Pharmacological Investigations of the PolC-type DNA polymerase III of the Human Gut Microbiome

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For the love of my life, Michelle – you are my world, you are my sunshine.

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Abstract

Broad-spectrum antibiotics that deplete the commensal bacteria of the human gut microbiome can leave a host organism susceptible to pathobionts through the loss of mechanisms collectively referred to as colonization resistance. The principal infectious disease that takes advantage of the antibiotic-mediated loss of colonization resistance, *Clostridioides difficile*, is also treated with antibiotics that may further harm and limit the recovery of these commensal microbiota, often leading to recurrent *C. difficile* infections (rCDI). Thus, the development of narrow-spectrum antibiotics for the treatment of *Clostridioides difficile* infection (CDI) that spares the commensal microbiota is a challenge at the crossroads of the human gut microbiome and antimicrobial resistance. One antibiotic target that has a phylogenetically restricted evolution is the PolC-type DNA polymerase III alpha-subunit (PolC), the essential, catalytic subunit of the Bacillota DNA replisome. Critically, the *polC* is devoid from the genomes of *Actinomycetota*, *Bacteroidoidota*, and *Pseudomonadota*, other bacterial phyla that comprise a large proportion of the human gut microbiome, making the PolC an attractive drug target for narrow-spectrum antibiotic development The small molecule competitive inhibitor of the PolC, ibezapolstat (IBZ), has entered clinical trials for the treatment of CDI. However, the impact of IBZ, a selective PolC inhibitor, on the human gut microbiome remained uncertain. While we observed orally administered IBZ was associated with an increased relative abundance of *polC*$^{-}$ *Actinomycetota* in healthy adults, we curiously observed an increased *polC*$^{+}$ *Lachnospiraceae* and *Oscillospiraceae* in adults with non-severe, non-recurrent CDI. Furthermore, we associate the abundance of these bacterial taxa with alterations in microbial bile acid biotransformation, a key mechanism of colonziation resistance to CDI. I hypothesized the observed increased abundance of Lachnospiraceae and Oscillospirace was due to phylogenetically distinct variations in the IBZ-binding pocket of PolC that decreased their antibiotic susceptibility, elevating their abundance in the human gut

microbiome during oral IBZ treatment of CDI. Using genomics and microbiology, I associate the *Lachnospiraceae* and *Oscillospiraceae* reduced susceptibility to IBZ with their naturally occurring amino acid substitutions near the IBZ-binding pocket of their PolC not found across the majority of the Bacillota phylum. Furthermore, these non-synonymous mutations are not found in publicly available genomes of globally circulating *C. difficile*. Together, these findings suggest IBZ for the treatment of CDI will not only inhibit the growth of *C. difficile*, but also restore colonization resistance via increased abundance of *Lachnospiraceae*- and *Oscillospiraceae*-mediated bile acid metabolism.

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List of Common Abbreviations

Abbreviation	Definition
CDI	*Clostridioides difficile* infection
PolC	PolC-type DNA Polymerase III alpha-subunit
DnaE	DnaE-type DNA Polymerase III alpha-subunit
IBZ	Ibezapolstat
1° BA	Primary Bile Acid
2° BA	Secondary Bile Acid
SCFA	Short-Chain Fatty Acid
MIC	Minimum Inhibitory Concentration
CFU	Colony Forming Units
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
NGS	Next-Generation Sequencing
LC–MS	Liquid Chromatography–Mass Spectrometry
ASV	Amplicon Sequence Variant
OTU	Operational Taxonomic Unit

Preface

I am the product of my father's social experiment to create a clinician-scientist through early childhood exposure to medical drama and science-fiction pop-culture. From an early age I was exposed to educational and entertaining media such as Bill Nye, the Science Guy (1993-1999), the Crocodile Hunter (1996-2004), House M.D. (2004-2012), Iron Man (2008), The Big Bang Theory (2007-2019), Contagion (2011), and the Martian (2015). What you may observe from this list is the *absence* of a specific direction towards any one of the four realms of science, technology, engineering and medicine (STEM). This was not by design, but rather my natural inclination. Following my college studies of biology, life steered me towards a particular topic of great interest to me that is more relevant than ever today – infectious diseases. I find a deep sense of purpose and meaning in the reduction and elimination of infectious diseases, not only to help those suffering today, but also to protect those who may face these threats tomorrow and forever on.

I chose to study *Clostridioides difficile* infection (CDI) for its burden, and scientific position at the intersection of the antimicrobial resistance crisis and the human gut microbiome revolution. This work highlights my efforts in The Garey Lab studying the PolC inhibitor, ibezapolstat (IBZ), a Gram-positive selective spectrum (GPSS) antibiotic in development for the treatment of CDI through a microbiome-sparing mechanism. Although not new to the literature, I grew to appreciate the critical role of the *'unculturable'* microbiota, Lachnospiraceae and Oscillospiraceae, for their protective role in biotransformation of host-derived bile acids and fermentation of dietary fiber to short-chain fatty acids (SCFA) against CDI.

I initially set out to determine the intra-Bacillota differences in PolC structure and function using electron cryogenic microscopy (cryo-EM) and rapid quench flow (RQF) enzyme kinetics. In particular, a focus would be given to the PolC of *C. difficile*, *Clostridium leptum* (representative species of Oscillospiraceae), *Blautia coccoides* (representative species of Lachnospiraceae). However, I stumbled many times at both trivial and non-trivial wet-lab steps. I recommend trainees seek mentors with direct hands on experience, and not attempt to accomplish these difficult methods on their own. I have learned through these failures the importance of experience in mentorship of scientists.

