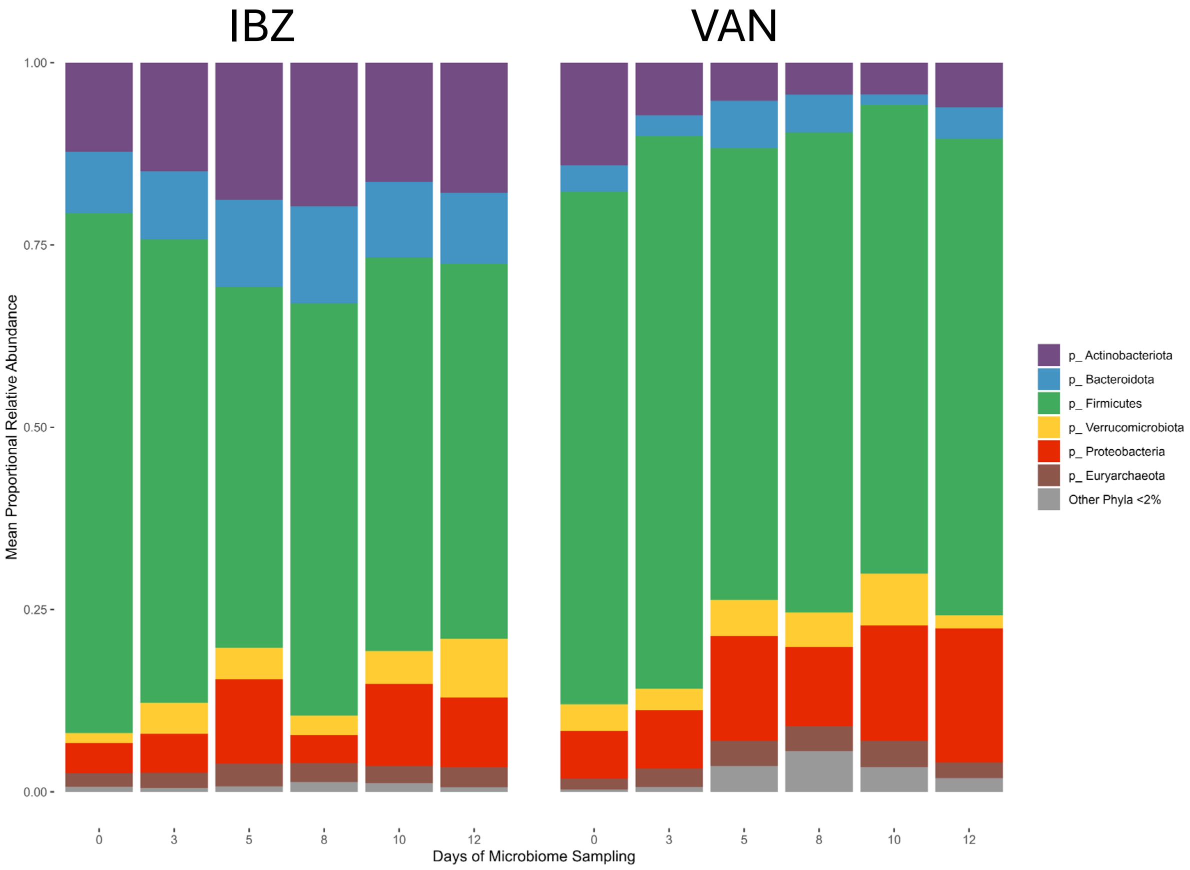


b. Fecal concentrations

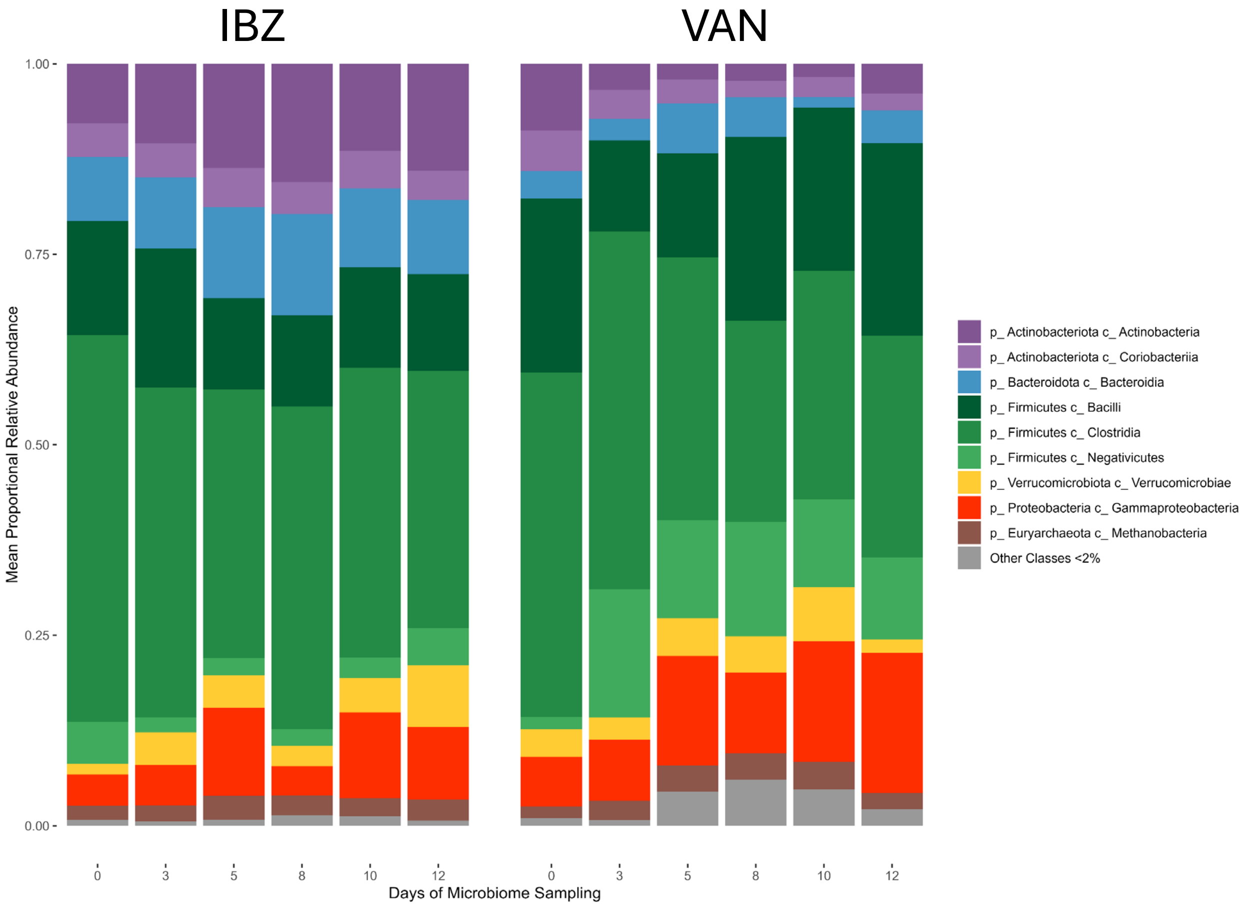


Microbiome. Favorable microbiome results were observed with IBZ

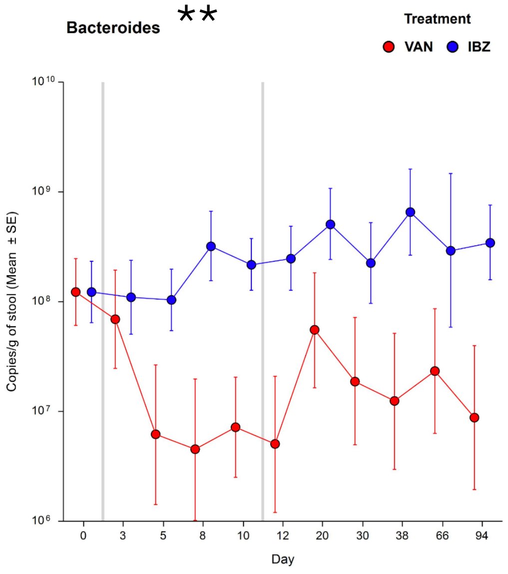
Phylum

