The Basis of Commensal Bacillota Resistance to a Novel PolC-type DNA Polymerase III Inhibitor, Ibezapolstat, and the "Narrower" Spectrum of Activity Towards *C. difficile*

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Training Program in Antimicrobial Resistance

UNIVERSITY of HOUSTON
COLLEGE OF PHARMACY

INSTITUTE OF BIOSCIENCES AND TECHNOLOGY
TEXAS A&M HEALTH SCIENCE CENTER

Clostridioides difficile infection (CDI): an Urgent Threat with Limited Narrow-spectrum Antibiotics

Vancomycin

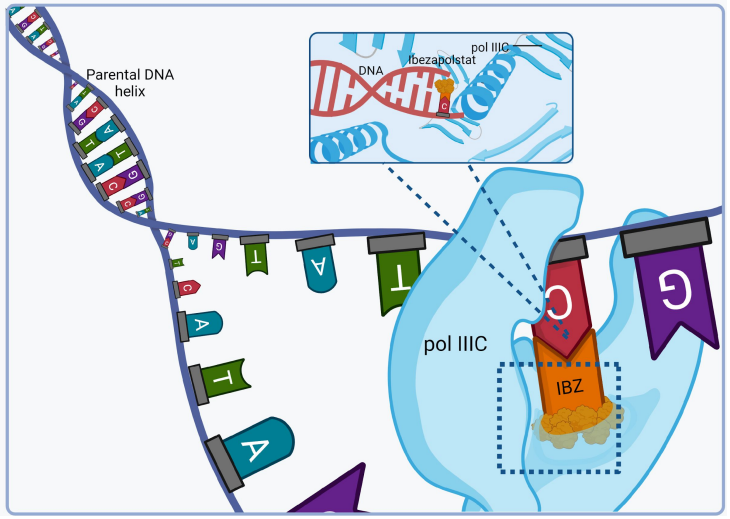
Fidaxomicin

_____ ?

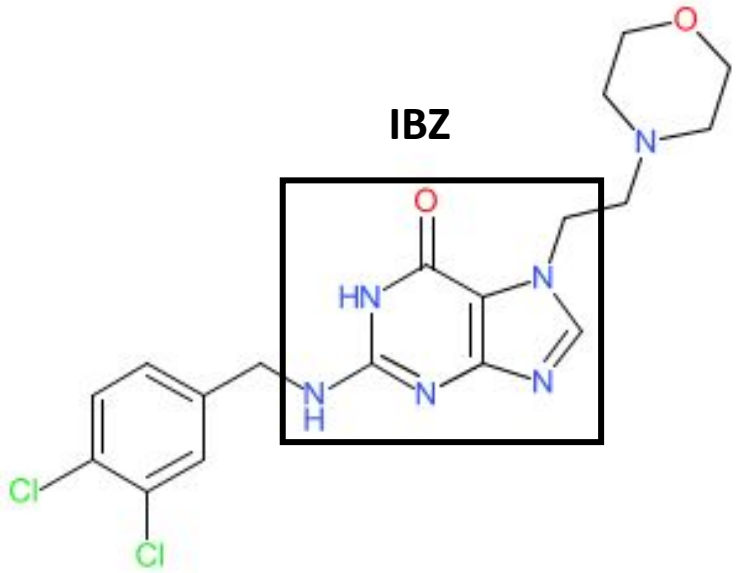
CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.
Johnson, S., Lavergne, V., Skinner, A.M., Gonzales-Luna, A.J., Garey, K.W., Kelly, C.P. and Wilcox, M.H. *Clinical infectious diseases* (2021)

Ibezapolstat (IBZ; ACX362E)

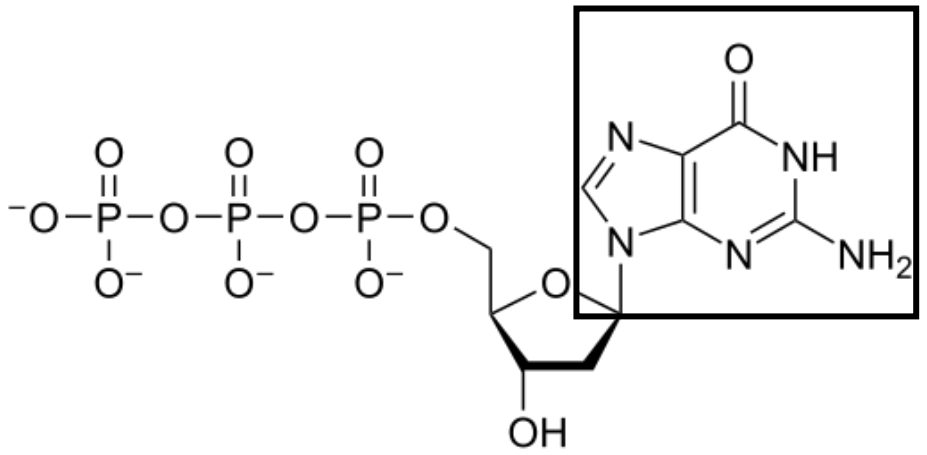
- Deoxyguanosine triphosphate **(dGTP)** analog
- Small-molecule *competitive* inhibitor of the PolC-type DNA Polymerase III **(PolC)**
- PolC is *essential* for replication of low G+C genome Gram-positive bacteria **(Bacillota)**
- **GPSS**™ (**G**ram **P**ositive **S**elective **S**pectrum) *narrow*-spectrum of activity



IBZ

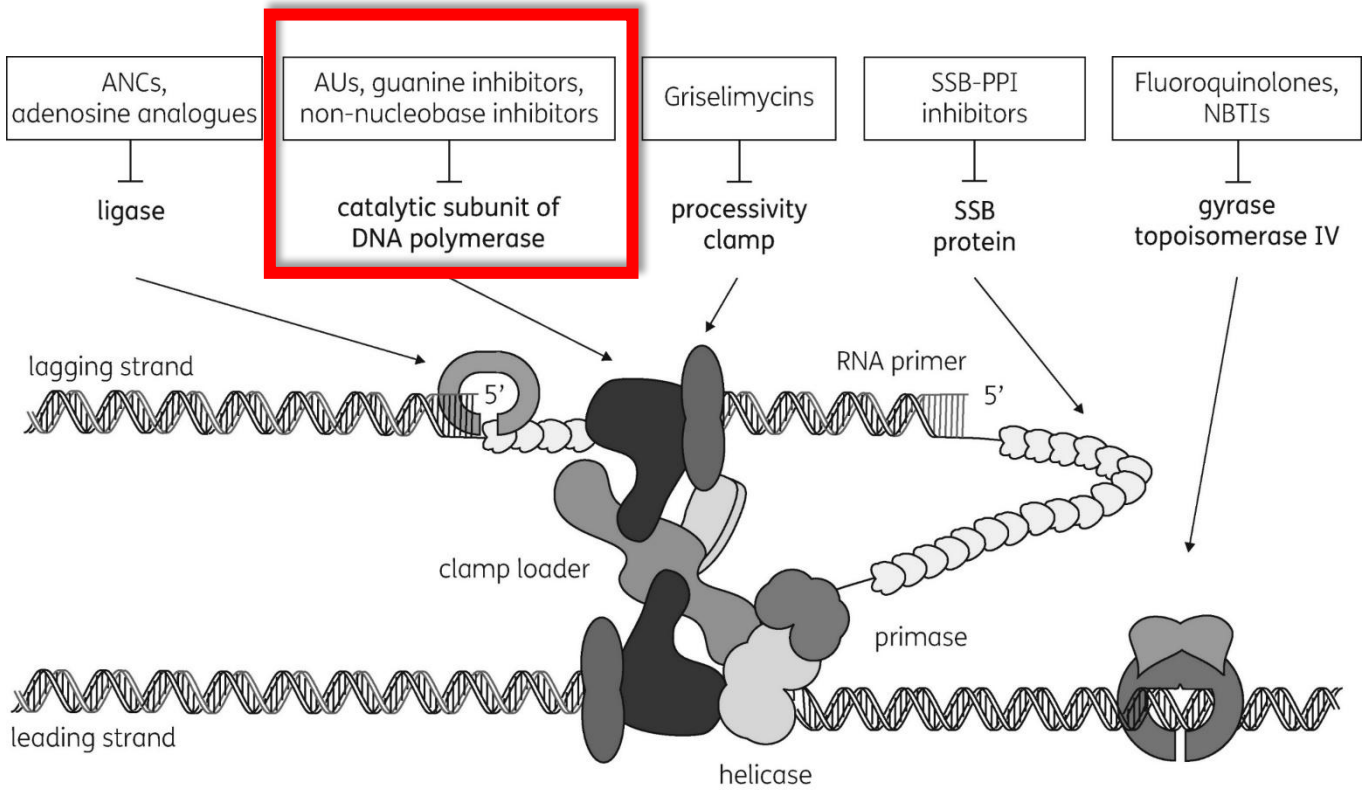


dGTP

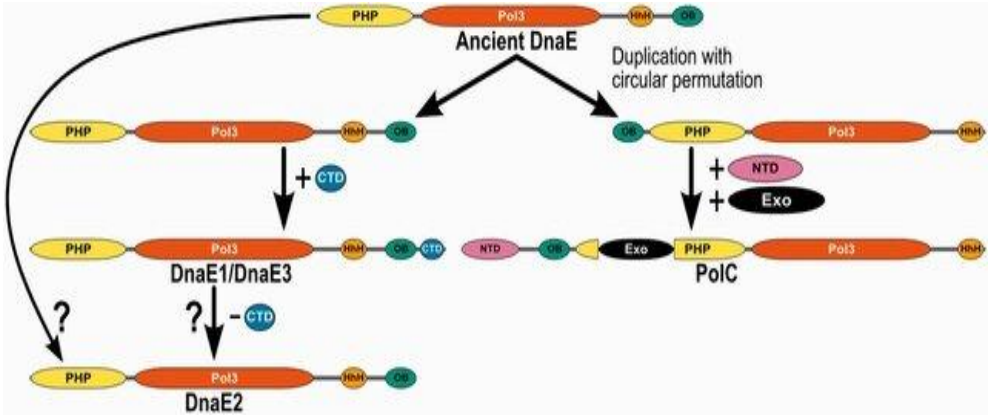


Xu et al. *Bioorg Med Chem* (2019)
https://www.nature.com/articles/d43747-021-00149-0
Figure by BioRender created by Avalon Starr

The PolC-type DNA Polymerase III **(PolC)** is the catalytic subunit of the **Bacillota Replisome**



'Ancient DnaE Hypothesis'