In light of these setbacks, I sought a different path: rather than focus on data generation, I sought to focus on data *analysis*. I reasoned this shift from upstream to midstream effort was still aligned with Ackoff's model of $data \Rightarrow information \Rightarrow knowledge \Rightarrow wisdom$. I initially struggled to move beyond the experimental foundations of Receptor Theory into areas not traditionally taught to pharmacists or pharmacologists, such as genomics, metagenomics, and metabolomics. Over time, as I identified my strengths, I became less constrained by these artificial boundaries between biological disciplines, focusing instead on the structure and visualization of data. Accordingly, throughout this document it can be assumed that my direct experimental contributions were minimal. My primary responsibilities lay in data analysis—curating both public and private datasets, writing and sharing reproducible code, and generating figures and datasets. This shift reflected a natural adaptation of my strengths and limitations to the needs and context of the study.

Chance favors the prepared mind.

– Louis Pasteur

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Introduction

Clostridioides difficile infection (CDI)[1–83] is the quintessential infectious disease at the intersection of the human gut microbiome[84–183] and the antimicrobial resistance crisis.[184–225] The obligate anaerobe,[226–228] *C. difficile*, is a toxigenic,[229–257] biofilm-producing,[258–298] spore-forming,[299–311] Gram-positive[312,313] pathobiont that germinates in the bile acid-rich upper duodenum,[314–322] and exploits the antibiotic-mediated depletion of commensal and symbiotic microbiota that otherwise confer host-protective mechanisms referred to as colonization resistance.[323–407] The current guideline-recommended[408] antibiotics for the treatment of CDI, vancomycin[408–439] and fidaxomicin,[440–465] have their limitations including the clinical efficacy of the former, the cost of the latter, and the early warning signs of resistance to both.[466–484] To address these concerns, continued investment into narrow-spectrum antibiotic development is warranted. To this end, the Centers for Disease Control and Prevention designates *C. difficile* as an Urgent threat.[75] However, the World Health Organization has failed to designate toxigenic *C. difficile* as a bacterial priority pathogen, raising concerns that investments in narrow-spectrum therapeutic development are sub-optimal.[83,184] Together, these concerns have renewed the public call for investment into narrow-spectrum antibiotics that spare (or even – restore) the human gut microbiome in the treatment of CDI.[73]

One particular target for the development of narrow-spectrum antibiotics is DNA replication. Although fluoroquinolones inhibit the DNA gyrase of bacteria upstream of the DNA replication fork, they are clinically considered broad-spectrum, associated with massive microbiome disruption, and have a highly

evolvable target, the *gyrA*-encoded DNA gyrase subunit A.[485] On the other hand, the essential catalytic subunit of the DNA replisome, the DNA polymerase III α-subunit (Pol III), has a few fundamental features for the development of narrow-spectrum antibiotics.[486,487] While most bacteria conduct template-directed DNA synthesis via the DnaE-type DNA polymerase III alpha-subunit (DnaE) encoded by the genes *dnaE1*, *dnaE2*, or *dnaE3*, a limited number of bacteria, including the *Bacillota* to which *C. difficile* belong, utilize the PolC-type DNA polymerase III alpha-subunit (PolC) encoded by the *polC* (formerly *dnaF*) gene. The evolutionarily distinguishing feature between the PolC and DnaE is the insertion of a *dnaQ* homolog (ε-subunit) within the polymerase and histidinol phosphatase domain of a *dnaE* in what is referred to as the "ancient *dnaE* hypothesis."[488] Critically, this ancient genetic event predates the radiance of bacteria, thus limiting the *polC* to *Bacillota*, *Fusobacteriota*, and *Mycoplasmatota*; importantly, it is not found in the genomes of *Actinomycetota*, *Bacteroidota*, or *Pseudomonadota*. Finally, not only is the PolC distinct from the DnaE, it is more highly conserved at functionally critical residues – indicating a more precise machine less tolerant to amino acid substitutions from non-synonymous mutations.[489]

As a consequence of the phylogenetically restricted evolution of the *polC*, the pre-clinical development of PolC-selective inhibitors led to the small molecule compound, ibezapolstat (IBZ, ACX-362E, 7-MorEDCBG), to be further evaluated for its candidacy as a therapeutic drug for the treatment of CDI that may spare the microbiota. Critically, IBZ is microbiologically active against *Bacillota*, including *C. difficile*, and inactive against *polC*$^{-}$ *Bacteroidota*, *Actinomycetota*, and *Pseudomonadota*. However, there exists not only pathogenic/pathobiont but also commensal/symbiotic *polC*$^{+}$ *Bacillota* that are critical mediators of colonization resistance to CDI. Specifically, *polC*$^{+}$ *Bacillota* sub-taxa – *Lachnospiraceae* (Clostridium cluster XIVa, or [Cl. coccoides] group) and *Oscillospiraceae* (formerly *Ruminococaceae*, Clostridium cluster IV, or [Cl. leptum] group) are critical mediators of colonization resistance to CDI via microbial biotransformation of host-derived bile acids and fermentation of dietary fiber to short-chain fatty acids that inhibit *C. difficile* growth and CDI recurrence. Because these host-protective families of bacteria, *Lachnospiraceae* and *Oscillospiraceae*, were historically referred to as the *unculturable* microbiota, they were not previously characterized for their susceptibility to IBZ. To address this scientific gap in the literature and unmet medical need, we identify the changes of the human gut microbiome associated with

orally administered IBZ for CDI, with attention to the *Lachnospiraceae* and *Oscillospiraceae* that play a critical role in bile acid biotransformation.