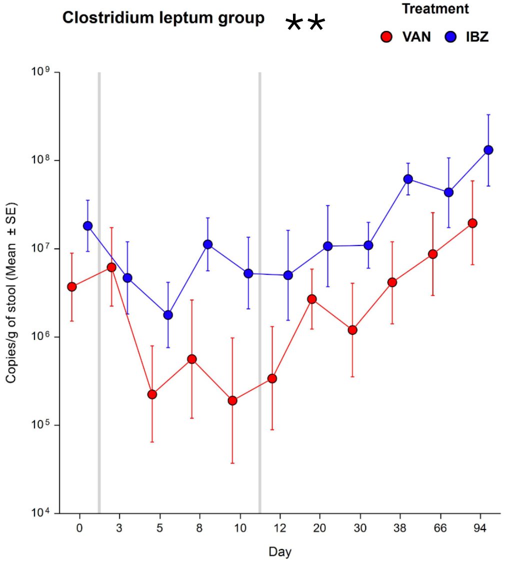


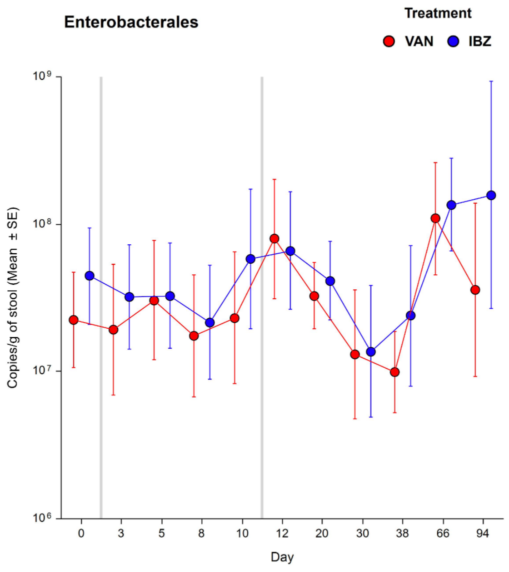
Class

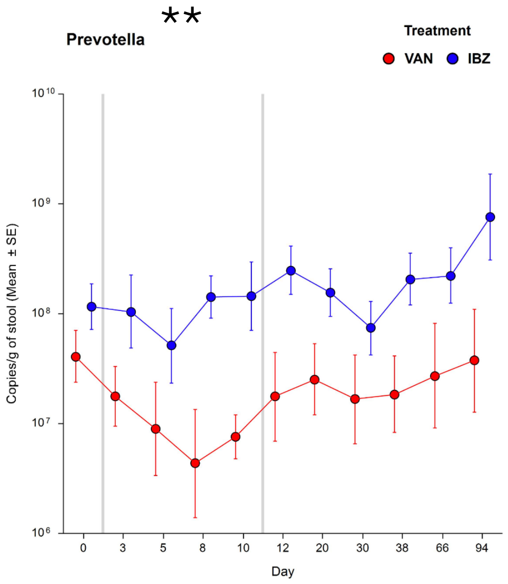


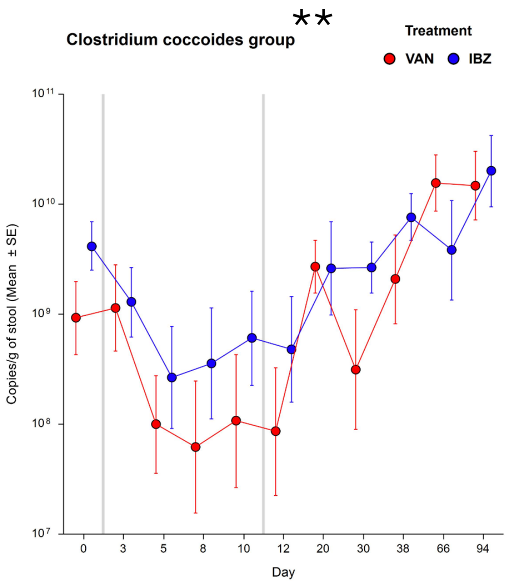
IBZ qPCR microbiome analysis

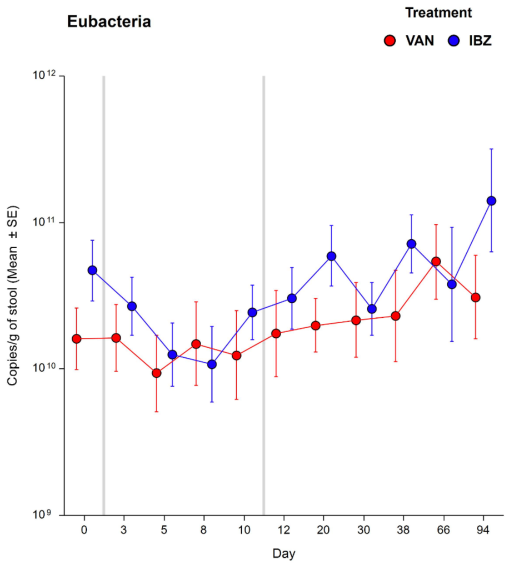