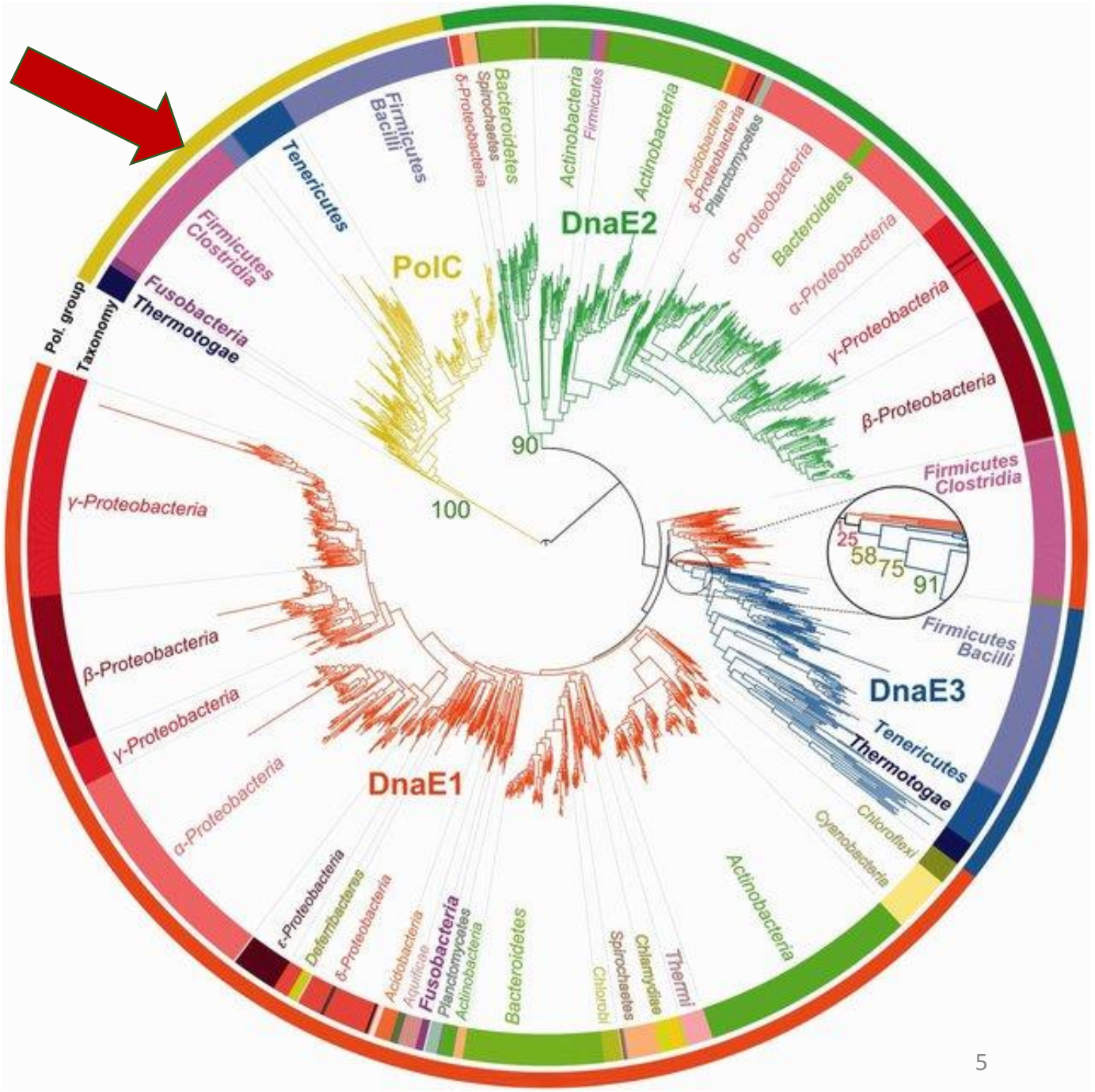


van Eijk, E., Wittekoek, B., Kuijper, E. J., & Smits, W. K. *Journal of Antimicrobial Chemotherapy* (2017).

Koonin EV, Bork P. *Trends Biochem Sci* 1996

polC is found in **Bacillota**,

not Proteobacteria, Bacteroidetes or Actinobacteria



Timinskas, K., Balvočiūtė, M., Timinskas, A., & Venclovas, Č. *Nucleic acids research* (2014)

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

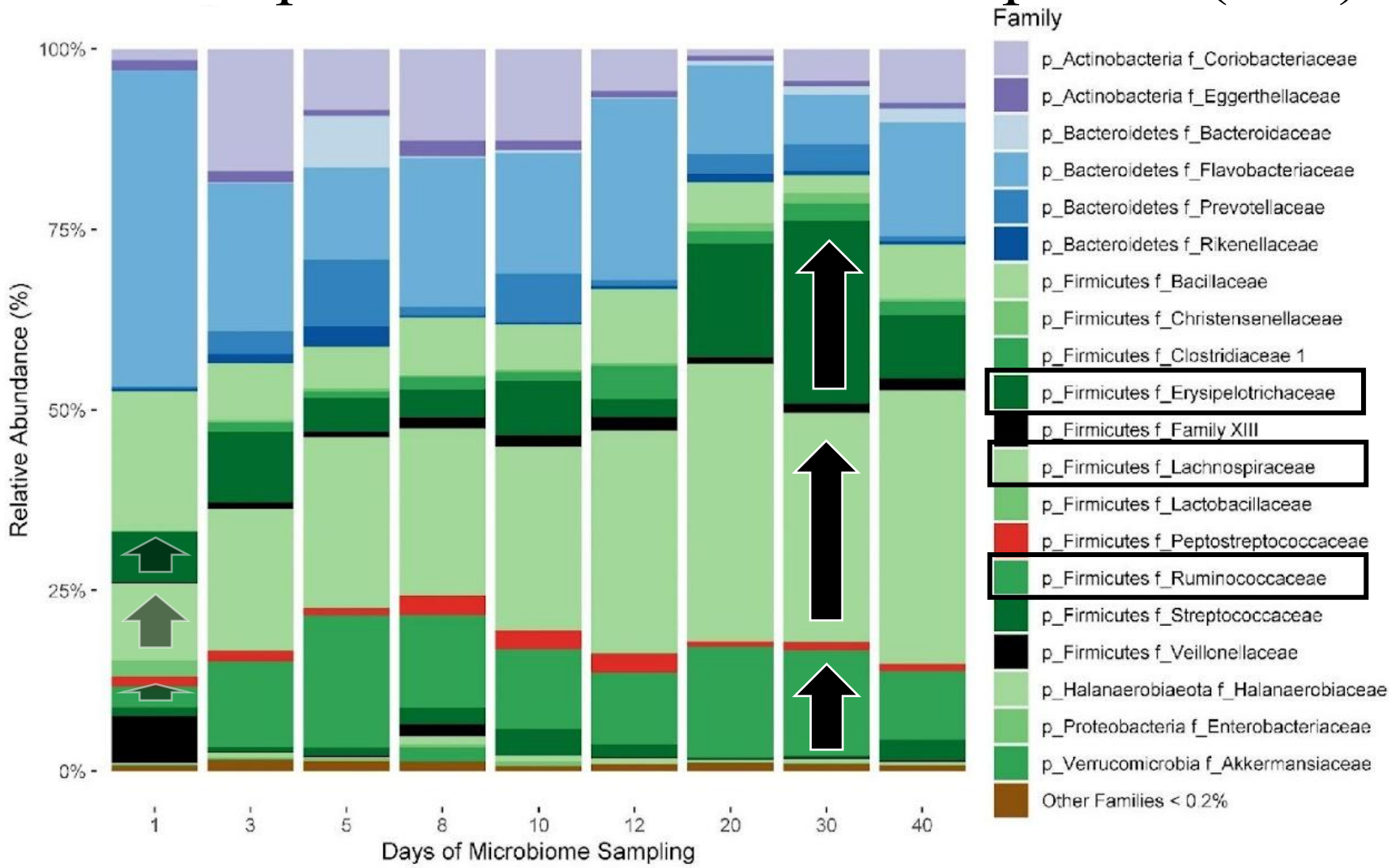
  - *****Microbiome evaluations predicted an anti-recurrence effects due *narrower* than expected spectrum of activity
    - *****McPherson et al. *Antimicrob Agents Chemother* 2022

- **Phase 2a** (n=10): *Completed*
  - *****10 of 10 patients experienced clinical cure with no recurrence.
  - *Narrower* than expected spectrum of activity also observed
    - *****Garey et al. Clin Infect Dis 2022

- **Phase 2b**: *Completed*
  - IBZ 450 mg twice daily vs. vancomycin 125 mg PO four times daily
  - ClinicalTrials.gov Identifier: NCT04247542
  - See poster being presented at lunch today

*In phase I/II studies, an IBZ selective spectrum of activity to certain Bacillota

Lachnospiraceae, **Ruminococcaceae** and **Erysipelotrichaceae** **increased** in CDI patients treated with Ibezapolstat (IBZ)



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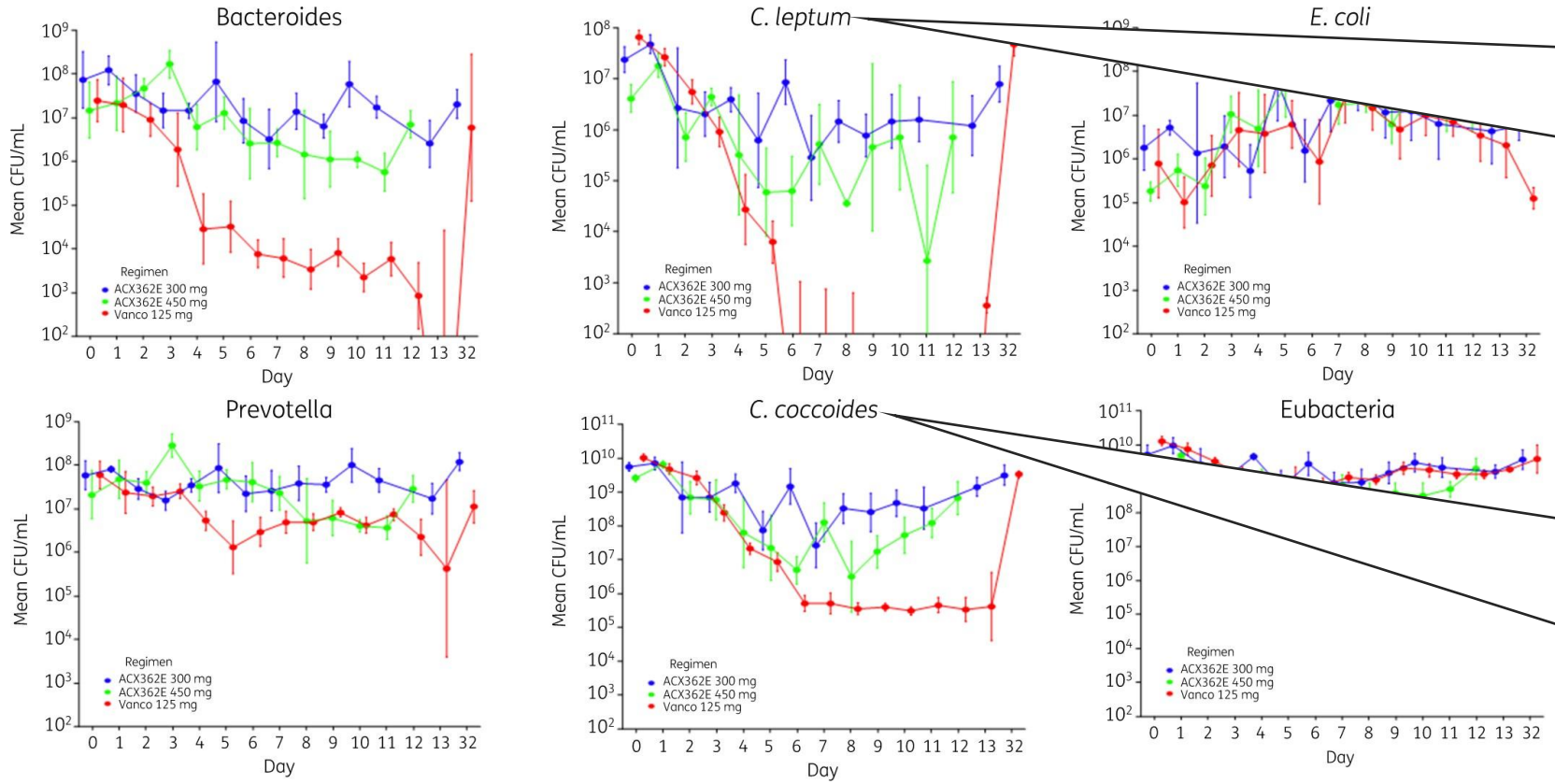
Garey KW, McPherson J, Dinh AQ, et al. *Clinical Infectious Diseases*. 2022.

IBZ-mediated Increases in Specific Sub-taxa of Commensal Bacillota Confirmed by qPCR



Many of Clostridium cluster IV (*C. leptum* group) taxonomically reassigned **Oscillospiraceae** (formerly Ruminococaceae)

Many of Clostridium cluster XIVa (*C. coccoides* group) taxonomically reassigned **Lachnospiraceae**

Garey KW, Begum K, Lancaster C, et al. *J Antimicrob Chemother*. 2020.