Bacterial DNA Replication

DNA replication is the fundamental biological process that ensures the accurate transmission of genetic information across generations of daughter cells for the inheritance of the central dogma of biology.[490–493] The earliest discoveries of enzymes that coordinate DNA replication were conducted in bacteria, including the model Gram-negative bacterium, *Escherichia coli*. For instance, in 1956, Arthur Kornberg discovered the first DNA polymerase capable of enzymatic DNA biosynthesis in *E. coli*, which he named DNA Polymerase I (Pol I) shared with Severo Ochoa the 1959 Nobel Prize in Physiology or Medicine.[494–497] In 1970, Thomas Kornberg (Arthur Kornberg's son) and Malcolm Gefter discovered the DNA Polymerase II (Pol II) and Pol III, also in *E. coli*.[498–500] For these reasons, Arthur Kornberg is generally considered the father of DNA replication.[501] In parallel to the discovery of Pol I, Pol II, and Pol III from *E. coli*, that of the model Gram-positive bacterium, *Bacillus subtilis*, were identified by Gass and Cozzarelli.[502] Eventually, the discovery of several types of DNA polymerases with nuanced differences led to their classification into families – A, B, C, D, X, and Y – based on phylogenetic sequence differences that confer their distinct structures and functions.[503–505] Eventually, subtle differences between Pol III of *E. coli*,[506] *B. subtilis*,[507] and *Staph. aureus*[508] would spark further curiosity into their subtle differences in structure and function. The Pol III would later be classified as a C-family DNA polymerase and recognized as the catalytic alpha (α)-subunit of the bacterial DNA replisome.[509]

It's important to remember these DNA polymerases do not function in isolation, but rather as a subunit of a larger multi-subunit "machine of machines" known as the DNA replisome.[510–513] Due to the importance of high fidelity DNA replication, the components of the DNA replisome are present across the tree of life.[514] The **DNA replisomes of bacteria** generally consist of either 12 or 13 subunits that begin their assembly at a single position on a bacterial chromosome, referred to as the OriC. The α **(alpha) subunits** of bacterial DNA replisome, the DNA-directed DNA polymerases (often simply referred to as DNA

polymerases), enzymatically incorporate nucleotides in (5' -> 3') leading- and (3' -> 5') lagging-strand DNA synthesis; the β_2-clamp (beta-clamp) re-anneals newly synthesized DNA strands to their template, forming the double-stranded DNA (dsDNA) duplex; the $\tau_3\delta\delta$' (gamma-complex, or clamp-loader complex [CLC]) loads the beta-clamp onto the DNA replisome and is comprised of 3:1:1 stoichiometry of subunits τ (tau), δ (delta), δ' (delta-prime); the ε (epsilon) proofreading exonuclease stabilized by the θ (theta) subunit is bound to the α subunit for the removal of misincorporated nucleotides; accessory subunits ψ (psi) and χ (chi) that mediate interactions with single-stranded binding (SSB) proteins on the lagging-strand trumbone of single-stranded DNA (ssDNA); DnaB (DNA helicase) separates dsDNA and forms the classical "replication fork;" DnaG (DNA primase) is a DNA-directed RNA polymerase that *de novo* synthesizes Okazaki fragments[515] to provide the essential 3' hydroxyl primer for a lagging-strand DNA synthesis. However, while the DNA replisomes differ considerably across different branches of life in their components, assembly, and function, they generally possess the essential catalytic subunit, the DNA polymerase.[509]

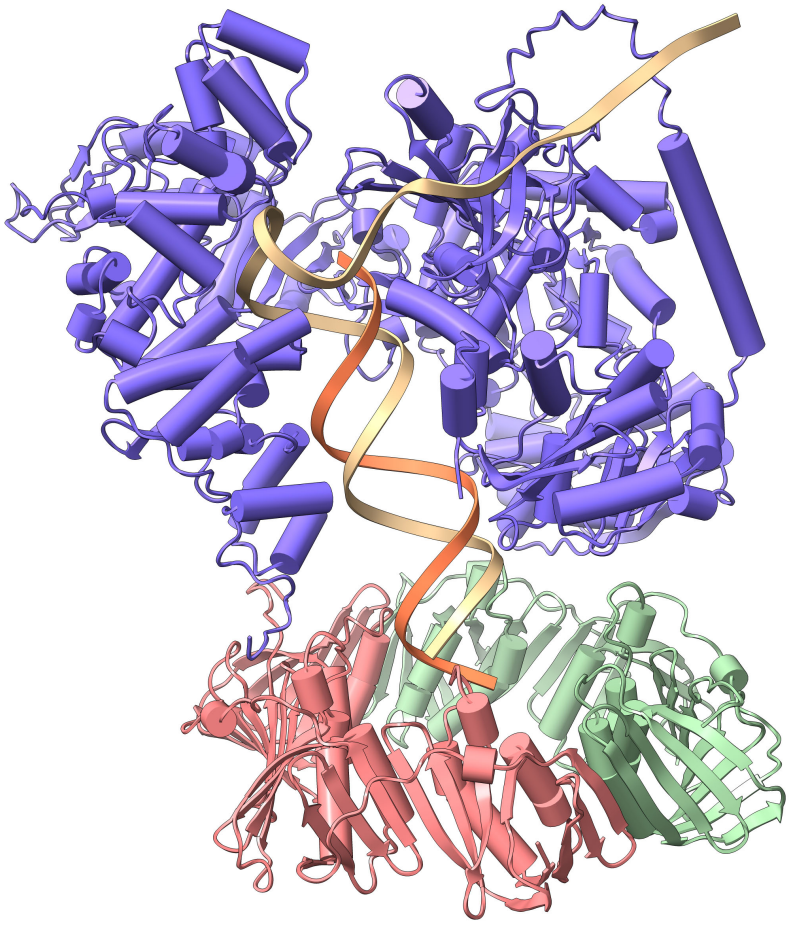
The PolC-type DNA Polymerase III alpha-subunit