Bacteroides **


Clostridium leptum group **


Enterobacterales


Prevotella **

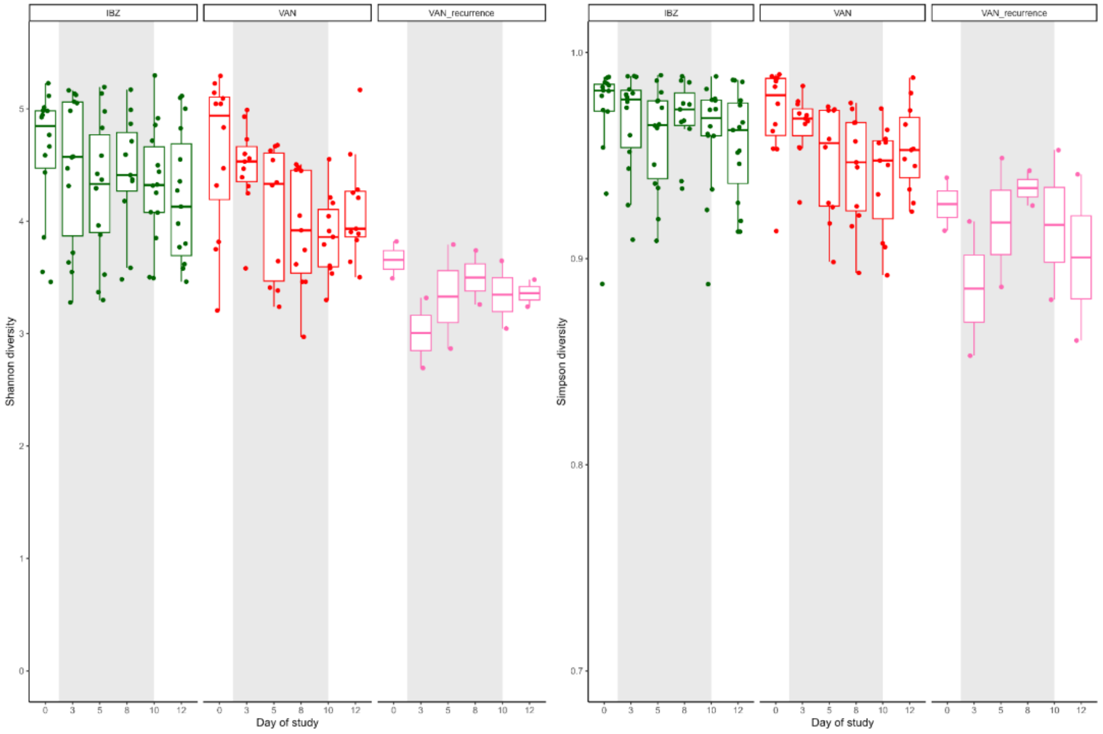

Clostridium coccoides group **


Eubacteria

**$p < 0.01$

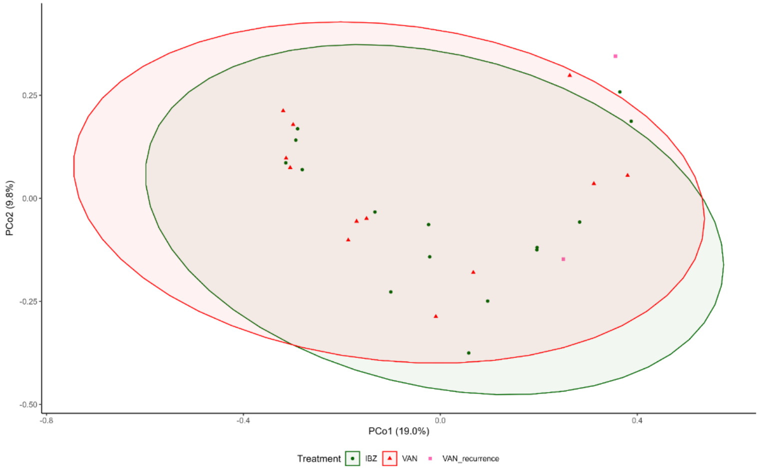