Specific Aims

The global aim of my project is to determine the narrower than expected spectrum of IBZ

- Determine the structure, binding pocket and drug-binding residues of the *C. difficile* PolC-IBZ complex
  - Cryogenic-electron microscopy (cryo-EM): Work in progress
  - In silico prediction: Presenting today
    - PolC Phylogenetics
    - In silico binding prediction

In silico Methods

- **PolC sequence relatedness**: mmSeqs2 MSA, Neighbor-joining, and Jukes-Cantor
- **3D protein structure prediction**: AlphaFold2 (ColabFold)
- **Cavity-detection blind drug docking**: CB-Dock2: CurPocket, AutoDock-Vina
- **Homology modeling**: CLC Genomics (Qiagen), Maestro (Schrodinger), UCSF ChimeraX

Sequence Relatedness of the PolC does **not** predict IBZ susceptibility

PolC Clade 3: Eubacteriales, Erysipelotrichales

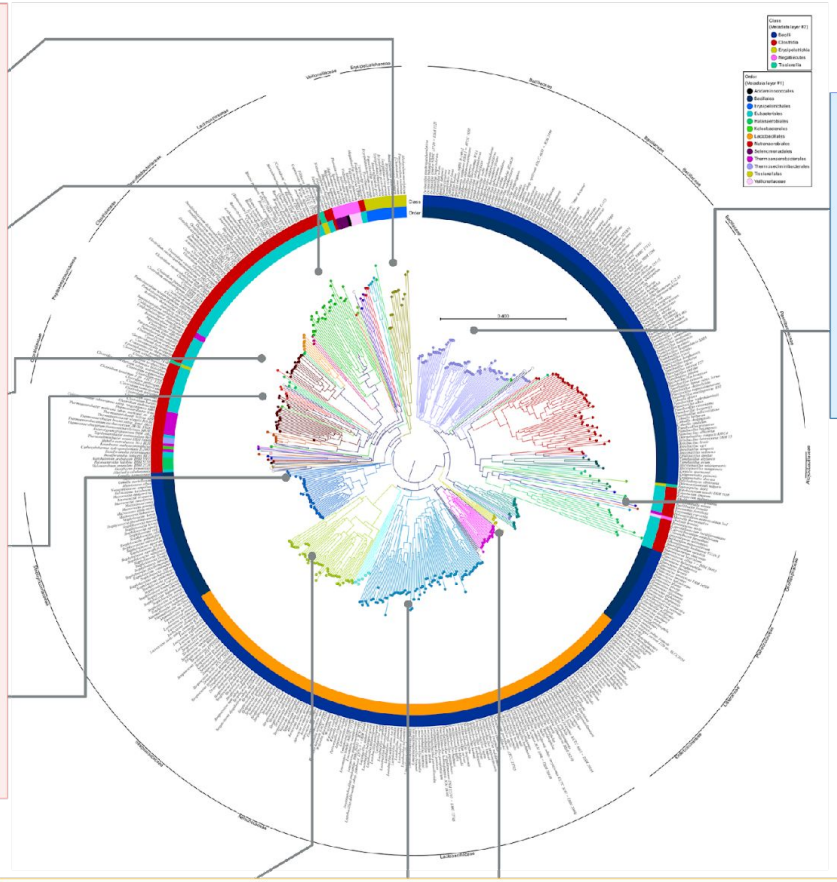
Erysipelotrichaceae/Coprobacillaceae
Thomasclavelia ramosa (formerly *E. ramosum*)
IBZ Non-susceptible (MIC >128 µg/mL)

Lachnospiraceae
Blautia coccoides (formerly C. coccoides)
IBZ Non-susceptible (MIC >128 µg/mL)

Peptostreptococcaceae
Clostridioides difficile
IBZ Susceptible (MIC <2 µg/mL)

Clostridiaceae
Clostridium butyricum
IBZ Susceptible (MIC 1 - 4 µg/mL)

Staphylococcaceae
Staphylococcus aureus
IBZ Susceptible (MIC 2 - 8 µg/mL)



PolC Clade 1: Bacillales (*and Oscillospiraceae)

Bacillaceae
Bacillus subtilis
IBZ Susceptible (MIC 0.25 - 1 ug/mL)

Oscillospiraceae (Ruminococceaeae)
Clostridium leptum
Pending susceptibility

Definition of *in vitro* Susceptibility: IBZ Susceptible = MIC < 8 µg/mL; IBZ Non-Susceptible = MIC ≥ 16 µg/mL

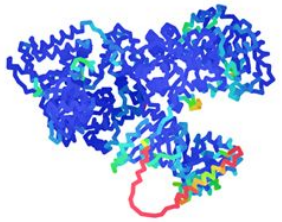
PolC Clade 2: Lactobacillales

Streptococcaceae
Streptococcus pneumoniae
Pending susceptibility

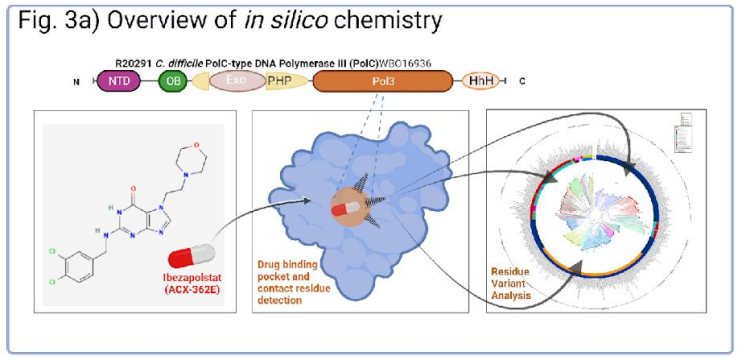
Lactobacillaceae
Lactobacillus acidophilus
Pending susceptibility

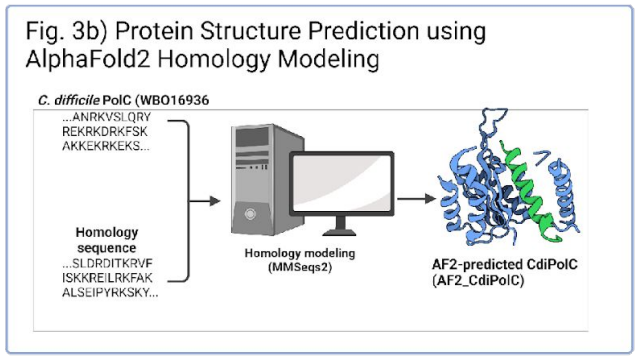
Enterococcaceae
Enterococcus faecalis
Enterococcus faecium
IBZ Susceptible (MIC 4 µg/mL)

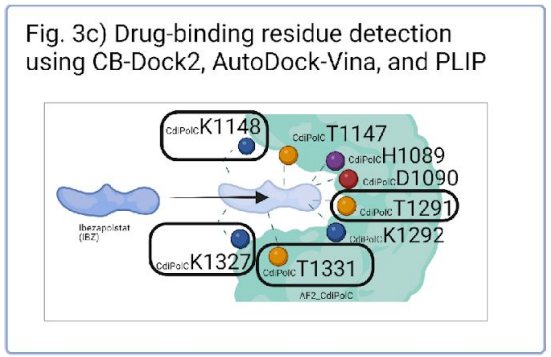
IBZ selectivity is likely related to unique binding **residues** present in select Bacillota taxa