The evolutionary history, divergence, and phylogenetic distribution of Pol III plays an essential role to the development of GPSS antibitoics. In *E. coli* strain K12, the Pol III is an 1,160 amino acid protein encoded by the *dnaE* gene.[516–518] Likewise, there exists a *dnaE* gene in *B. subtilis* that encodes a Pol III of comparable sequence, structure, and function: the *dnaE* gene of *B. subtilis* strain 168 encodes a 1,115 amino acid Pol III that also performs 5' – 3' (forward) DNA synthesis, but lacks 3' – 5' proofreading exonuclease activity.[519] However, unbeknownst to researchers at the time was the ancient evolutionary divide between *E. coli* of *Pseudomonadota* and *B. subtilis* of *Bacillota*: **the *polC* gene of low-GC, Gram-positive bacteria (Bacillota) encodes a second essential Pol III with a distinguishing *dnaQ* homolog insertion within the polymerase and histidinol phosphatase (PHP) domain**.[520–524] The *polC*-encoded PolC was identified in related low-GC, Gram-positibe bacteria, including *Staphylococcus aureus*[525] *Enterococcus faecalis*,[526] *Streptococcus pyogenes*,[527] and *Clostridioides difficile*. To distinguish these two Pol IIIs, I choose to use their gene – protein nomenclature: *dnaE* – the DnaE-type DNA Polymerase III alpha-subunit

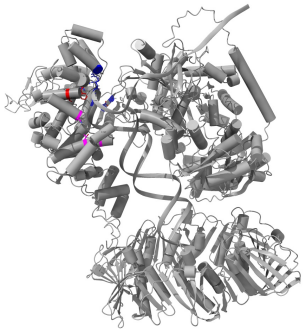
(DnaE); *polC* – the PolC-type DNA Polymerase III alpha-subunit (PolC).[528]

The earliest works in the PolC-type DNA polymerase III alpha (α)-subunit were conducted in the model Gram-positive bacterium, *B. subtilis*. The literature sometimes interchangeably used the terms *dnaF* and *polC* not only due to differences in methodological approach, but also a critical distinction between *B. subtilis* and *E. coli*. Methodologically, temperature-sensitive mutants with impaired DNA replication had mutations that mapped to the *dnaF* locus,[529–531] whereas strains with PolC inhibitor resistance had mutations that mapped to the *polC* gene.[532–539] Perhaps the contemporary naming of genes created confusion when it was unclear whether Pol III-related genes in *B. subtilis* would either sequentially follow that of *E. coli* (*dna**E*** of *E. coli*, and *dna**F*** of *B. subtilis*), or the sequential discovery of Pol I, II and III as *pol**A***, *pol**B***, *pol**C***. The improved understanding of the PolC encoding region would follow investigations into HPUra-resistant azp-12 laboratory strain of *B. subtilis* that possessed a T-to-G transversion in the *polC* gene.[540–543] Not long thereafter, the *B. subtilis polC* gene would be cloned,[543] confirmed by sequencing,[520–522] and its encoded PolC – purified.[522]

To maintain clarity, the literature and antibiotic development effort would greatly benefit from unification of the nomenclature to the *polC* gene and PolC protein. It is highly advisable to not misconstrue the **"C"** between the C-family and PolC, as this can potentiate confusion. It is recommended that nomenclature avoid the use of "Family C", "DNA Polymerase C" or "Pol IIIC" in writing. Additionally, it should be noted that DnaE-type Pol IIIs encoded by *dnaE* should never be referred to as a PolC, PolC-type or DNA Polymerase C to denote their classification to the C-family of DNA polymerases. Furthermore, the *dnaE* genes have been further sub-divided into the classifications as *dnaE1*, *dnaE2*, and *dnaE3*.[489,544] Finally, the bioinformatic databases sometimes use *dnaF*, or "Gram-positive type;" both terms are generally considered outdated and advised against. In summary, these recommendations aim to clarify the nomenclature as the *polC*-encoded PolC-type DNA polymerase III alpha-subunit (PolC) and the *dnaE*-encoded DnaE-type DNA polymerase III alpha-subunit (DnaE).