Focus on CDI Recurrence. Alpha and Beta Diversity

a. Alpha diversity plots

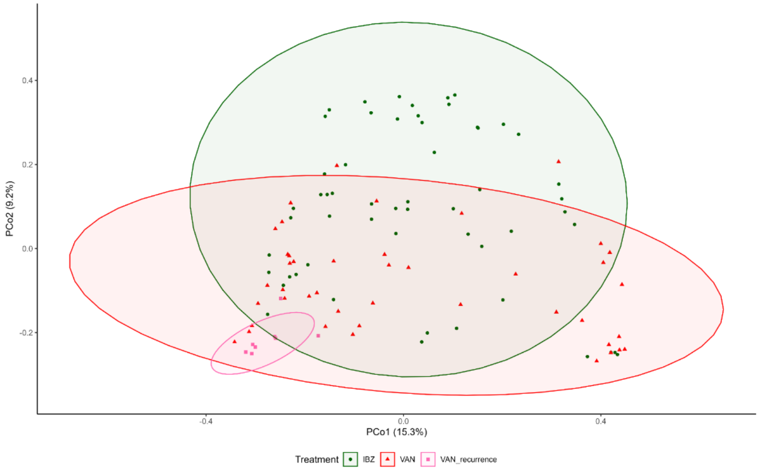


b. Beta diversity plots

Baseline Classical (Metric) Multidimensional Scaling of Beta-Diversity
by Bray-Curtis Dissimilarity