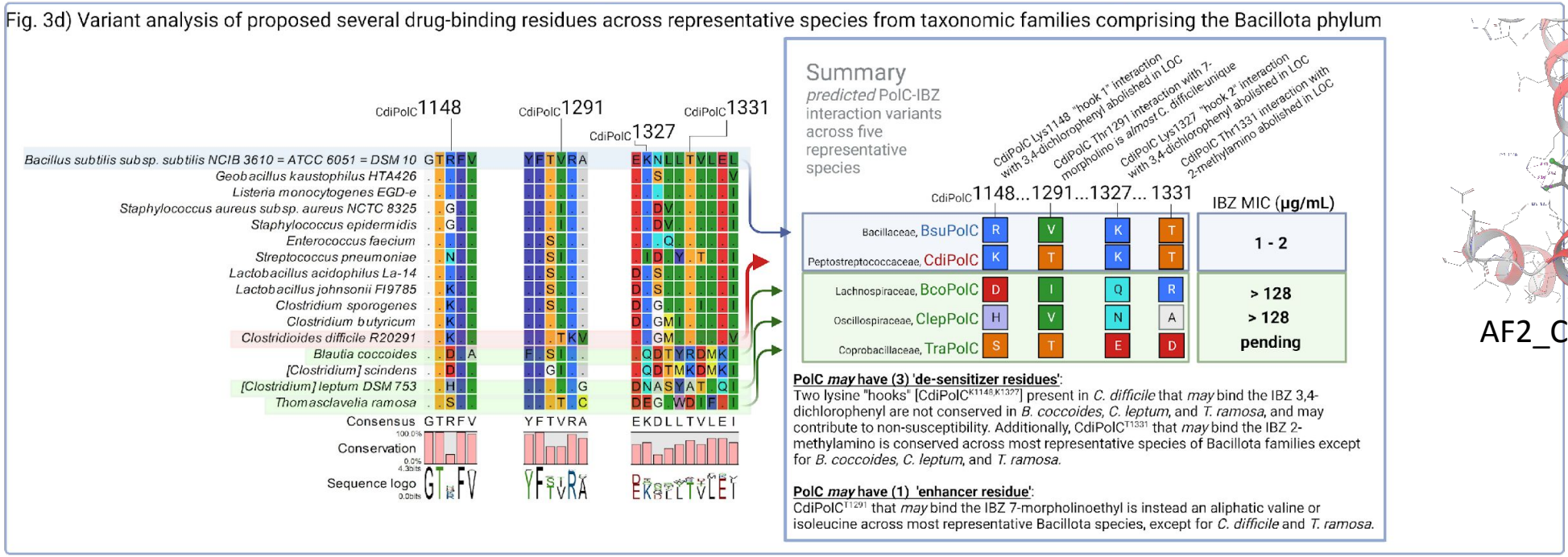


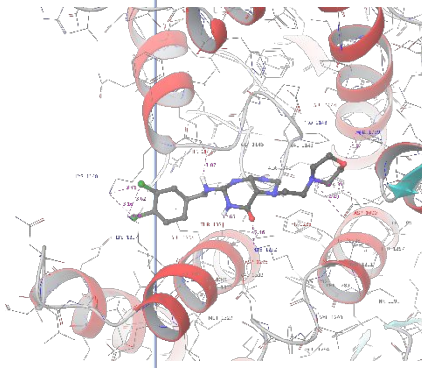
AF2_CdPolC







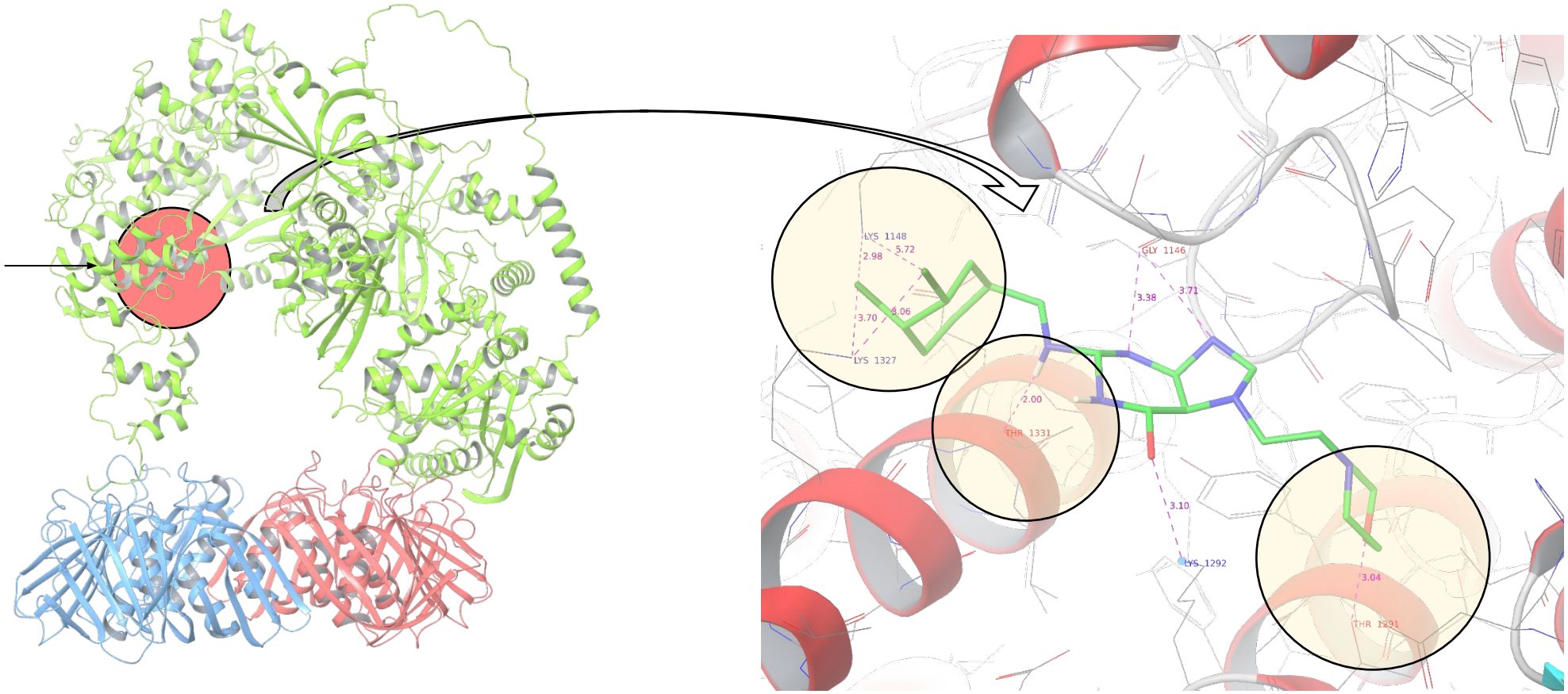




AF2_CdPolC_ADV_IBZ

In silico (predicted) IBZ drug-binding residues are conserved across *most* Firmicutes except **Lachnospiraceae**, **Ruminococcaceae**, **Erysipelotrichaceae**

Site of *Bacillus subtilis* HPUra resistant mutant (*azp-12)* in the catalytic pocket

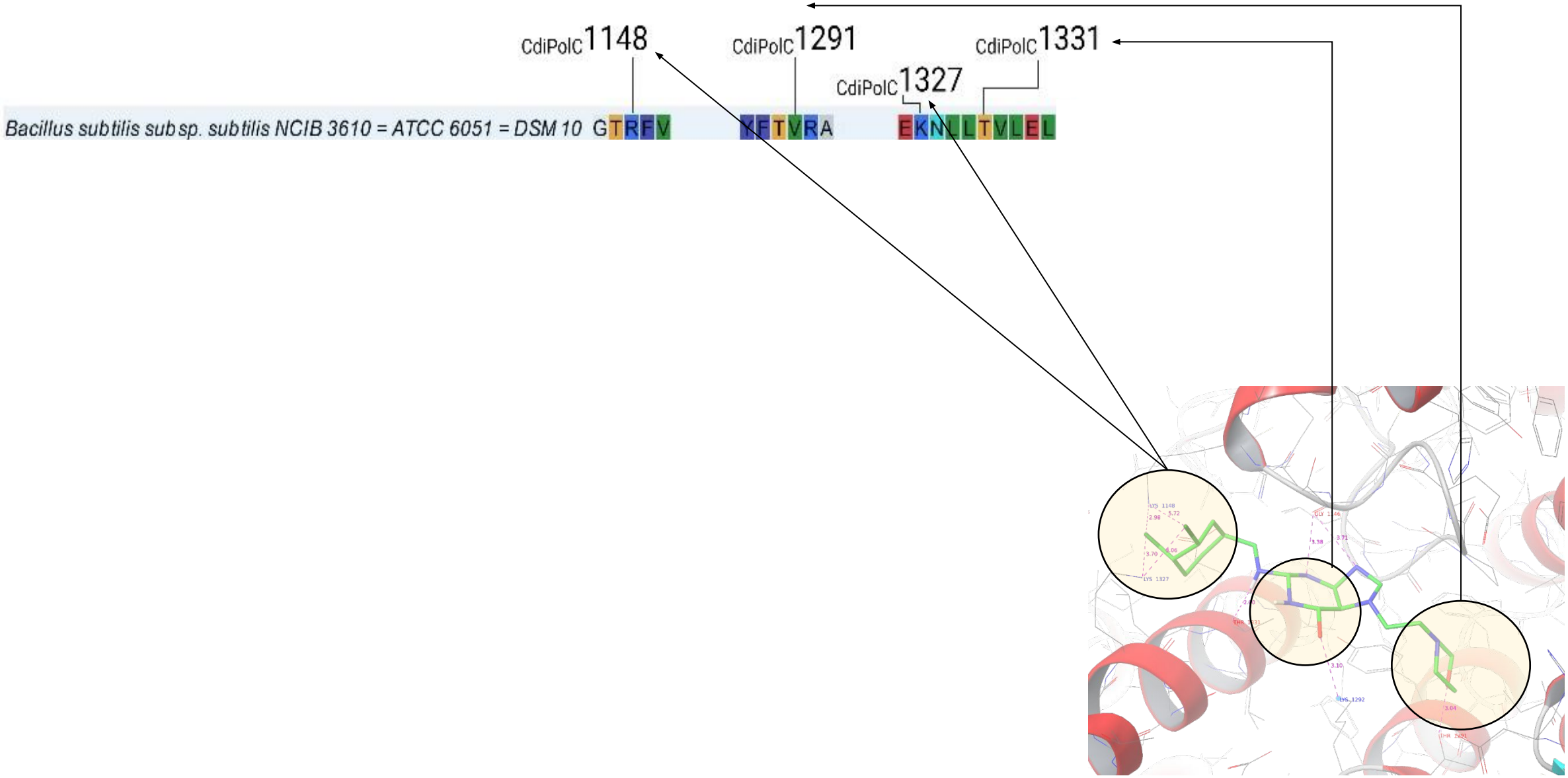


CB-Dock2 [CurPocket + AutoDock-Vina] of IBZ to AF2-CdiPolC

Liu Y, Yang X, Gan J, Chen S, Xiao ZX, Cao Y. *Nucleic Acids Res*. 2022

Jumper J, Evans R, Pritzel A, et al. *Nature*. 2021

Eberhardt J, Santos-Martins D, Tillack AF, Forli S. *Journal of Chemical Information and Modeling*. 2021



Lachnospiraceae: *Blautia coccoides;* [Clostridium] scindens
Ruminococcaceae: [Clostridium] leptum
Erysipelotrichaceae: Thomasclavelia ramosa

CB-Dock2 [CurPocket + AutoDock-Vina] of IBZ to AF2-CdiPolC

My hypotheses after *in silico* studies

1. The ***C. difficile*** **PolC** catalytic pocket contains a **'sensitizer residue'** (CdiPolC^{Thr1291})that increases the relative potency of IBZ towards *C. difficile*
2. Lachnospiraceae, Oscillospiraceae, and Erysipelotrichaceae PolC catalytic pockets contain **'de-sensitizer residues'** (CdiPolC^{Lys1148}, CdiPolC^{Lys1327}, CdiPolC^{Thr1331}) that confer IBZ **non-susceptibility**

Conclusions

- Using in silico modeling, we were able to predict the narrower than expected spectrum of activity of IBZ
  - Sensitizer and de-sensitizer residues were identified
- MIC work to date has helped confirm these predictions
- Cryo-EM work to validate this work ongoing

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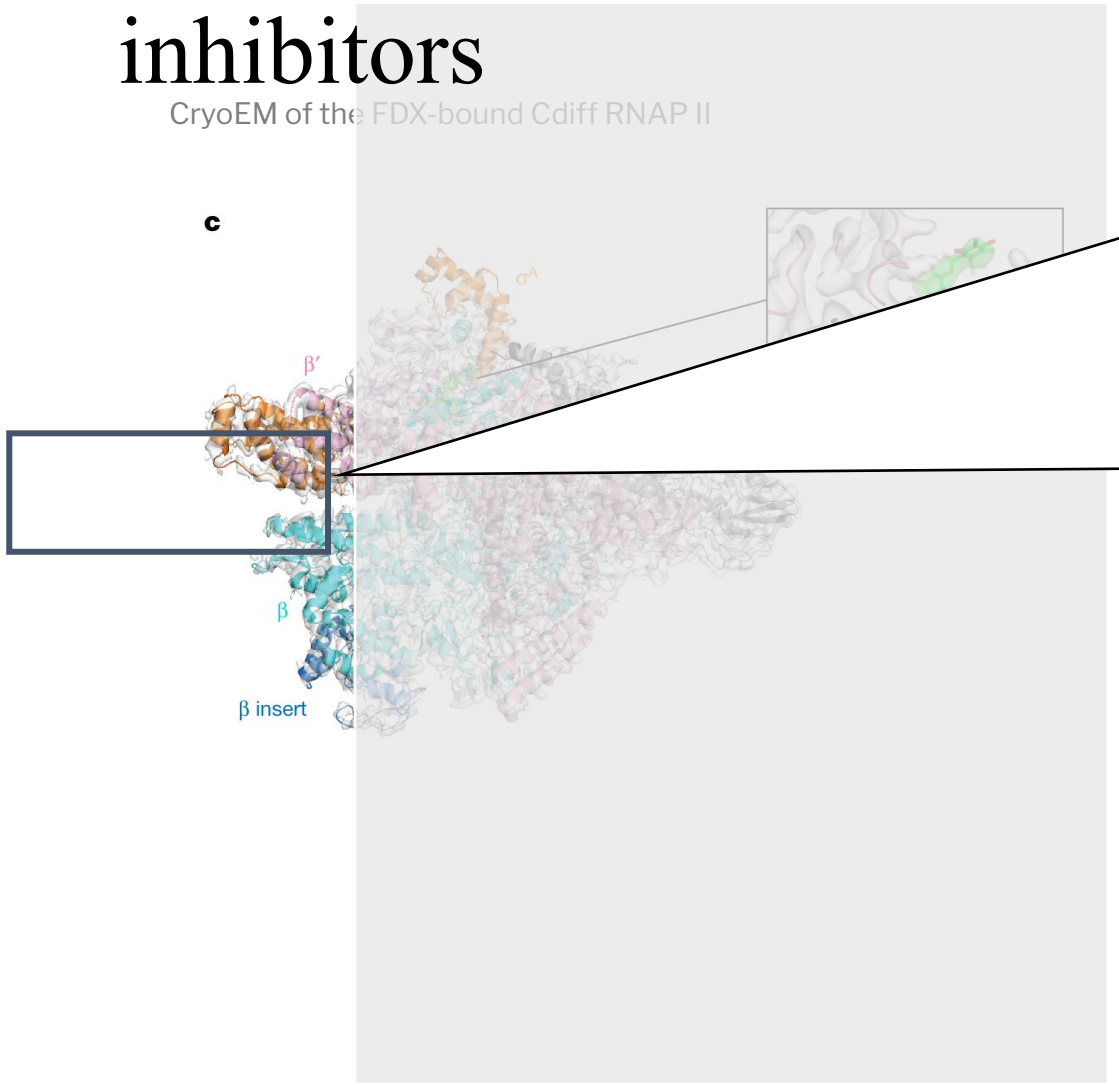
QUANTITATIVE BIOMEDICAL SCIENCES

NIH National Institute of Allergy and Infectious Diseases

Comparative Structural Biology to determine the Gram-positive selective spectrum activity of PolC inhibitors

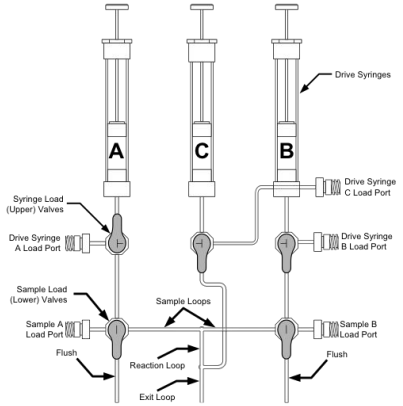


Peptostreptococcaeae	?	?
Bacillaceae	?	?
Lactobacillaceae	?	?
Lachnospiraceae	?	?
Ruminococcaceae	?	?
Erysipelotrichaceae	?	?