(a)



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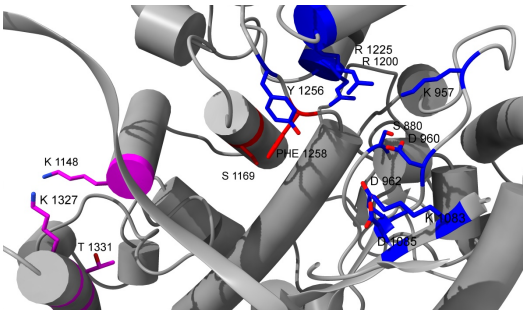

(c)

Figure 1.1: *C. difficile* PolC·β_2·dsDNA complex. (a) The *C. difficile* PolC in complex with doule-stranded DNA and the beta-clamp homodimer, generated by AlphaFold3 homology modeling[545–549]. (b) macroscopic view of residues of interest; (c) microscopic view of residues of interest: lachnospiraceae/oscillospiraceae variant (pink), associated with PolC inhibitor resistance (red),[550,551] and the catalytic residues (blue).[552]

Structure

The structure of the PolC-type DNA polymerase III alpha-subunit (PolC) was determined by Evans *et al* from that of *Geobacillus kaustophilus* (PolC^{Gka}) using x-ray cyrstallography.[552] They found the PolC^{Gka} forms the classical right-handed structure of a DNA polymerase, including the fingers, palm, and thumb that "wrap" around DNA. The prominent domains of the PolC include the oligonucleotide binding (OB) domain, the polymerase and histidinol phosphatase (PHP) domain, the fingers-, palm- and thumb domains, the duplex bindingβ-binding domain, and the proofreading 3' -> 5' exonuclease (Exo) domain.The similarities and differences between the structures of PolC- and DnaE-type DNA polymerase III alpha-subunits have been previously reviewed.[553] Crucially, **the distinguishing feature of the PolC that differentiates it from the DnaE is the presence of an Exo domain inserted within the Polymerase and Histidinol Phosphatase (PHP) domain**.[488] This exo domain is evolutionarily related to the *dnaQ*-encoded ε (epsilon)-subunit of *E. coli*. Another distinguishing feature of the PolC is the N-terminal domain (NTD), which phylogenetic studies suggest originates from the V domain of the DNA polymerase III τ (tau) subunit.[554]

Function

DNA replication relies on the formation of a phosphodiester bond between the nucleophilic 3' hydroxyl of the terminal nucleotide on the growing single-stranded DNA and the electrophilic alpha (α)-phosphate of an incoming deoxyribonucleotide triphosphate (dNTP).[555,556] This reaction occurs within the active site of DNA polymerases, coordinated by Chargaff's rules of base pairing with the DNA template strand,[557] acidic residues such as aspartate and glutamate that stabilize the catalytic environment,[558] and divalent cations (e.g., Mg^{2+}) that facilitate pyrophosphate departure.[556]

Although the fundamental catalytic mechanism is conserved across DNA polymerases[559], subtle structural differences among enzymes influence replication fidelity ,[509,560–562] base substitution error rates, and consequently, evolutionary rates[563]. The structural and functional relationships that govern template-dependent nucleotide incorporation have been reviewed.[509,564] Mechanistically, DNA and RNA poly-

merases share a common catalytic strategy for template-directed nucleotide addition[555], but differ in substrate discrimination between deoxyribonucleotides (dNTPs) from ribonucleotides (NTPs) based on the ribose 2' hydroxyl group.[565–568] DNA polymerases also require the ribose to adopt the 3'-endo sugar pucker conformation to correctly position the 3' hydroxyl for catalysis.[569] This combination of proper orientation of the absence of a 2' hydroxyl, the 3'-endo hydroxyl, and nitrogenous base facilitates the pyrophosphorolysis at the alpha (α)-phosphate, allowing for the metal-coordinated extrusion of the beta- (β)/gamma (γ)-inorganic pyrophosphate (PP_i). Additionally, accurate base pairing according to Chargaff's rules (A –- T/U, G –- C) ensures fidelity in template recognition.[557] These combined elements—ribose conformation and base identity—are coordinated through an induced-fit mechanism that underlies substrate specificity and high-fidelity DNA (and RNA) synthesis.[570]

Current evidence supports **the function of the PolC is to replicate the chromosomal DNA at both leading- and lagging-strands of DNA**.[507,510,571–573] Early observations noted the contribution of DnaE to lagging-strand DNA synthesis; from this, early hypotheses inferred the PolC was responsible for only leading-strand DNA synthesis.[524] However, recent inveestigations into the dynamics of *B. subtilis* DNA replisomes suggest the DnaE (encoded by *dnaE3*) synthesizes a short stretch of lagging-strand DNA synthesis, following the DnaG primase, before a dynamic exchange and handoff to PolC;[507,510,571–573] Third, there is evidence to support the essentiality of the β-subunit, sometimes referred to as the processivity factor, not only increases the affinity of PolC to DNA, but also the rate of polymerization.[574,575] Taken together, these data support the PolC coordintes both leading- and lagging-strand DNA synthesis in the DNA replisome of *B. subtilis* and related bacteria.