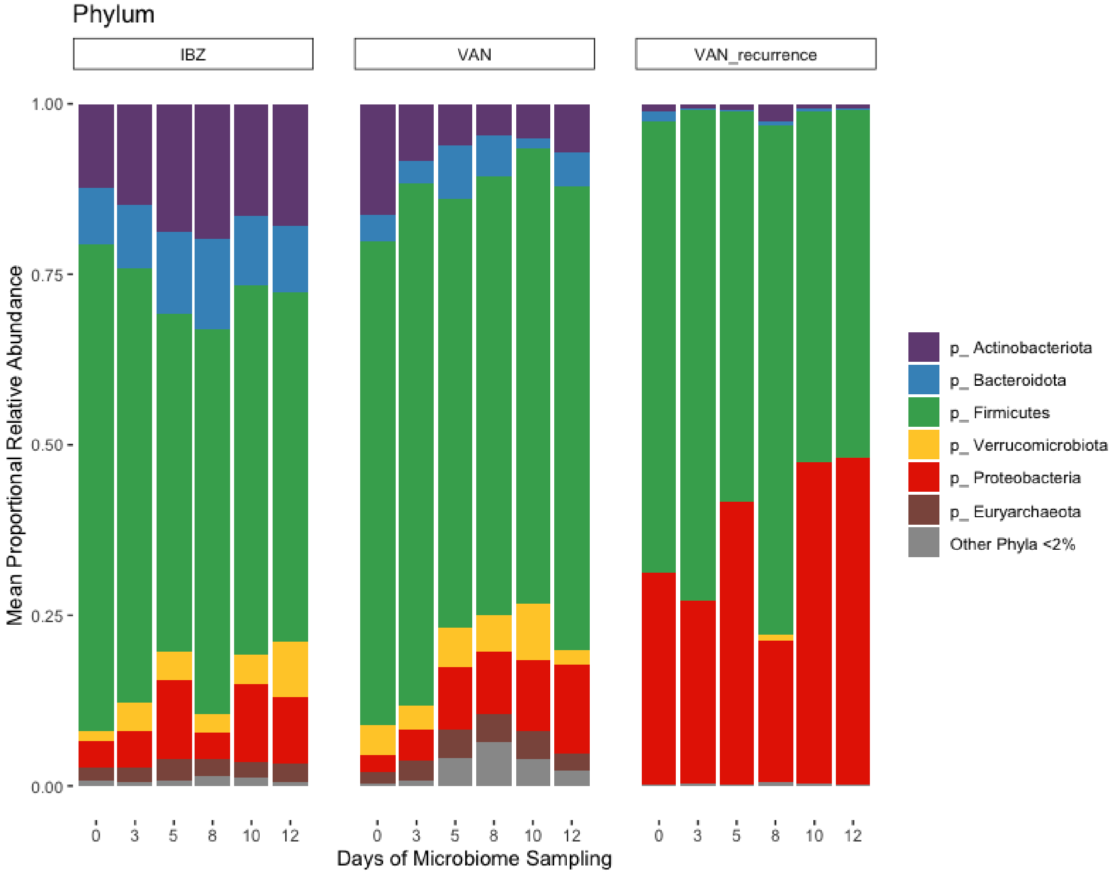


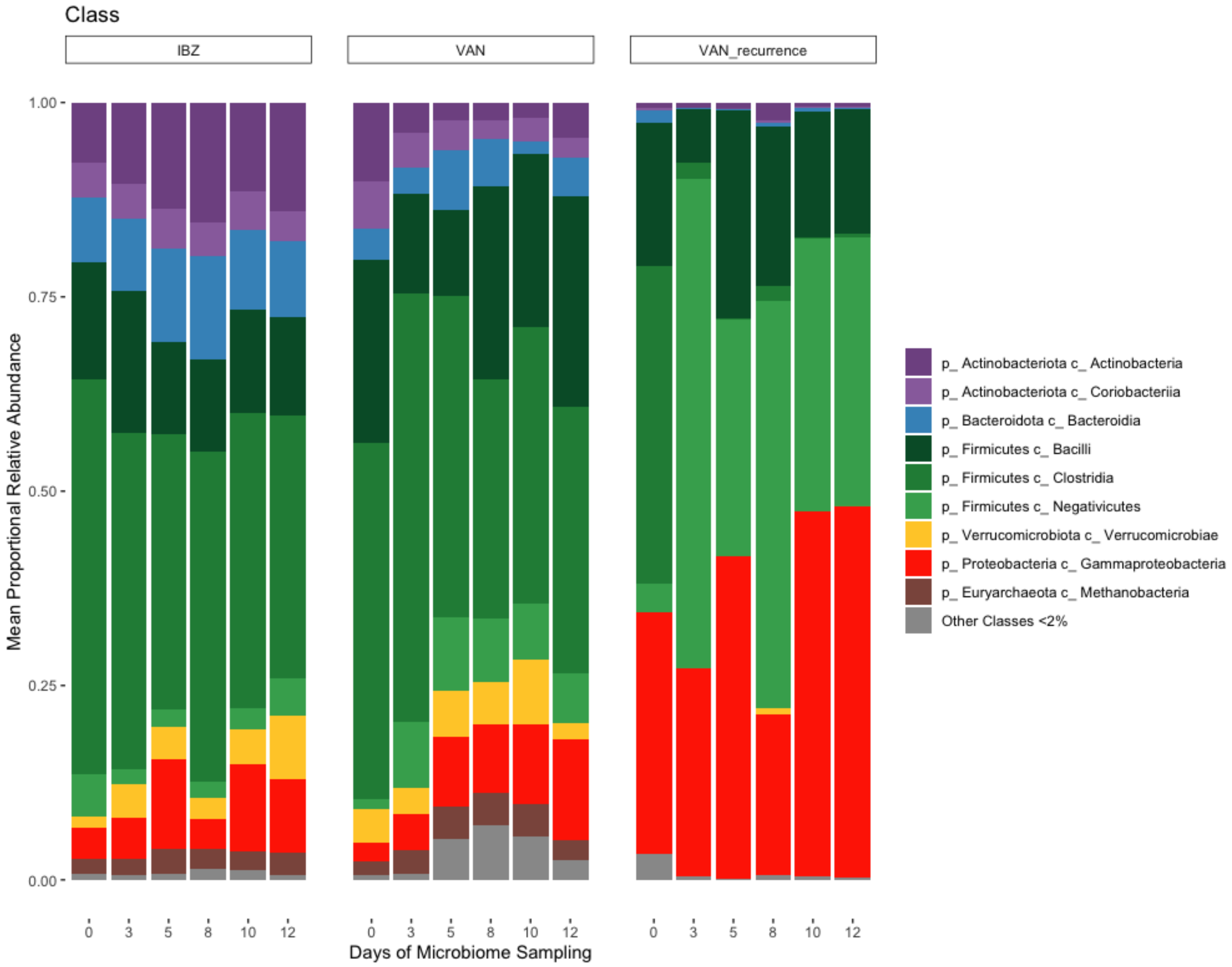
Day 5-12 Classical (Metric) Multidimensional Scaling of Beta-Diversity
by Bray-Curtis Dissimilarity