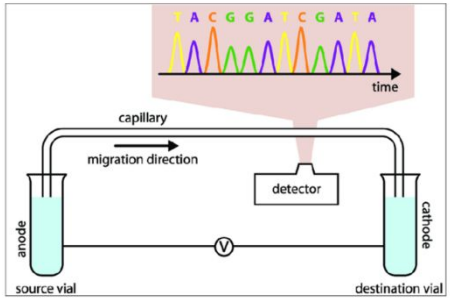
K84

Cao X, Boyaci H, Chen J, Bao Y, Landick R, Campbell EA. *Nature*. 2022

Enzyme Kinetics of Inhibition

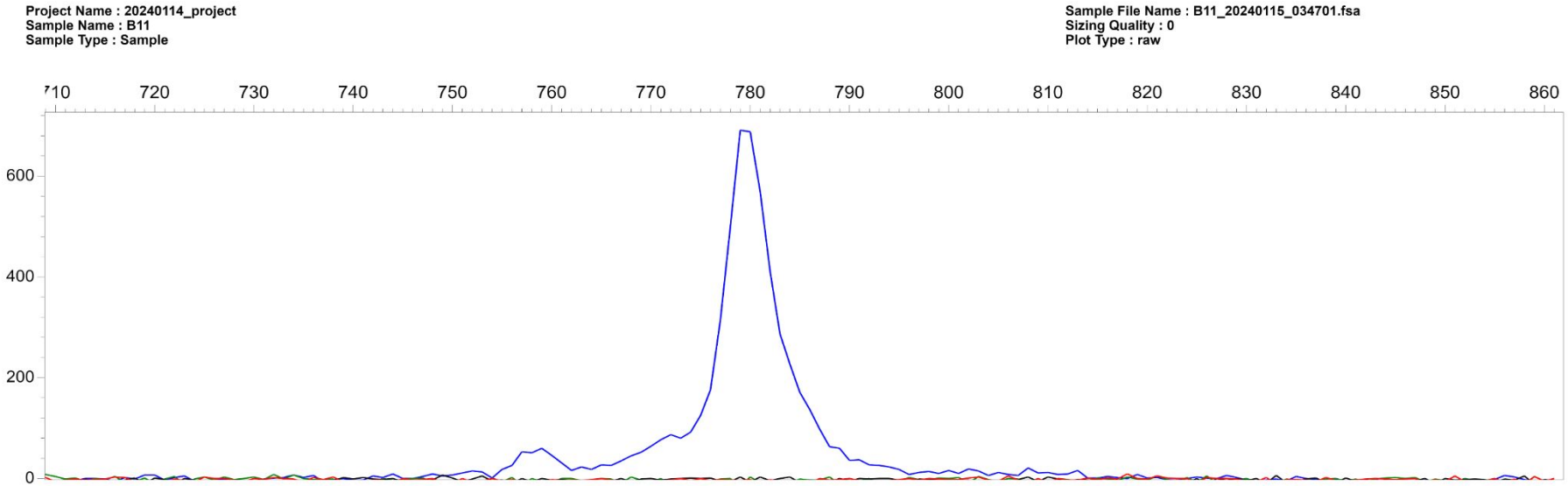


Rapid Quench Flow (RQF)
<u>milli</u>second-resolution



Fluorescence Capillary Electrophoresis: <u>single</u>-nucleotide incorporation resolution

<u>Stay tuned</u>



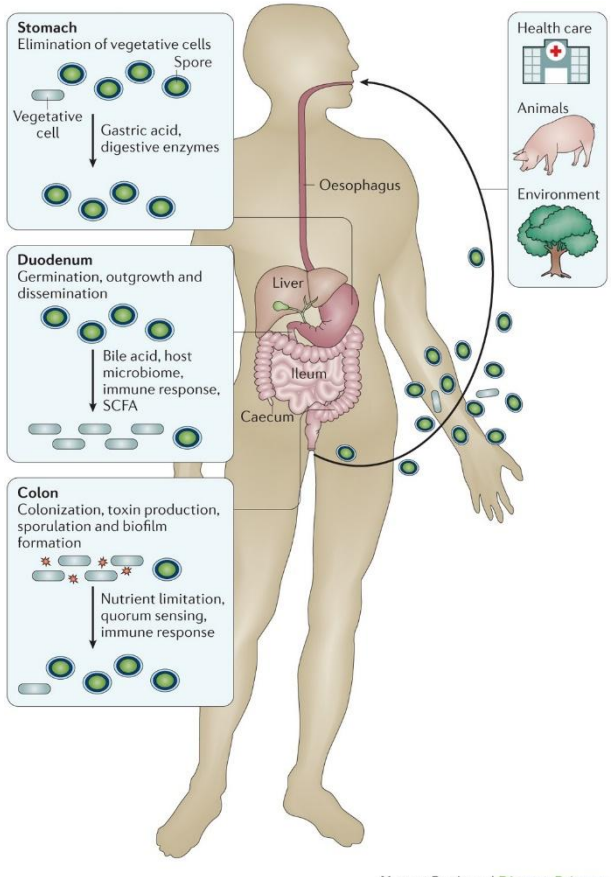
Copeland RA. *Wiley*. 2013
Johnson KA, Goody RS. *Biochemistry*. 2011.
Arunlakshana O, Schild HO. *British Journal of Pharmacology and Chemotherapy*. 1959.

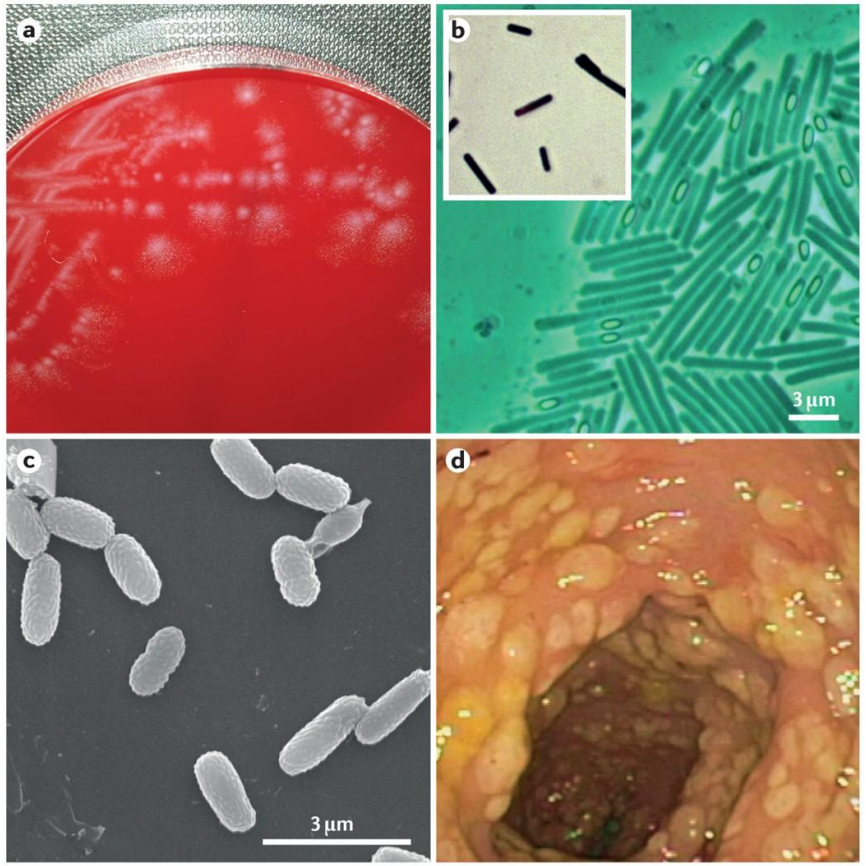
Structural Biology of PolC Pharmacology

- In collaboration with the University of Texas McGovern School of Medicine cryo-EM core facility

Stay tuned

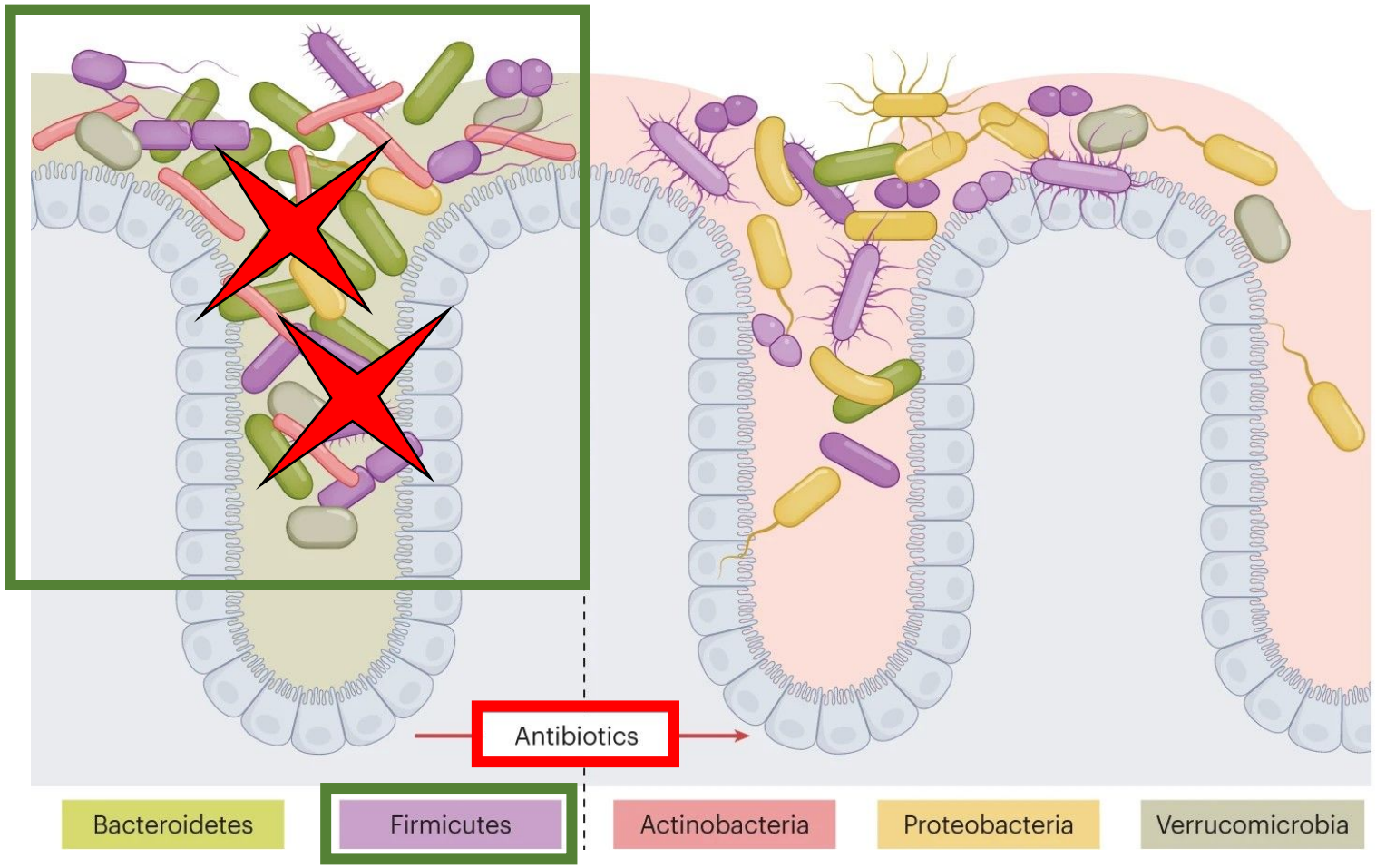
Clostridioides difficile is a Gram-positive **Bacillota pathobiont** that leads to antibiotic-associated diarrhea, pseudomembranous colitis, colectomy and death





