Elucidating the Gram-Positive Selective Spectrum Activity of Ibezapolstat; Secondary Analysis from the phase 2a trial

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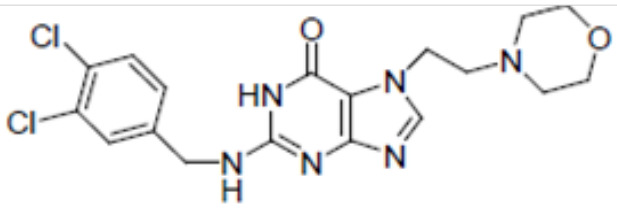
Ibezapolstat (IBZ; ACX362E)



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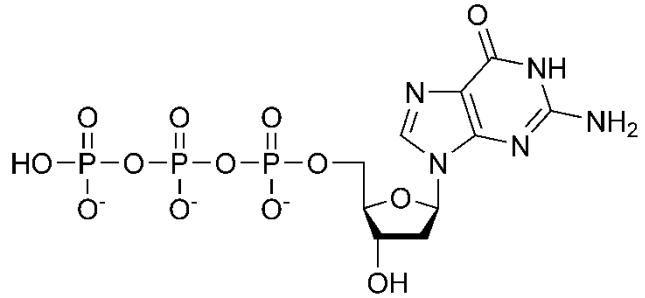
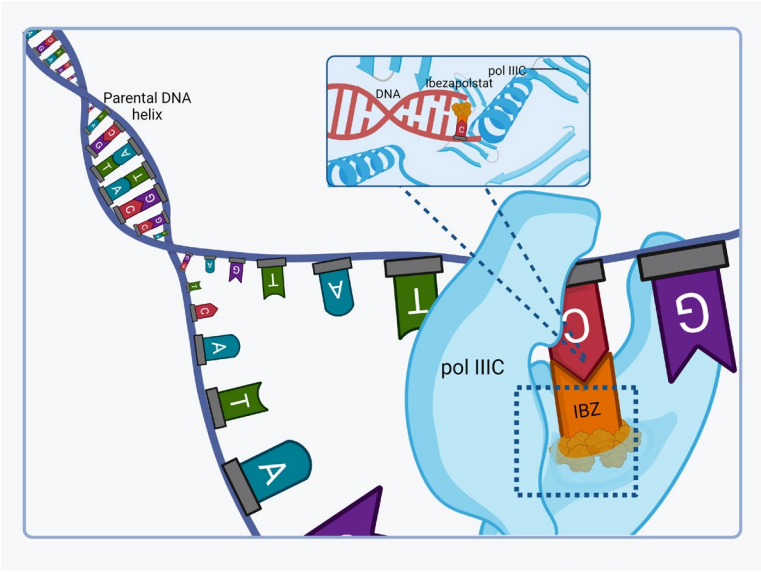


dGTP

- Ibezapolstat: small-molecule inhibitor of DNA pol IIIC enzyme based upon competitive inhibition of dGTP (guanosine analog)
  - DNA pol IIIC: essential for replication of low G+C content Gram-positive bacteria (Firmicutes)
  - Novel mechanism of action GPSS™ (**G**ram **P**ositive **S**elective **S**pectrum)



Xu et al. Bioorg Med Chem. 2019 Aug 1;27(15):3209-3217;
https://www.nature.com/articles/d43747-021-00149-0
Figure by BioRender created by Avalon Starr