The complete kinetic cycle of the *Staph. aureus* PolC ($PolC^{Sau}$) has been previously elucidated by the Pata lab using rapid quench flow enzyme kinetics.[550,576–578] They showed the first step of the ${}_{Sau}PolC$ complete kinetic cycle – the binding to DNA and formation of the $PolC \bullet DNA_n$ complex – occurs with a calculated K_D^{DNA} of 0.18 μM; the second step – nucleotide binding (*e.g.* dGTP) and formation of the $PolC \bullet DNA_n \bullet dNTP$ complex at the template position (T_0) – occurs with a calculated K_D^{dGTP} of 14.4 μM; the third step – phosphodiester bond formation[556,579] – occurs with a k_{pol} (rate of polymerization) of approximately 1,200 s^{-1},[550,578] and dGTP-specific k_{pol}/K_D^{dGTP} (catalytic efficiency) of 92 $\mu M^{-1}s^{-1}$, increas-

ing the length of the DNA by +1 in the complex $PolC \bullet DNA_{n+1} \bullet PP_i$. Following phosphodiester bond formation, the release of the PP_i (inorganic pyrophosphate) is initially slow, approximately 20 s^{-1}, much too slow for reported processive rates s^{-1}. Uniquely, the Pata lab reported the rate of PP_i release is increased to as high as 1,230 s^{-1} by the next incoming, correctly paired nucleotide $(PolC \bullet DNA_{n+1} \bullet PP_i \bullet dNTP)$.[578] The investigators posit the next incoming dNTP displaces the $\beta-, \gamma-$phosphate and Mg^{2+} at the catalytic aspartate residues of the active site. Finally, the translocation of the polymerase to the next template position (T_{+1}) requires the bonds between the $PolC \bullet DNA_{n+1}$ to be broken and reformed; However, how the finger domain transitions between the 'open' or 'closed' state to facilitate this increased processive speed remains yet to be elucidated. To analogize, the weak affinity between the PolC and DNA is not similar to the European locomotive freight train that exerts a significant force on its tracks, but rather the Japanese bullet train that hovers just 10 cm above its tracks using magnetic levitation and more smoothly propels itself forward using momentum rather than force.[580]

Mutations that result in target modifications is a major mechanism of antimicrobial resistance.[199] For example, the genetic changes associated with PolC inhibitor resistance was first reported in the HPUra-resistant laboratory strain of *B. subtilis*: $polC^{Bsu^{azp\text{-}12}}3523^{T\Delta G} \Rightarrow \underline{T}CA\Delta\underline{G}CA \Rightarrow 1175^{Ser\Delta Ala}$.[543,551,581] More recently, investigators have uncovered the genetic and functional consequences of *polC* mutations that confer resistance to 3 -ethyl-4'-methylanilino)uracil (EMAU), a chemical class of PolC inhibitors. Previous reports indicate the emergence of *polC* mutations that confer resistance, such as $PolC^{1261Phe\Delta Leu}_{Sau}$, occur with a frequency of 7.4 $\times 10^{-10}$. This mutation, in particular, was associated with >13- to 26-fold increased minimum inhibitory concentrations (MICs) and >1,000-fold increased K_i (apparent inhibition constant) to 3(4-hydroxybutyl)-6-(3-ethyl-4-methylanilino)uracil (HB-EMAU).[582] More recently, the related compound of 2-methoxyethyl-6-(3 -ethyl-4'-methylanilino)uracil (ME-EMAU) has a calculated $K_D^{ME-EMAU}$ (dissociation constant of ME-EMAU) of 0.014 μM to $PolC_{Sau}$; The HB-EMAU resistant $polC^{Sau^{Smith}}1261^{Phe\Delta Leu}$ confers a 1.6-fold reduction in K_D^{DNA} (DNA binding), a 5.2-fold reduction in K_D^{dGTP} (dGTP binding), an 8.5-fold reduction in k_{pol} (rate of polymerization), and a 1.6-fold reduction in k_{pol}/K_D^{dGTP} (dGTP catalytic efficiency).[550] These very low rates of non-synonymous mutations in the *polC* that are associated with reduced PolC binding and inhibition by PolC inhibitors, but with markedly elevated fitness defects, suggest

the PolC is not only an essential enzyme but also potentially promising antibiotic drug target.

CAN POLC BE A TARGET FOR NARROW-SPECTRUM ANTIBIOTICS?

Evolution

The quest to understand the origins of life generally involves the comparative genomics analyses of DNA replication in the oldest form of life – bacteria. However, while much of our current knowledge on bacterial DNA replication stems from experiments in the model Gram-negative bacterium, *Escherichia coli*, some literature suggests *Bacillus subtilis* of *Bacillota* (formerly *Firmicutes*), the most ancient form of bacterial life, might serve as a better model to understand the evolutionary origin of DNA replication.[489,583] Across most bacteria, the Pol IIIs are encoded by *dnaE* genes, sub-typed as either *dnaE1*, *dnaE2*, or *dnaE3*. However, there does exist the distinct divergence of the *polC*, found in *Bacillota*, *Fusobacteriota*, and *Mycoplasmatota*.[489] For our purposes – **where did the *polC* come from?** The origin of the *polC* gene is thought to be a *dnaE* homolog that evolved in what is referred to as the "ancient dnaE hypothesis." Bacterial comparative genomic studies between *E. coli*, *B. subtilis* and more by Koonin and Bork provided the early observations into the key similarities and differences between the *polC* from the *dnaE*, leading to what is known as the **"ancient dnaE hypothesis"** which generally states the *polC* diverged from the ancestor homolog dnaE, but with the distinguishing feature of an inserted exonuclease domain from a *dnaQ* homolog of the epsilon subunit.[488] A larger analysis of approximately 2,000 bacterial genomes by Timinskas *et al* extended this theory further by proposing a most-probable evolutionary pathway including (1) duplication with circular permutation that moved the oligonucleotide binding (OB) to the N-terminal; (2) the insertion of the N-terminal domain upstream of the OB domain; (3) the distinguishing *dnaQ* homolog exonuclease insertion within the poylmerase and histidinol phosphatase (PHP) domain.[489]