Smits, W., Lyras, D., Lacy, D. *et al. Nat Rev Dis Primers* (2016)

Oral Antibiotics Damage the **Human Gut Microbiome**



Urgent threat

Clostridioides difficile

Carbapenem-resistant Enterobacteriales

Serious threat

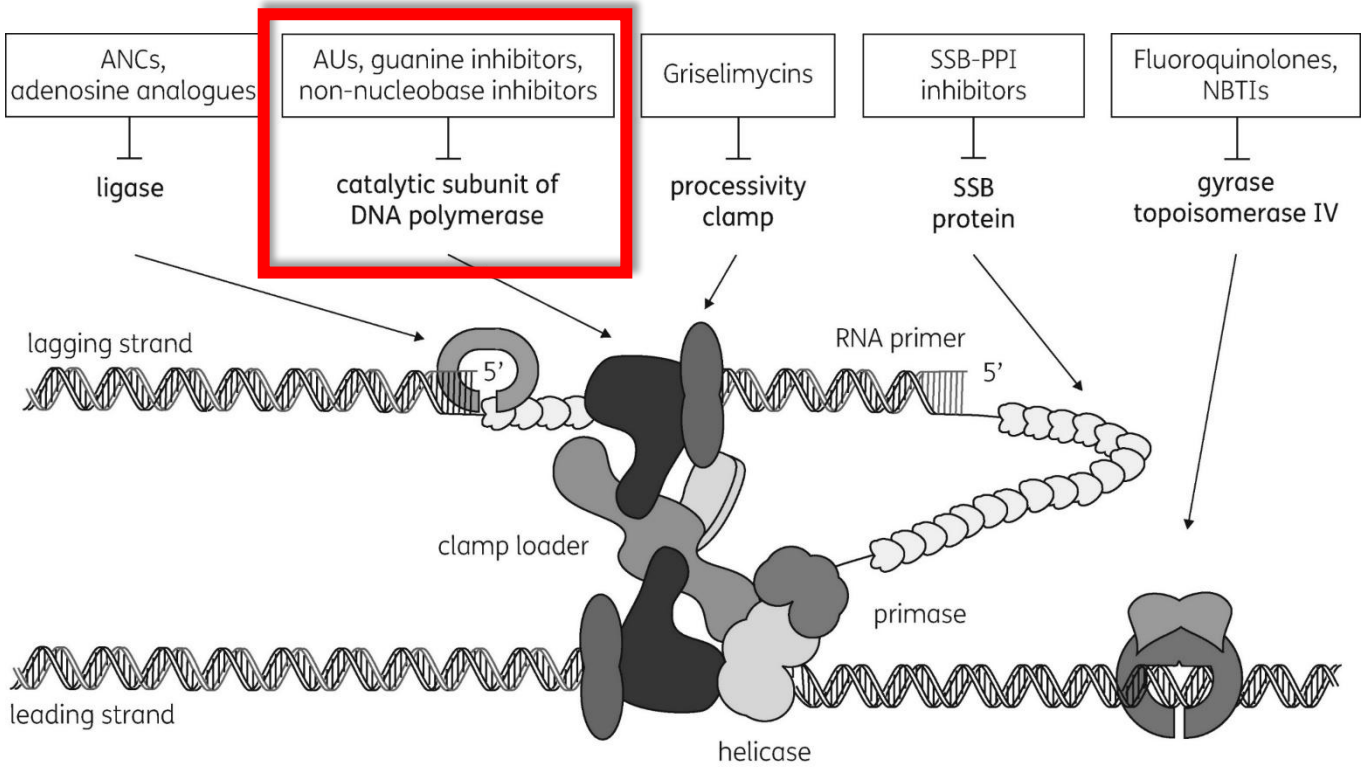
ESBL-producing Enterobacteriales

Vancomycin-resistant *Enterococcus*

Drug-resistant *Campylobacter*, *Salmonella* and *Shigella*

Fishbein, Mahmud, & Dantas, *Nat Rev Microbiol* (2023).

The PolC-type DNA Polymerase III (**PolC**) is the catalytic subunit of the **Firmicute** Replisome



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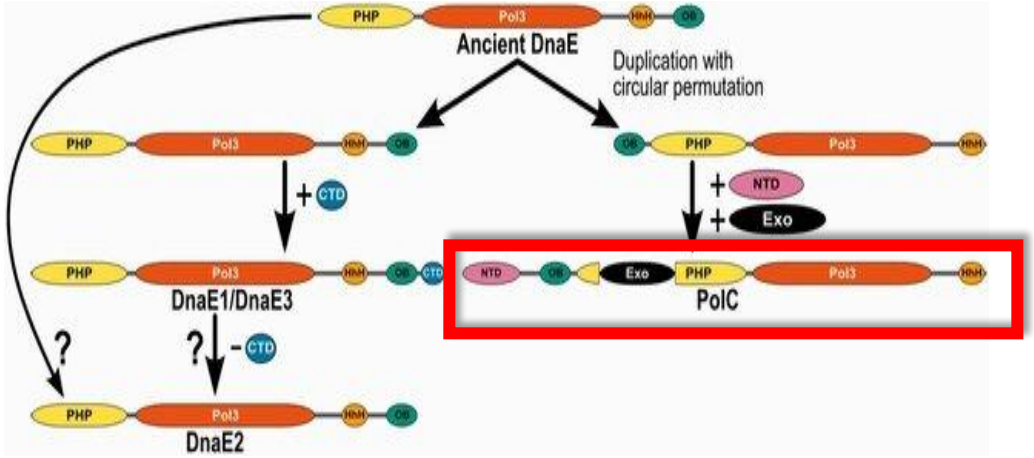
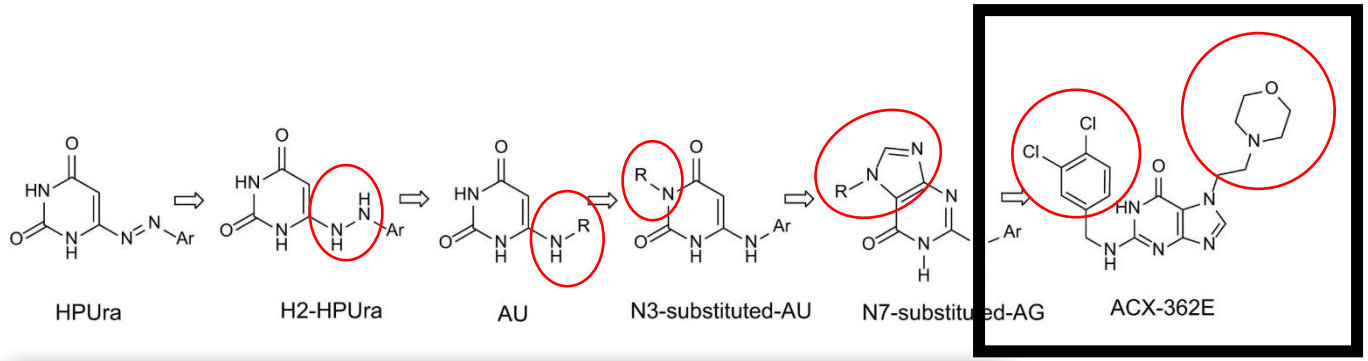


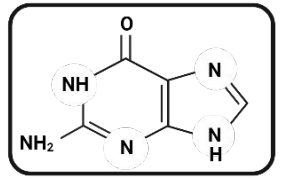
van Eijk, E., Wittekoek, B., Kuijper, E. J., & Smits, W. K. *Journal of Antimicrobial Chemotherapy* (2017).

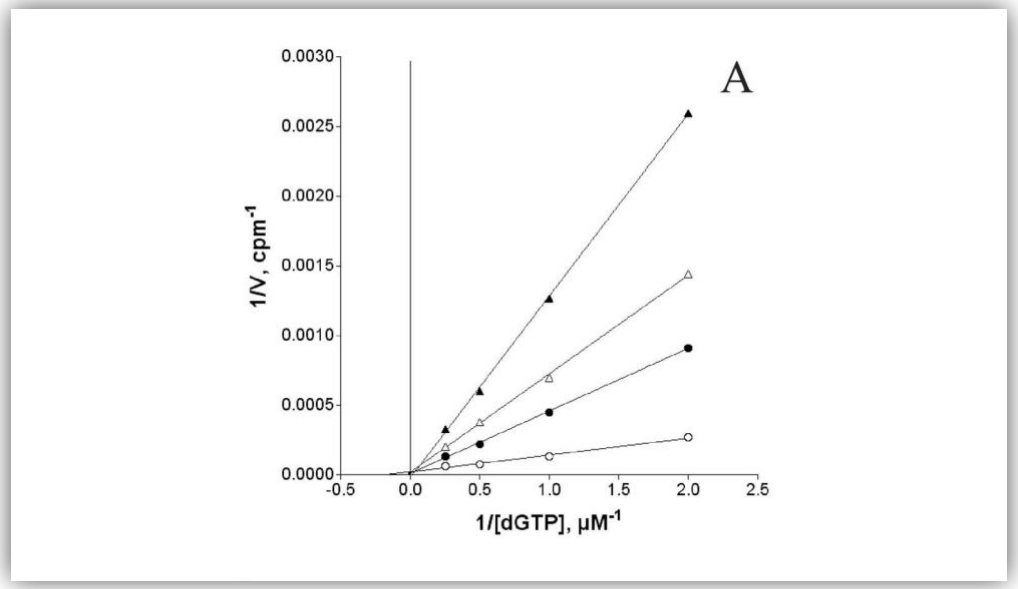
Koonin EV, Bork P. *Trends Biochem Sci* 1996

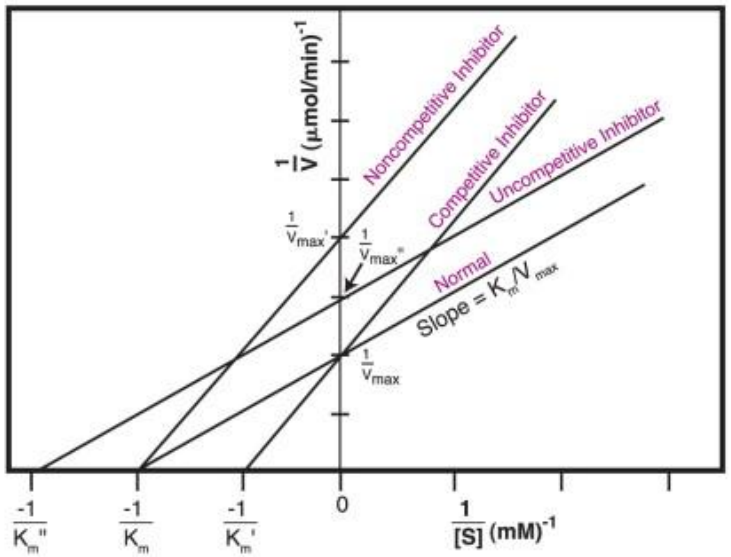
Ibezapolstat (IBZ) is a dGTP-mimetic inhibitor of the *C. difficile* PolC



Guanine





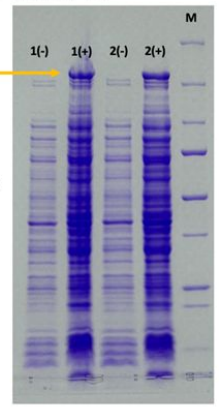


Xu, Silverman, Yu, Wright & Brown, 2019.