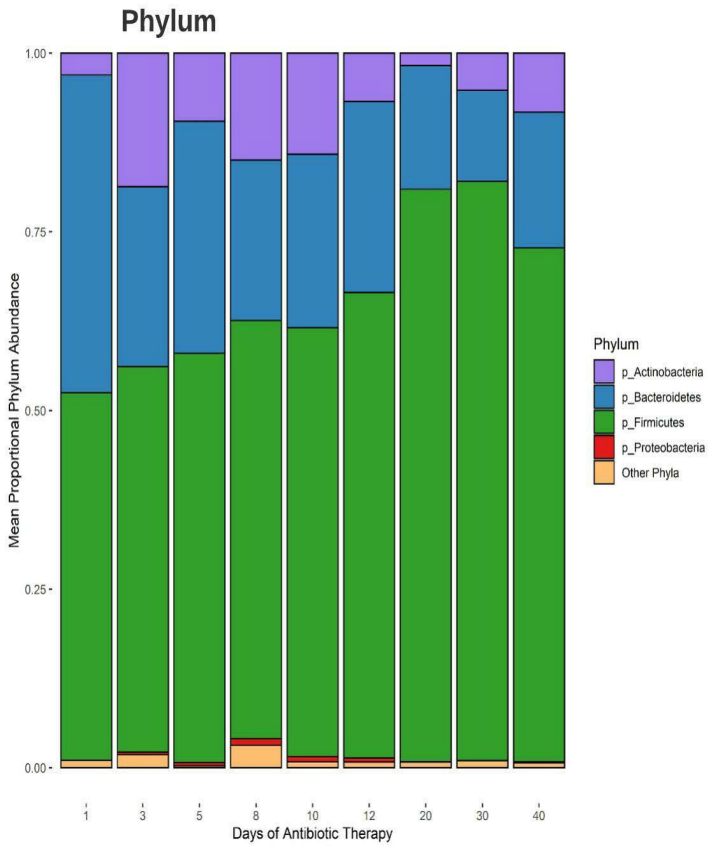
IBZ Clinical update

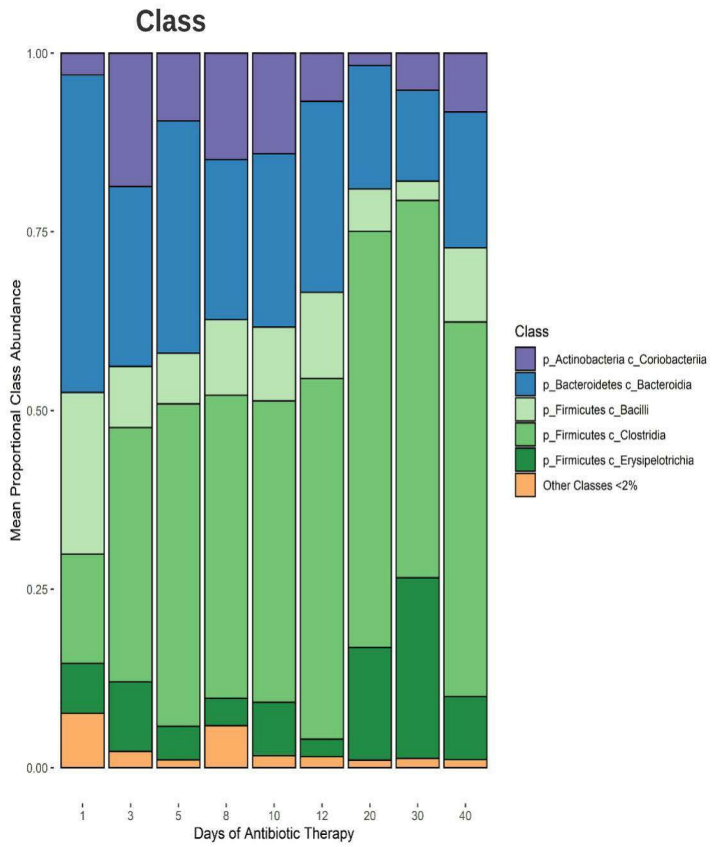
- Phase 1, Healthy Volunteer: Completed
  - IBZ 450 mg twice daily chosen for phase 2 studies
    - Garey et al. *J Antimicrob Chemother* 2020.
  - Microbiome evaluations predicted an anti-recurrence effects
    - *McPherson et al. *Antimicrob Agents Chemother* 2022
- Phase 2a (n=10): Completed
    - *Garey et al. Clin Infect Dis 2022
- Phase 2b: Enrolment complete (n=32)
  - IBZ 450 mg twice daily vs. vancomycin 125 mg PO four times daily
  - ClinicalTrials.gov Identifier: NCT04247542