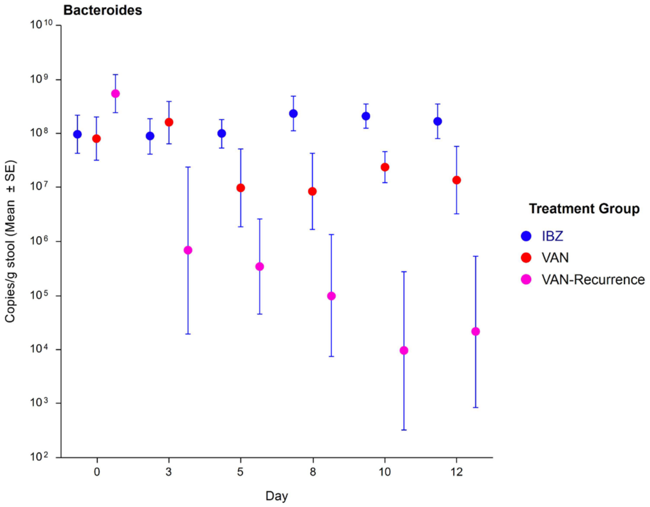
IBZ: n=16; VAN (no recurrence): n=12; VAN (recurrence: n=2)

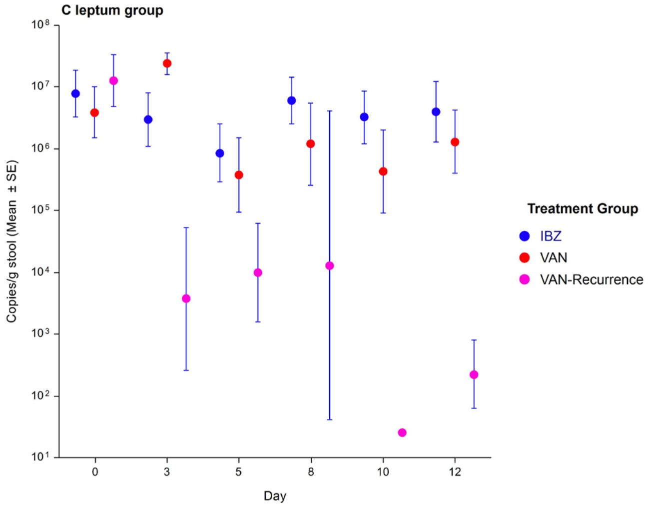
CDI recurrence associated with marked microbiome disruption

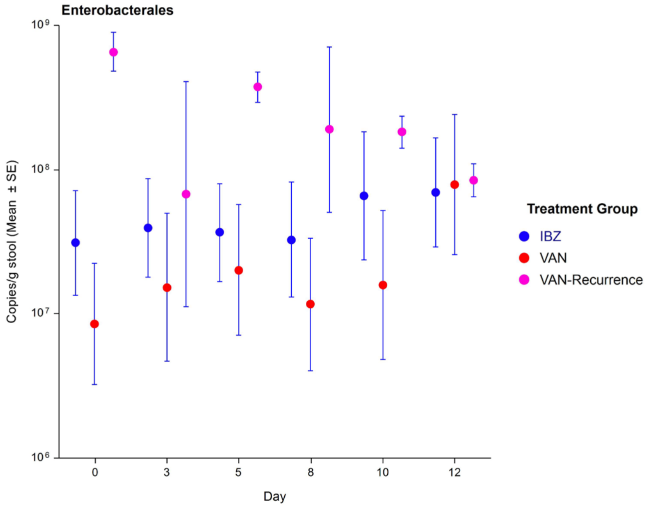


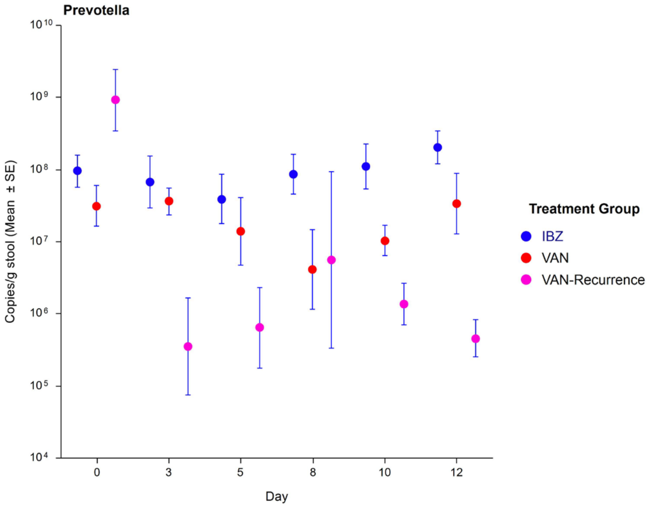


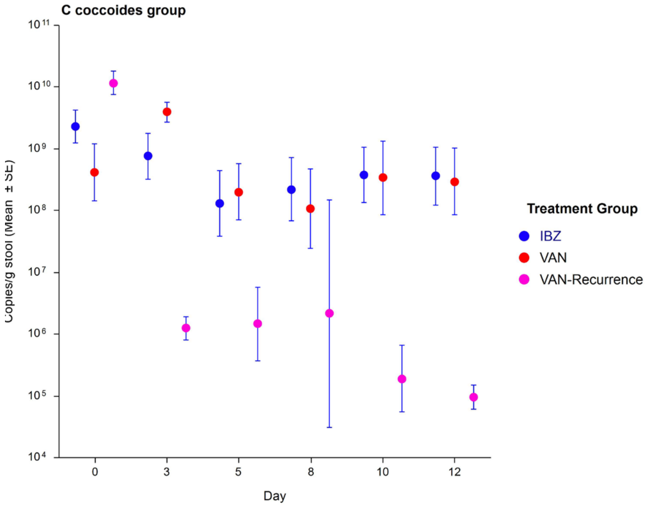
CDI recurrence associated with marked microbiome disruption