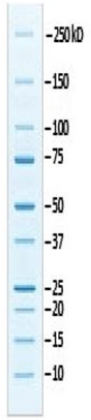
Torti, A., Lossani, A., Savi, L., Focher, F., E Wright, G., C Brown, N., & Xu, W. C. *Current enzyme inhibition* (2011)

C. difficile PolC and DnaN beta sliding-clamp expression and purification

POL C = 162,307 Da

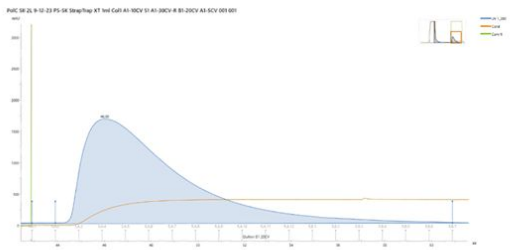


- (-) = Uninduced Sample, (+) = Induced sample
- The expression level of POL C is evident



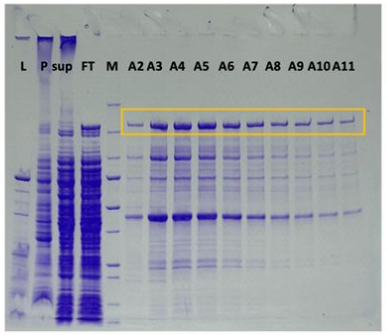
Project : DNA Polymerase III-POL C purification

Method: StrepTrap XT 1ml column, RUN 1

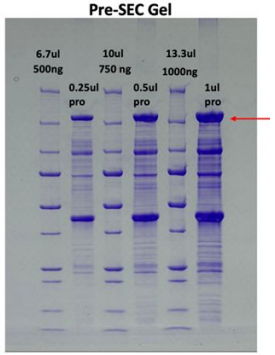


- Single peak , fractions from A2-B7
- Did Nanodrop and Gel run

POL C= 162 kDa

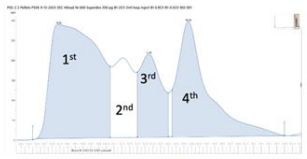


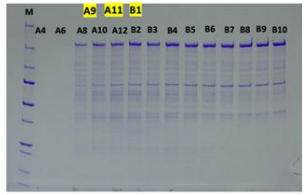
Pre-SEC Gel

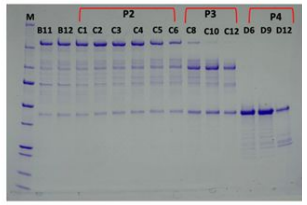


POST SEC- Peaks, Concentration, and GEL

Post-SEC peak, Superdex 200 pg



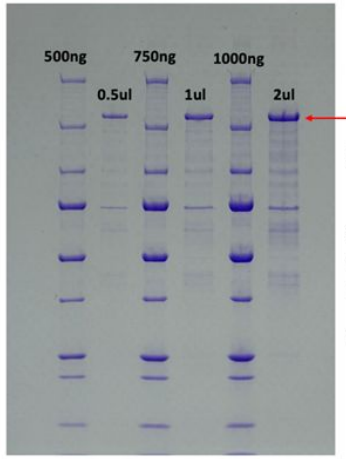




- 4 PEAKS, peak of concern 1st
- Ran two Gel, 1st Gel contained all sample fractions of 1st peak
- 2ND Gel contains all fractions from 2nd peak, and 3 sample fractions from 3rd and 4th peak

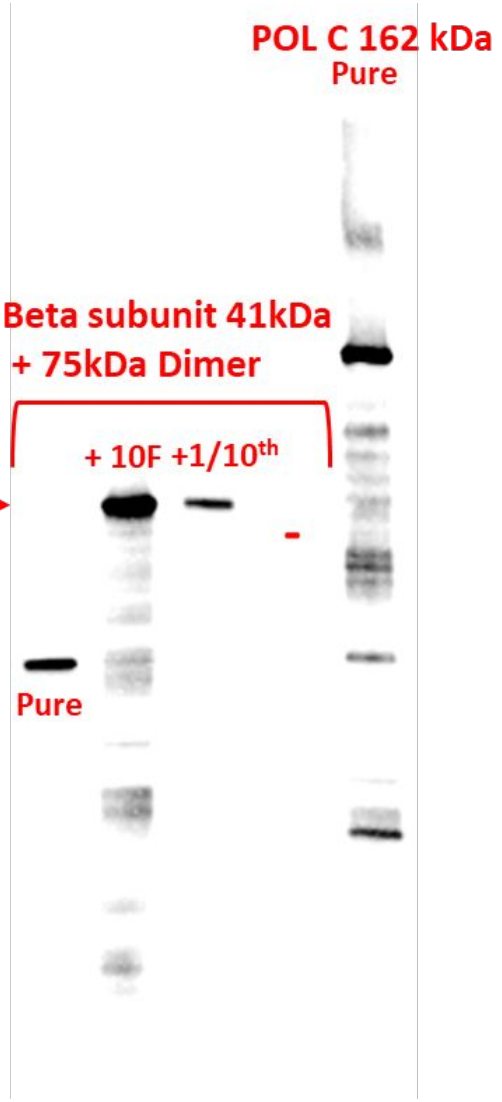
#POOLED 19 Fractions , A8- A12, B1-B12, C1 and C2 = 19 ml

POLC = 162 kDa

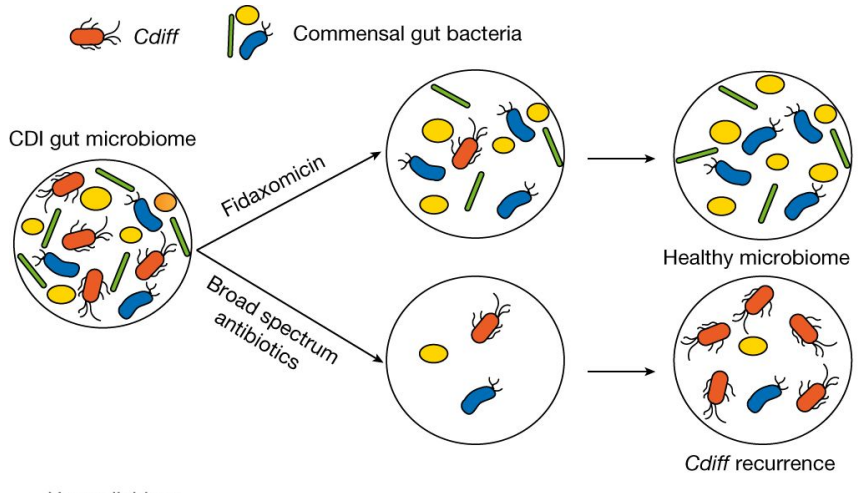


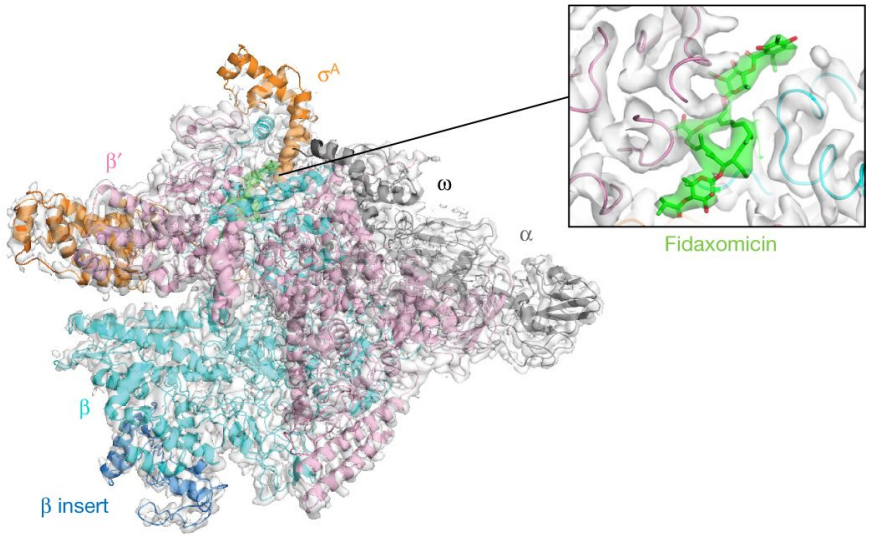
- 0.5ul, 1ul and 2ul are protein amount

So, finally, the protein was diluted, and established concentration = **1 mg/ml**
= 23 tubes, each having 100ul