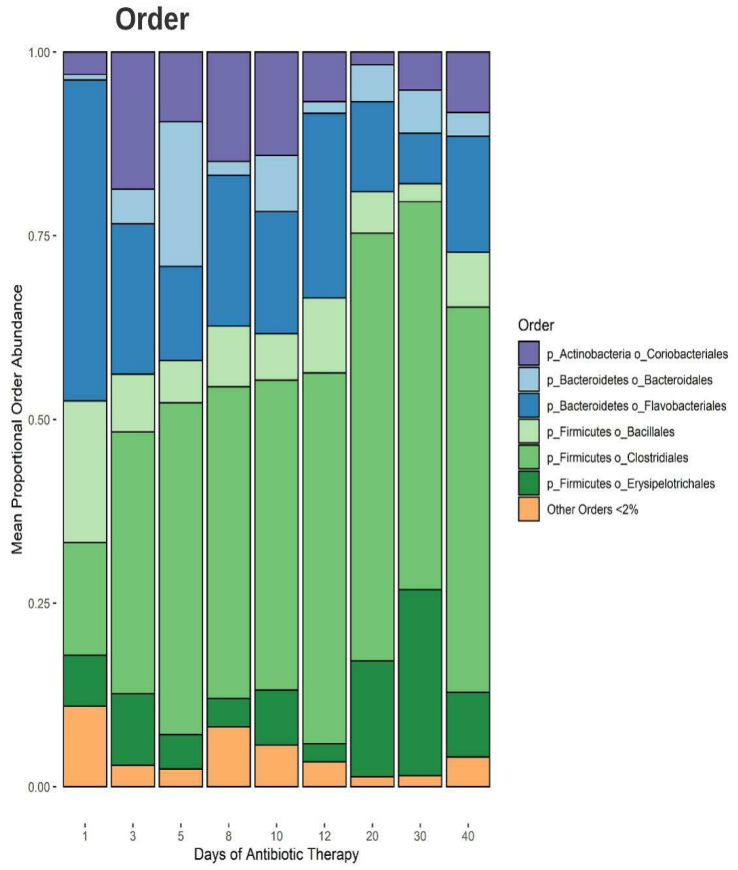
*In phase I/II studies, a IBZ selective susceptibility to certain Firmicutes was noted

Phase 2a. Open label CDI study

Increased proportion of Firmicutes was observed on ibezapolstat therapy, Clostridiales most common taxa