Critically, how widespread is the *polC* across the bacteria commonly found in the human gut mi-

crobiome? The bacteria of the human gut microbiome, although varying considerably by diet, age and geography,[111,169,584] generally consists of the *Bacillota*, *Bacteroidota*, *Actinomycetota* and *Pseudomonadota*.[115,139,585,586] The analysis by Timinskas *et al* showed not only the conservation of *polC* across *Bacillota*, but also its absence from *Bacteroidota*, *Actinomycetota* and *Pseudomonadota*.[489] Notably, the *polC* is also found in *Fusobacteriota* and *Mycoplasmatota*, but these taxa do not typically achieve relative abundances in the colon of healthy individuals or adults with CDI.

The presence of *polC* in one of the four bacterial phyla provides the basis for narrow-spectrum antibiotic drug development. While this is correct, it is worth noting the *Bacillota* comprise a considerable and diverse proportion of the human gut microbiota, with some of the most prominent taxa including classes – *Bacilli* and *Clostridia* [*p_Bacillota*]; orders – *Bacillales*, *Lactobacillales* [*c_Bacilli*]; *Eubacteriales*, *Lachnospiralales*, and *Erysipelotrichales* [*c_Clostridia*]; families – *Bacillaceae*, *paenibacillaceae*, and *Staphylococcaceae* [*o_Bacillales*]; *Enterococcaceae*, *Lactobacillaceae*, and *Streptococcaceae* [*o_Lactobacillales*]; *Clostridiaceae*, *Peptostreptococcaceae*, *Oscillospiraceae* [*o_Eubacteriales*]; *Lachnospiralales* [*o_Lachnospiraceae*]; *Erysipelotrichaceae*, *Coprobacillaceae* [*o_Erysipelotrichales*].

Many microbiologists and clinicians are trained and aware of the classical species of *Bacillota*, such as the *B. subtilis* of *Bacillaceae*, *Staph. aureus* of *Staphylococcaceae*, *E. faecium* of *Enterococcaceae*, *Strep. pneumoniae* of *Streptococcaceae*, and *C. difficile* of *Peptostreptococcaceae*. However, the previously "unculturable" microbiota,[587] largely including the *Lachnospiraceae* and *Oscillospiraceae*, are not only difficult to culture, but also difficult to phylogenetically assign, as evidenced by the polyphyletic genera of [*Ruminococcus*] and [*Clostridium*]. For example, [Cl.] butyricum [*f_Clostridiaceae*], [Cl.] *scindens* [*f_Lachnospiraceae*], and *Thomoaslcavelia ramosa* [*f_Coprobacillaceae*] (formerly [Cl.] *ramosum*) can be phylogenetically assigned to distinct sub-taxa of *Bacillota*. Likewise, so can *Mediterraneibacter gnavus* [*f_Lachnospiraceae*] (formerly [Ru.] *gnavus*), [Ru.] *torques* [*f_Lachnospiraceae*], and [Ru.] *bromii* [*f_Oscillospiraceae*] also be phylogenetically distinct. While these taxonomic and nomenclature changes may pose a challenge, they should not deter their study for the importance of their role in colonization resistance to pathobionts of the human gut microbiome, such as *C. difficile*. These taxa are not only difficult to study and important to the treatment of CDI, but also potentially innocent bystanders

depleted by PolC inhibitor therapy.

The aforementioned *Lachnospiraceae* and *Oscillospiraceae* specifically contribute to the host-protective mechanisms of colonization resistance to CDI through biotransformation of host-derived bile acids and fermentation of dietary fibers to short-chain fatty acids. However, they too possess the *polC*, making them potentially innocent bystanders of PolC inhibitors that aim to eliminate *C. difficile* and treat CDI. The susceptibility of *Lachnospiraceae* and *Oscillospiraceae*, once referred to as the unculturable microbiota,[587] to PolC inhibitor-mediated depletion remains yet to be fully elucidated, and the primary objective of my works described herein. The susceptibility of these taxa to PolC inhibitors was not previously studied as the development of PolC inhibitors ***pre***dates their culturability.[487,587] What you may be surprised to learn is that these taxa *may* be less susceptible to a PolC inhibitor, ibezapolstat, through amino acid substitutions in the PolC active site that leads to reduced susceptibility *in vitro* and expansion *in vivo*.[588–590] Furthermore, these amino acid substitutions that *may* confer reduced susceptibility in *Lachnospiraceae* and *Oscillospiraceae*, are devoid from thousands of publicly available *C. difficile* genomes, suggesting the future efficacy of IBZ for the treatment of CDI.

THE POLC HAS A UNIQUE EVOLUTIONARY DIVERGENCE. SO WHAT?