Inspiration from Fidaxomicin

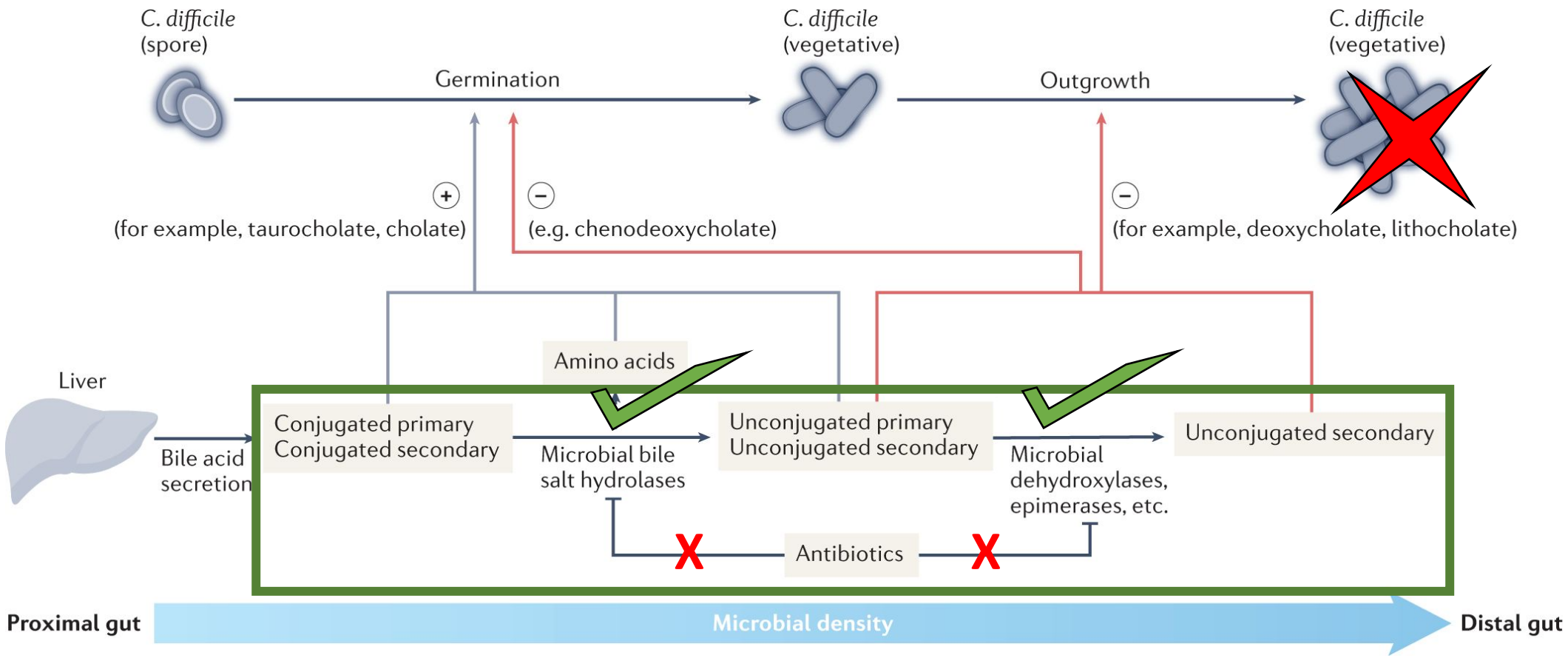




CryoEM of the FDX-bound Cdiff RNAP II

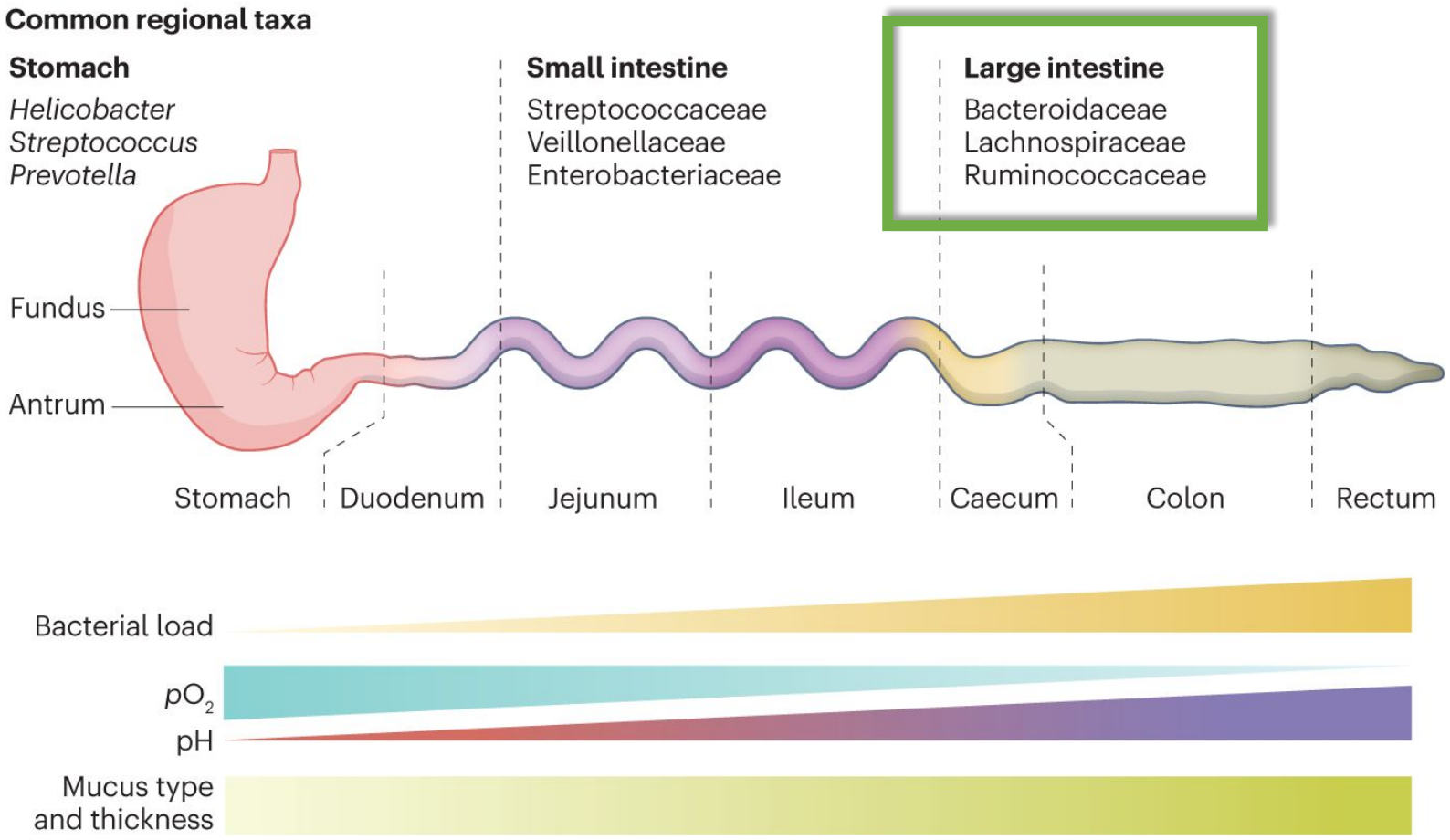
Cao X, Boyaci H, Chen J, Bao Y, Landick R, Campbell EA. *Nature*. 2022

Microbial Bile Salt Metabolism **Suppresses** *C. difficile* Germination and Outgrowth



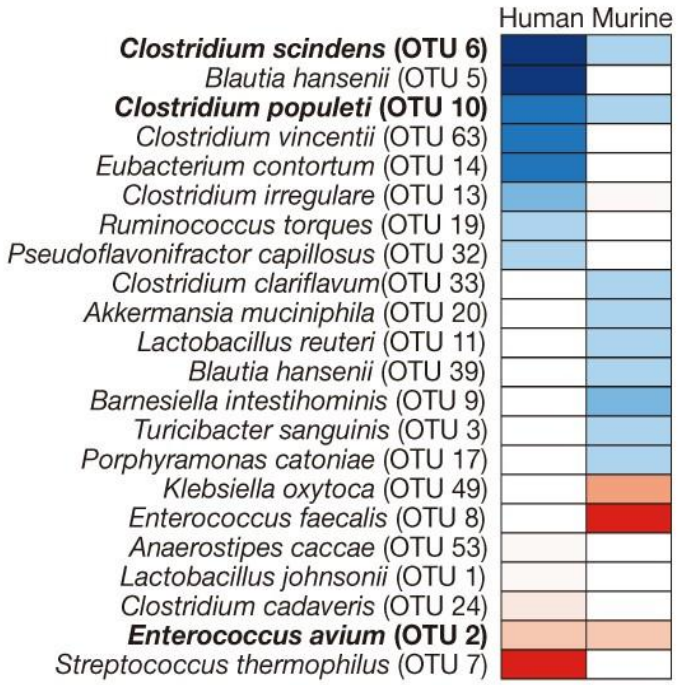
Schnizlein, M.K., Young, V.B. *Nat Rev Gastroenterol Hepatol* (2022)

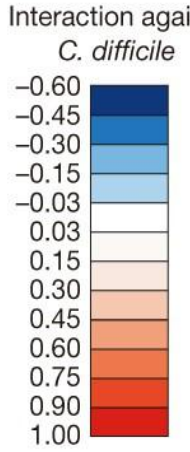
Lachnospiraceae and **Ruminococcaceae** are the dominant Bacillota of the Large Intestine

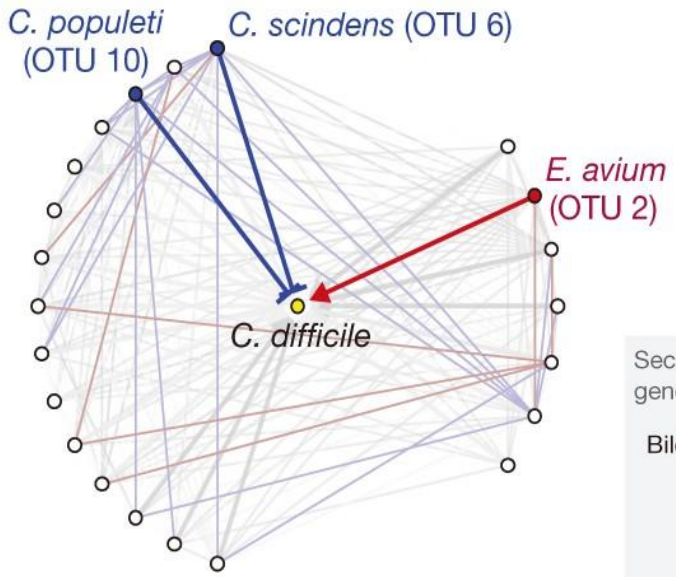


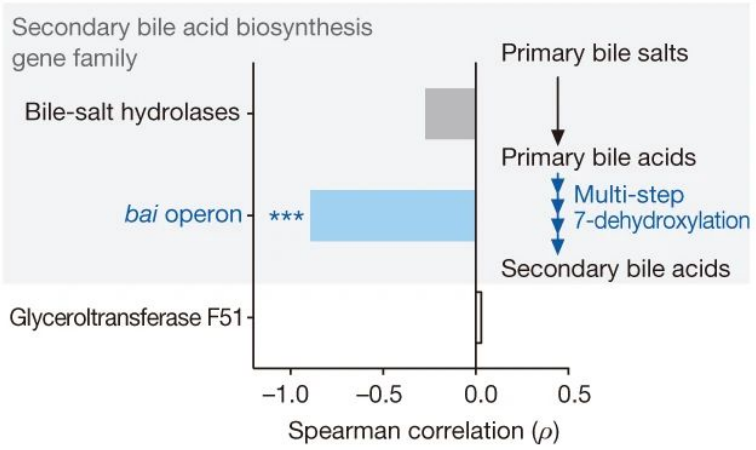
McCallum, G., Tropini, C. *Nat Rev Microbiol* (2023)

Lachnospiraceae (*C. scindens*) protects against CDI via bile acid metabolism



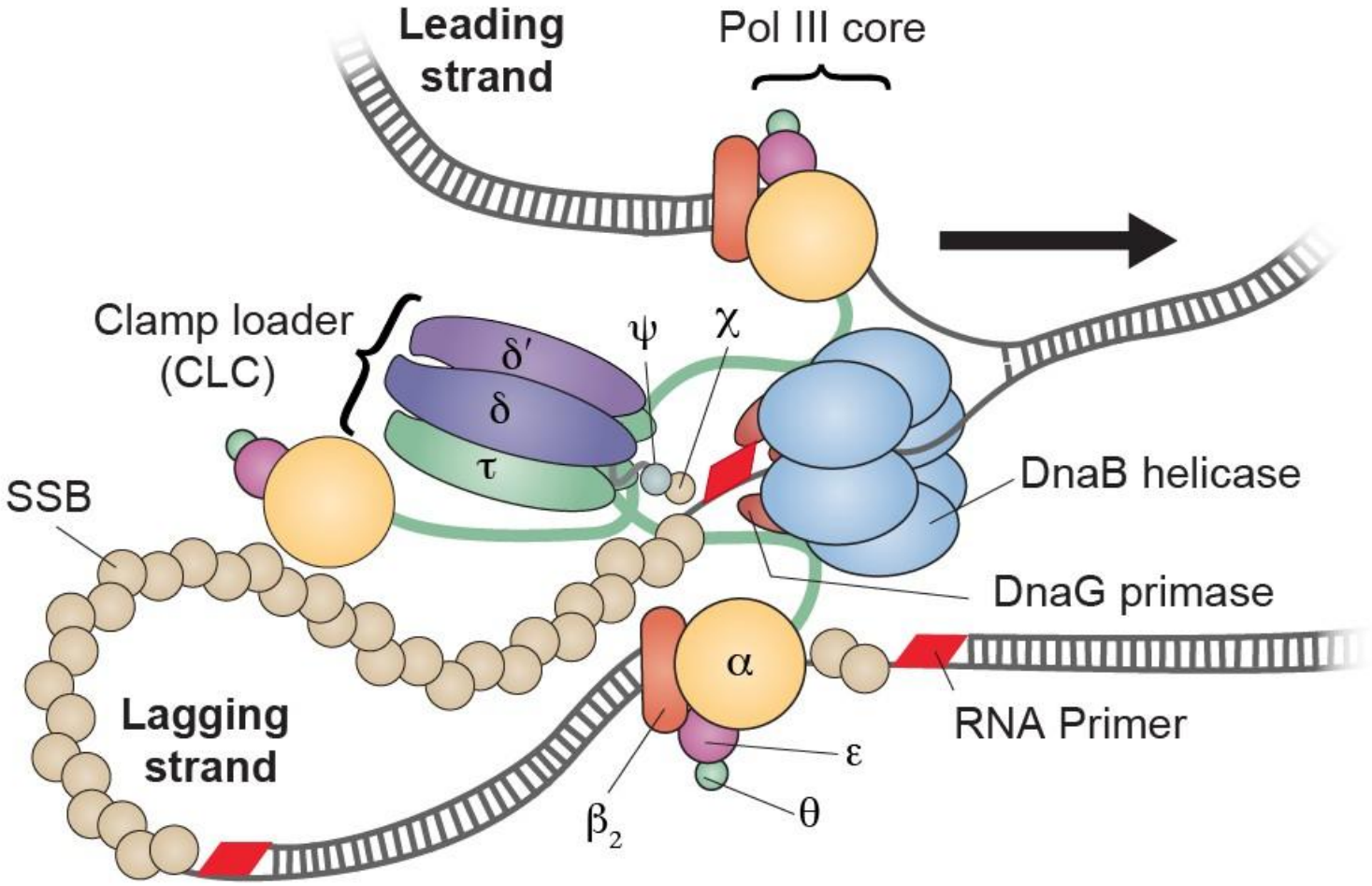






Buffie, C., Bucci, V., Stein, R. et al. *Nature* (2015)

Targeting the Bacterial Replisome



Lewis, Jacob S., et al *Elife* 6 (2017): e23932.