Spectrum of activity: Ibezapolstat vs. vancomycin

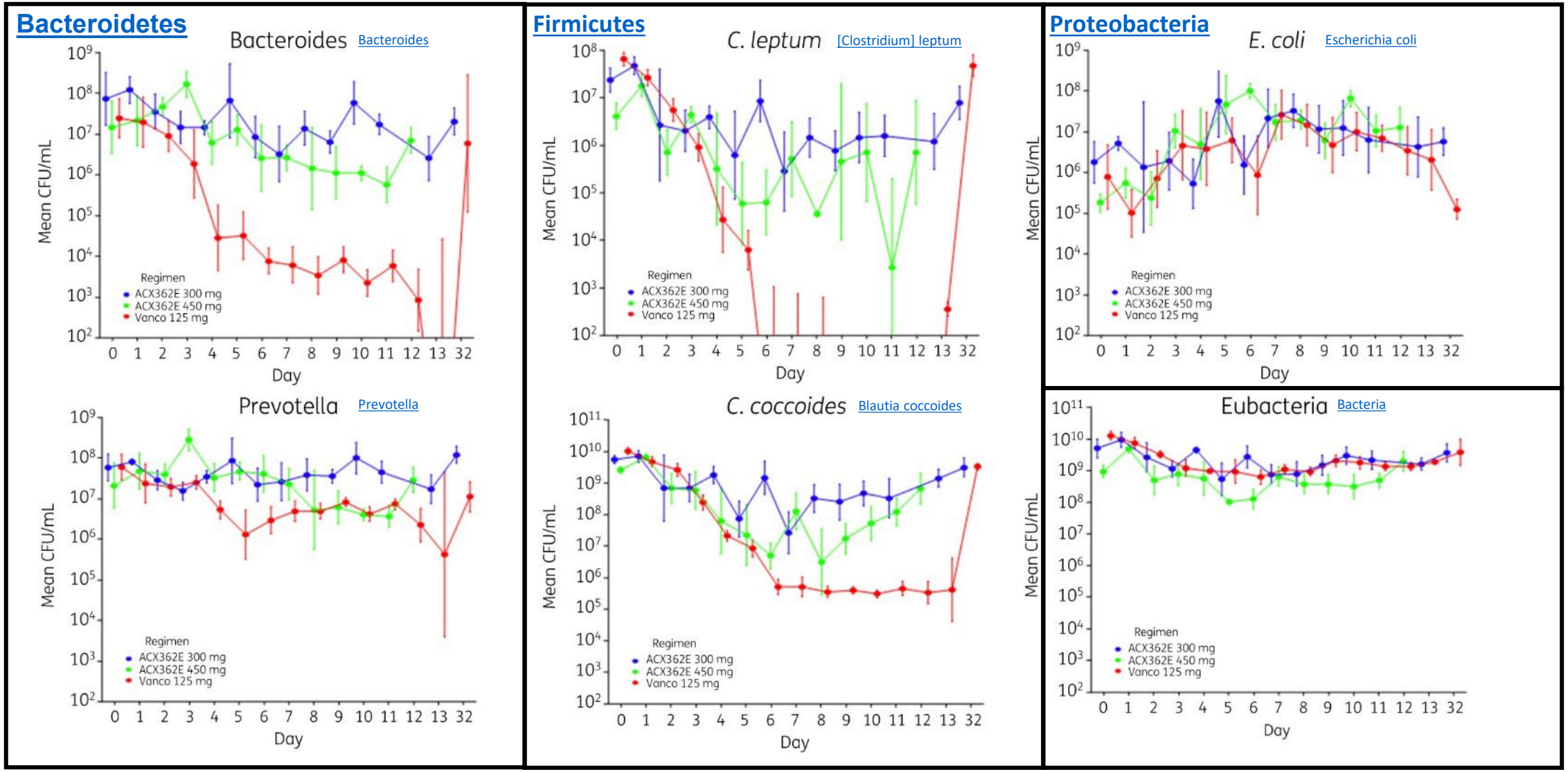
Expected

Phylum	Antibiotic activity	
	Ibezapolstat	Vancomycin (oral)
Actinobacteria	No	Yes
Bacteroidetes	No	Yes
Firmicutes	Selective	Yes
Fusobacteria	No	No
Proteobacteria	No	No

Study Objectives and Brief Methods

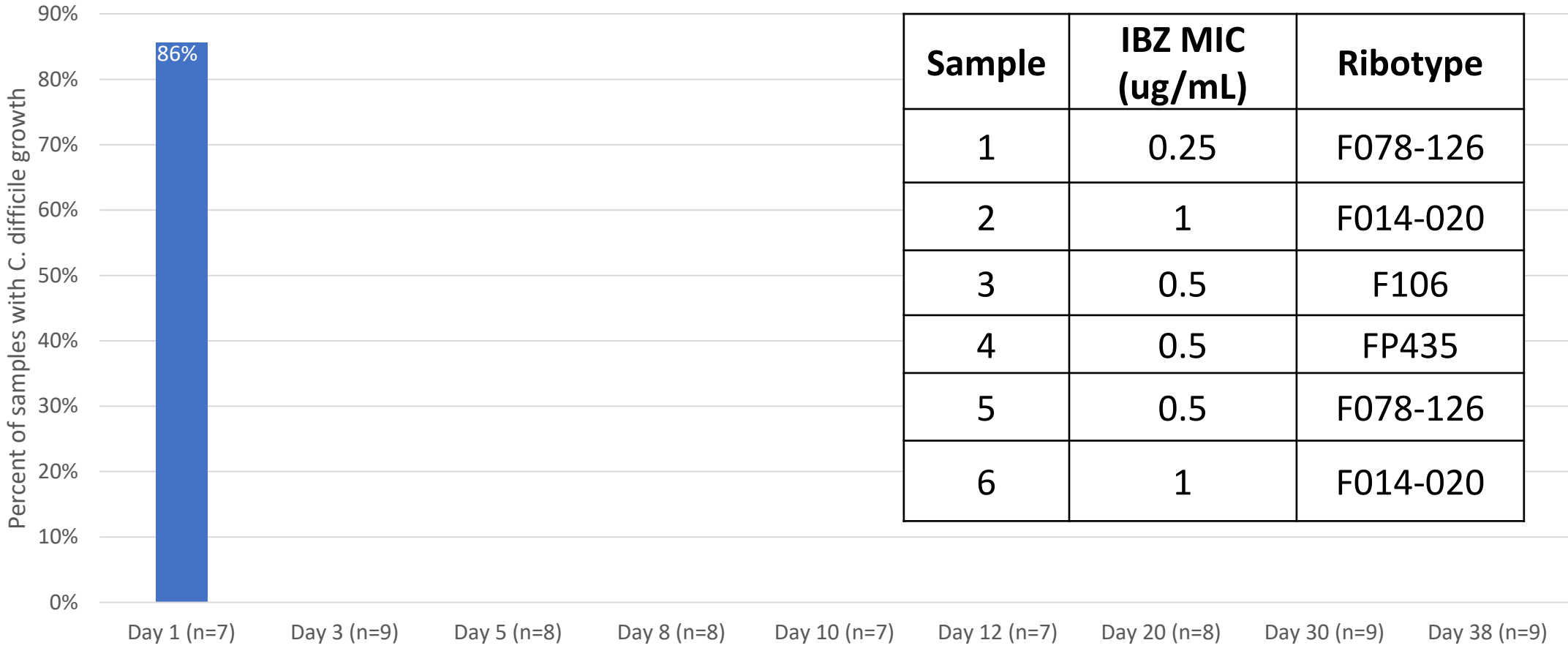
- To better understand IBZ Firmicute Selectivity
  1. Are the concentrations (quantitative) of Firmicutes decreasing with therapy?
     - Phase I data, qPCR of relevant taxa
  2. Are there IBZ MIC differences between Firmicute species in the gut microbiome of CDI patients?
     - Identify Firmicute species isolated from phase 2a samples over time with IBZ MIC agar dilution testing
  3. Does evolutionary phylogeny predict IBZ Firmicute selectivity?
     - Phylogenetic analysis of PolC from *C. difficile* genomes downloaded from the NCBI.