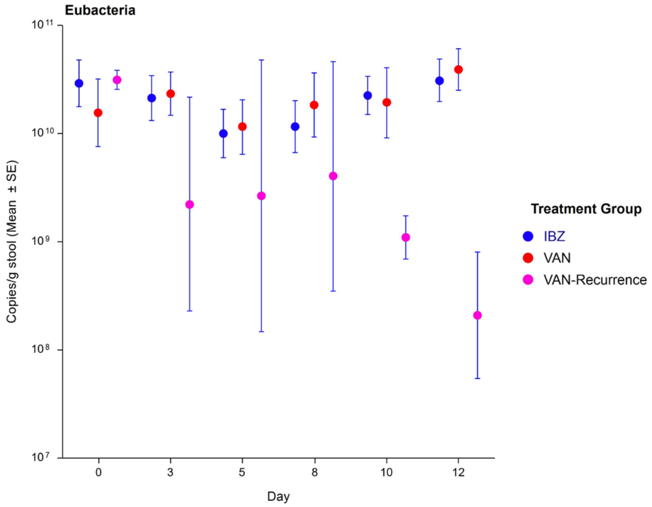




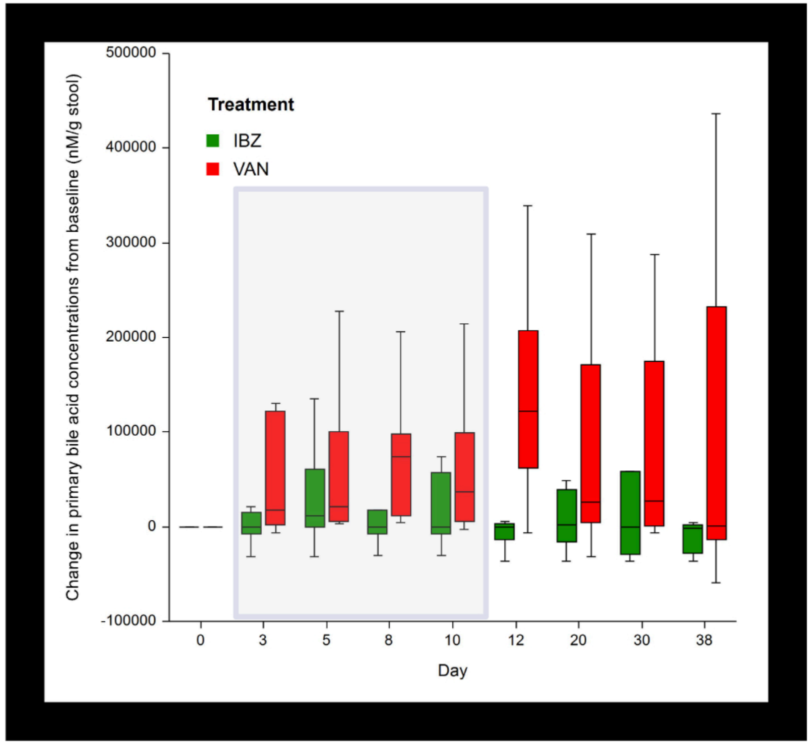


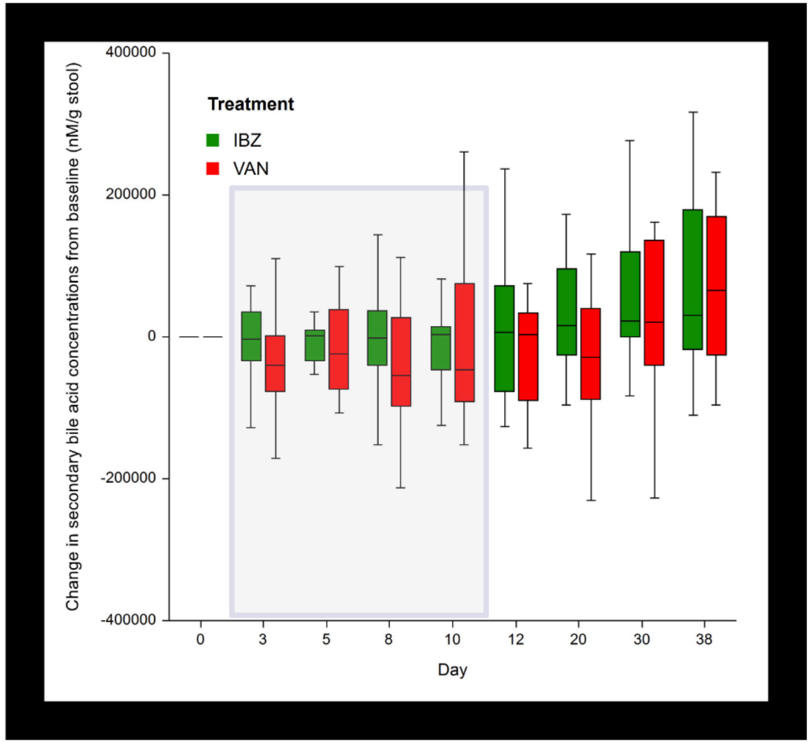


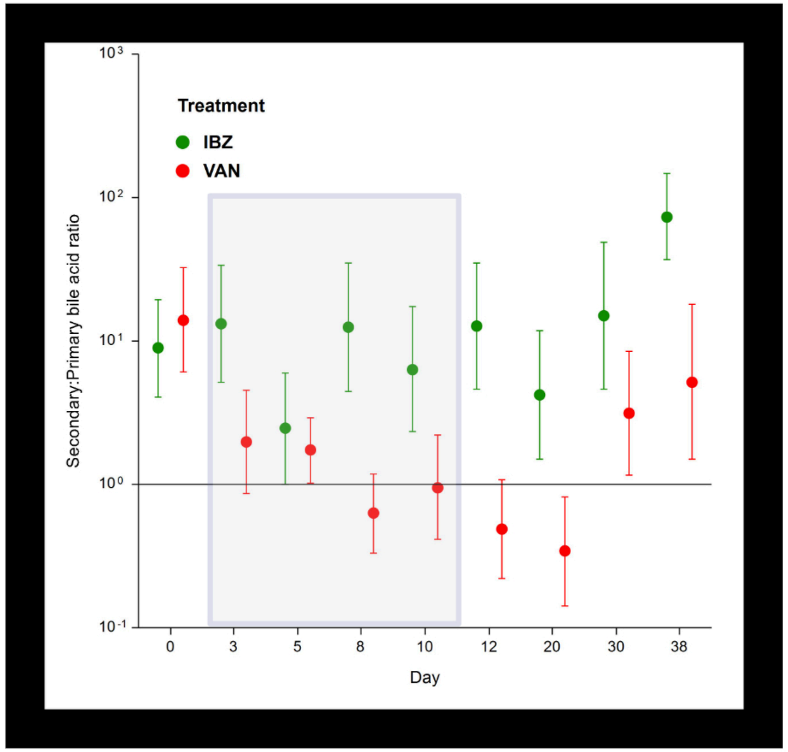




Change in bile acid homeostasis in CDI patients given ibezapolstat (IBZ) vs. vancomycin (VAN)


a. Change in primary bile acids


b. Change in secondary bile acids


c. Secondary to primary bile ratio

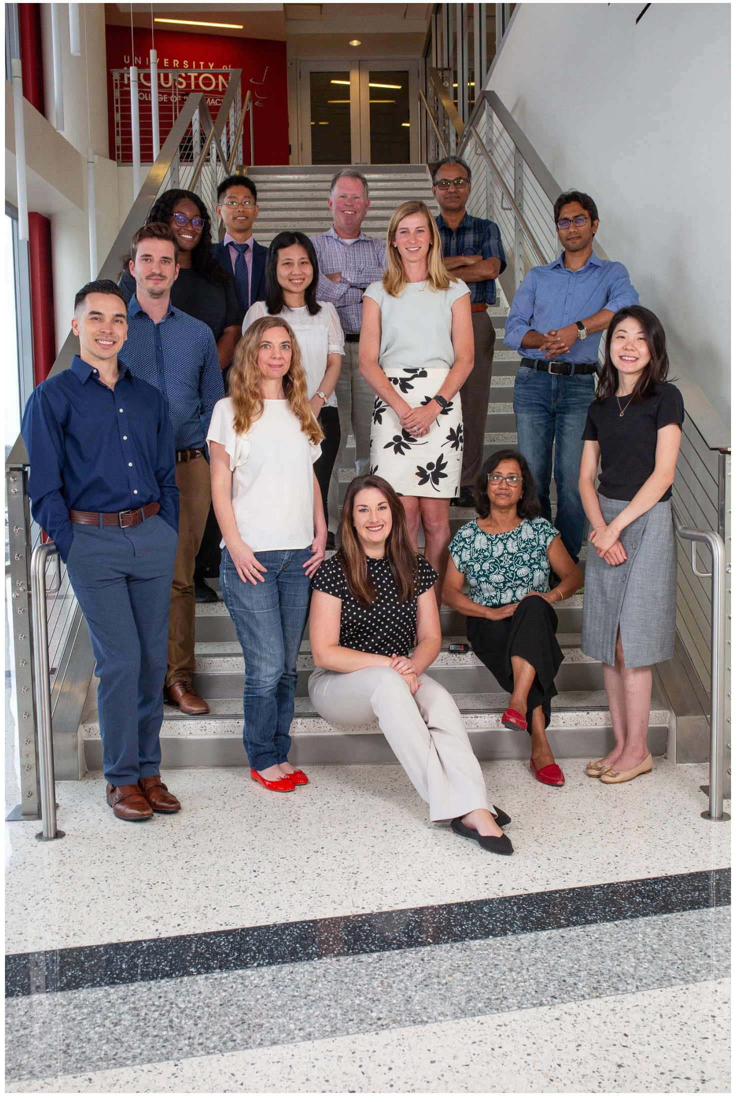
Conclusions

- IBZ had a clinically comparable cure rates and safety profile to VAN
  - No CDI recurrence vs. 2 VAN-treated patients
- IBZ had favorable PK and microbiome results to VAN
  - Higher colonic / low systemic concentrations
  - Fewer cases of persistently positive *C. difficile*
  - Increased Actinobacteria and beneficial Bacillota (Firmicutes)
  - Favorable effects on bile acid homeostasis

- These results warrant further development in phase 3 trials.

Acknowledgements

UH Center for Infectious Diseases and Microbiome Research



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