Q1. Relevant Firmicutes less disrupted with IBZ vs. vanco (qPCR results)



Q2. Phase 2a study: Ibezapolstat had potent *C. difficile* activity

All samples underwent a 48-hour enrichment step with taurocholate prior to plating on CCFA plates.



Sample	IBZ MIC (ug/mL)	Ribotype
1	0.25	F078-126
2	1	F014-020
3	0.5	F106
4	0.5	FP435
5	0.5	F078-126
6	1	F014-020

IBZ selectivity: MIC studies

CACHET
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Symbiotic Bacterial Strain Biobank. The biobank of commensal bacterial strains contains over 1,000 strains of bacteria cultured from **healthy donors**. The strains have been sequenced and their entire genomes are available for academic investigators who want to test the ability of these different strains to provide health benefits or disease resistance.

https://www.chicago-cachet.org/facility-cores/microbiomecore/

Stool samples from the IBZ Phase 2a study.

MIC testing results demonstrated selective activity vs. Clostridiales

Symbiotic Bacterial Strain Biobank (E Pamer)

Phylum, Class	Family	Species	N tested	IBZ MIC (ug/mL)
Actinobacteria, Actinomycetia	Bifidobacteriaceae	Bifidobacterium longum	2	>100
Actinobacteria, Coriobacteriia	Coriobacteriaceae	Collinsella aerofaciens	1	100
Firmicutes, Clostridia	Lachnospiraceae	[Clostridium] hylemonae	1	100
Firmicutes, Clostridia	Lachnospiraceae	[Clostridium] scindens	2	50
Firmicutes, Clostridia	Lachnospiraceae	[Clostridium] symbiosum	2	0.5
Firmicutes, Clostridia	Lachnospiraceae	Blautia obeum	1	1
Firmicutes, Clostridia	Lachnospiraceae	Blautia obeum	1	>100
Firmicutes, Clostridia	Lachnospiraceae	Coprococcus comes	2	>100
Firmicutes, Clostridia	Lachnospiraceae	Dorea formicigenerans	1	100
Firmicutes, Clostridia	Lachnospiraceae	Dorea formicigenerans	1	0.78
Firmicutes, Clostridia	Lachnospiraceae	Dorea longicatena	1	>100
Firmicutes, Clostridia	Lachnospiraceae	Dorea longicatena	1	3.125
Firmicutes, Clostridia	Lachnospiraceae	Lachnoclostridium pacaense (2)	2	>100
Firmicutes, Clostridia	Lachnospiraceae	Roseburia faecis	1	>100
Firmicutes, Clostridia	Lachnospiraceae	Roseburia faecis	1	25
Firmicutes, Clostridia	Lachnospiraceae	Roseburia inestinalis	1	100
Firmicutes, Clostridia	Lachnospiraceae	Tyzzerella nexilis	2	>100

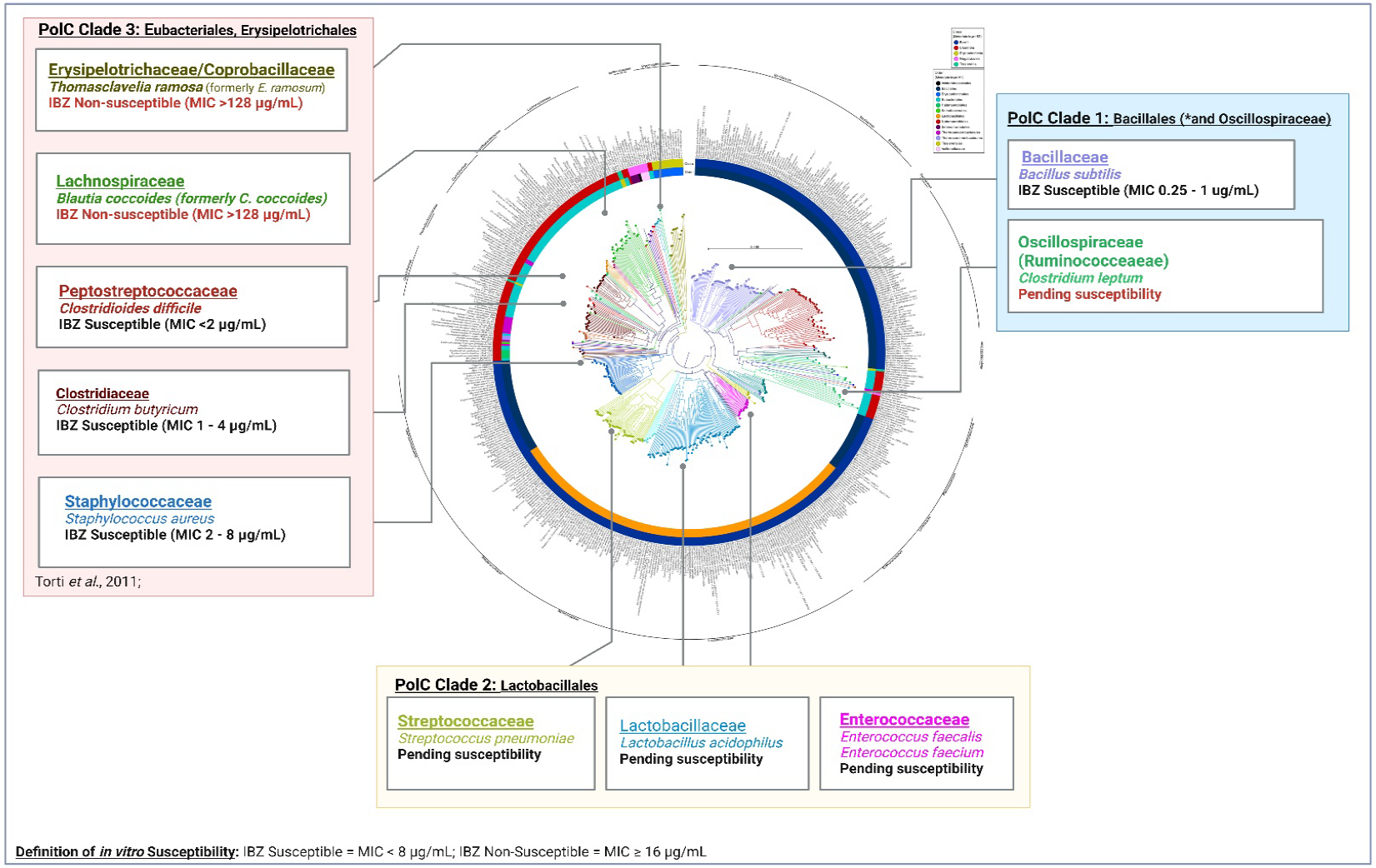
Samples from Phase 2a Clinical Trial

		IBZ MIC (ug/mL)		
Species	N	med	min	max
Blautia sp.	1	12.5		
Clostridium butyricum	5	12.5	1.56	100
Clostridium subterminale	1	0.78		
Clostridium tertium	2	12.5	3.125	12.5
Coprococcus sp	1	25		
Dorea longicatena	1	3.125		
Enterococcus avium	2	12.5	12.5	12.5
Enterococcus durans	1	100	100	100
Enterococcus faecalis	5	12.5	12.5	50
Enterococcus mundtii	1	12.5		
Enterococcus pseudoavium	1	100		
Erysipelatoclostridium ramosum	5	6.25	3.125	100
Flavonifractor plautii	1	25		
Lachnoclostridium pacaense	1	25		
Longibaculum sp.	1	50		
Melissococcus plutonius	1	6.25		
Pediococcus acidilactici	1			
Romboutsia hominis	1			
Streptococcus parasanguinis	1			
Unknown Firmicute	3	12.5	1.56	100

Clostridiales with differing IBZ susceptibility was observed in PH1 and PH2a studies

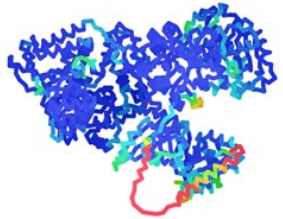
Q3. Can we predict the mechanism for this selectivity?

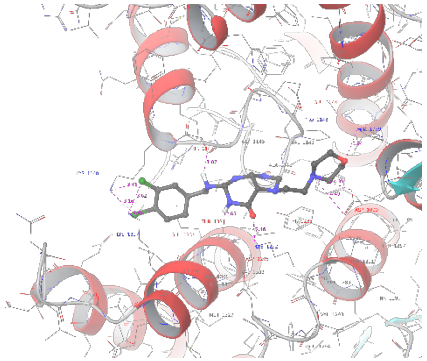
Phylogenetics of the PolC does not predict IBZ susceptibility

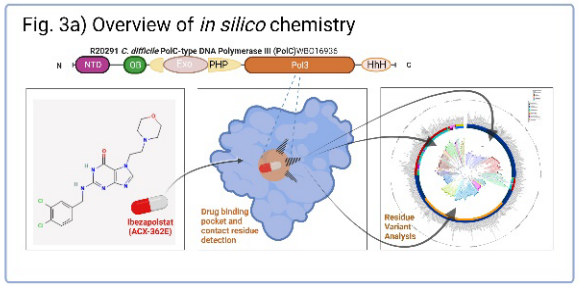


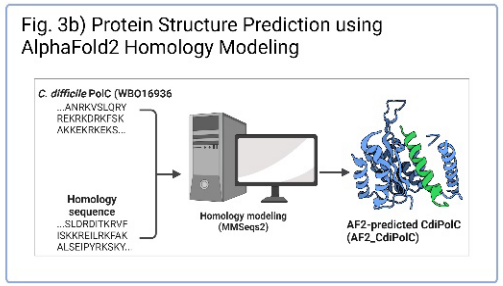
IBZ selectivity is likely related to unique binding sites present in select Firmicute strains. Will need confirmation

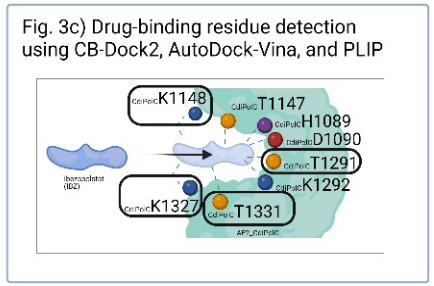
AlphaFold2: an artificial intelligence (AI) system that can **predict** three-dimensional (3D) structures of proteins from amino acid sequences with atomic-level accuracy.

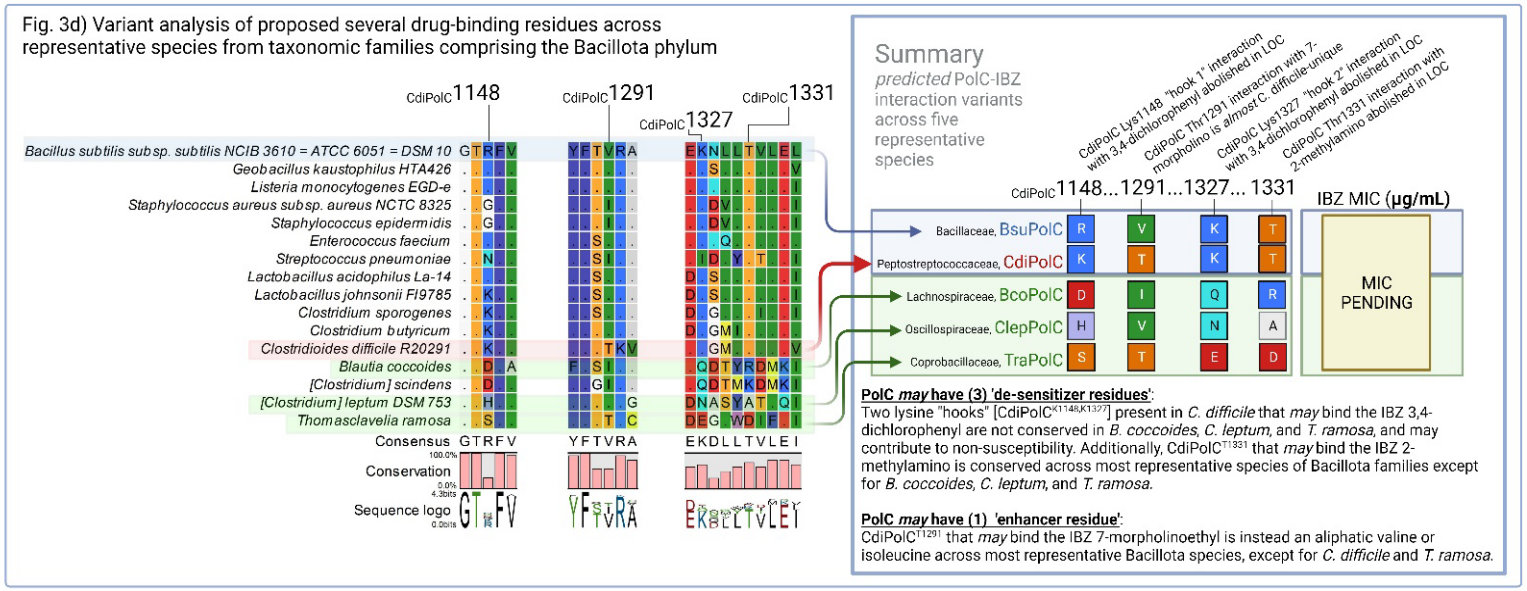










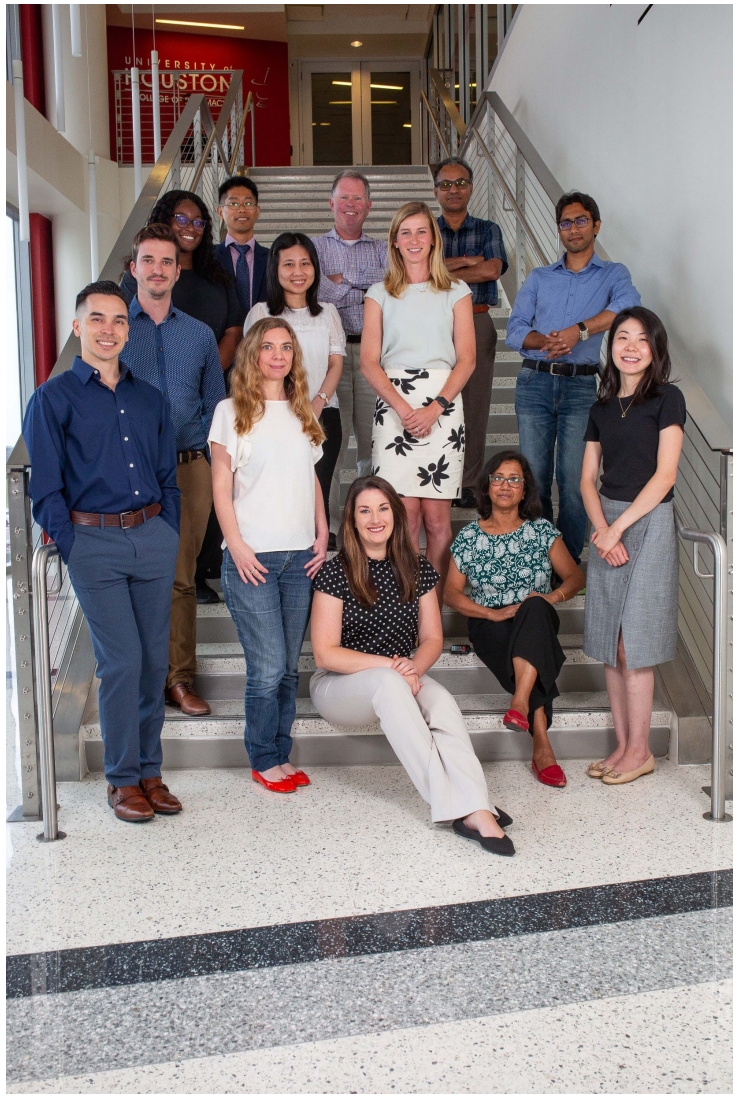


Conclusions

- IBZ is a selective inhibitor of the PolC enzyme in clinical trials for CDI
    1. Selectivity for certain Firmicutes identified by Illumina sequencing and confirmed by qPCR
    2. MIC testing showed variable susceptibility amongst different Firmicutes
    3. In silico prediction modeling identified potential binding sites that help explain selectivity
- All these results support continued development of IBZ

Acknowledgements

The Garey Lab



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