Honestly, why should a pharmacologist care about the ancient evolutionary origins of DNA replication? **"Just tell me if the drug worked or not."** While this is the common quip of the busy clinician, the drug development pipeline has a twenty-first century duty to develop antibiotics not only with activity against pathogens, but also inactivity against the commensal and symbiotic bacteria of the human gut microbiome.[73] Historically, antibiotic targets were evolutionarily conserved across bacteria and devoid from (or considerably divergent in) the genomes of eukaryotic mammalian lineages to limit undesirable toxicities. These might include the beta-lactam antibiotics that target bacterial cell wall biosynthesis, likely contributing to the general tolerability of these class of antibiotics, with some exceptions. **However, understanding the phylogenetically distribution and divergence of an antibiotic target across bacteria**

that comprise the human gut microbiome is critical to the development of narrow-spectrum antibiotics. In the setting of *C. difficile* infection, the inactivity of antibiotics against the *Lachnospiraceae* and *Oscillospiraceae* is of critical importance to the promotion or restoration of colonization resistance via bile acid biotransformation and dietary fiber fermentation. Hence, understanding the evolutionary history of the *polC* is critical to the development of Gram-positive selective-spectrum (GPSS) antibiotics that not only treat CDI but also preserve or perhaps even restore colonization resistance to CDI.

The phylogenetically restricted evolution of the PolC-type DNA polymerase III alpha-subunit (PolC) across the bacteria of the human gut microbiome has led to the development of the first-in-class small molecule PolC inhibitor, ibezapolstat (IBZ), for the treatment of Clostridiodies difficile infection (CDI).[486,487] The gene of the target, *polC*, is generally limited to the *Bacillota* and devoid from the genomes of *Actinomycetota*, *Bacteroidota*, and *Pseudomonadota*. Together, these four bacterial phyla comprise the majority of bacteria in the human gut microbiome, and support the hypothesis that PolC inhibitors would be narrow-spectrum antibiotics for the treatment of *polC*$^{+}$ *Bacillota* infectious diseases caused by *Staph. aureus*, *E. faecium/faecalis*, and *C. difficile*.[489] Similar to the phylogenetically distinct susceptibility of human gut bacteria to fidaxomicin,[368] an RNA polymerase inhibitor for the treatment of CDI,[447,451,454] we anticipated IBZ would be a ***more***-narrow spectrum antibiotic that preserves even more bacteria of the human gut microbiome.[73,589,591,592]

To fully appreciate the metagenomic spectrum of activity of IBZ, a deeper understanding of the phylogenetic distribution and divergence of the *polC* is essential. While the *polC*$^{+}$ may be devoid from three of the four bacterial phyla that comprise the majority of the human gut microbiome, the divergence of *polC* across the sub-taxa of *Bacillota* remains poorly understood. For instance, the *Bacillota* families of *Lachnospiraceae* and *Oscillospiraceae* are critical mediators of host-protective colonization resistance against the human gut pathobiont, *C. difficile*, via microbial biotransformation of host-derived bile acids and fermentation of dietary fiber to the four-carbon SCFA, butyrate, may be further innocent bystanders of PolC inhibitor-mediated depletion. The loss of these taxa and their host-protective mechanisms as a result of other antibiotic classes have been associated with loss of colonization resistance to CDI.[366,400,401]

We originally anticipated the IBZ-mediated depletion of *polC*$^{+}$ *Lachnospiraceae* and *Oscillospiraceae*,

and potentially the loss of their host-protective mechanisms. However, the *in vitro* antibiotic susceptibility of these taxa have not been thoroughly evaluated due to their difficulty to culture, earning the well-deserved name of the (previously) "unculturable microbiota."[587] Furthermore, the microbiome recovery following antibiotic exposure is considered a complex phenomenon that is individualized to an individual's baseline composition, diet and environment.[35,359,593–595] While these were valid considerations, we were surprised to observe IBZ increased the relative abundance of *Actinomycetota* in healthy adults,[588] and *Lachnospiraceae* and *Oscillospiraceae* in CDI patients.[589] From these observations, we asked **"can IBZ increase the colonization resistance to CDI via restoration of the human gut microbiome, principally the *Lachnospiraceae* and *Oscillospiraceae* that play a critical role in colonization resistance to CDI?"**

Herein we describe the taxonomic differences in IBZ pharmacophore across a selection of commensal microbiota, *polC*$^{+}$ *Lachnospiraceae* and *Oscillospiraceae*, that was not previously studied; while IBZ has activity for *C. difficile*, these investigations described herein suggest it might be possible to explore the differences in drug binding pockets between pathogens and symbionts to not only spare- but possibly restore the human gut microbiome. The chapters of this work describe the impact of IBZ on the metagenomics and metabolomics of the human gut microbiome (Appendix B), the mechanism of restoration of the human gut microbiome through amino acid differences in the PolC active site of *Lachnospiraceae* and *Oscillospiraceae* (chapter 4), and the absence of predicted PolC inhibitor resistance across globally circulating strains of *C. difficile* (chapter 5).

Ibezapolstat

The development of selective PolC inhibitors were instrumental in the determination of the *polC* gene encoding the PolC-type DNA polymerase III alpha-subunit (PolC), made possible by 6(p-Hydroxyphenylazo)-uracil (HPUra).[502,532,581,596,597] Over time, the starting uracil moiety was modified for increased potency and selectivity to the PolC of *B. subtilis* by chemists with training in structure-activity relationships (SAR), eventually leading to substituted-anilouracils,[598,599] and anilouracil-fluoroquinolone hybrids.[600,601] However, a few limitations of these pyrmidine mimetics, including including poor clinical response, associated

toxicity, and antibiotic resistance shifted the focus of attention to the development of guanine (purine) mimetics. Beginning with an N2-(3 ,4 -dichloro)-benzyl-guanines (DCBGs) The addition of a morpholino group to the N7 position, yielded the N7-morpholinobutyl-DCBG (7-MorBDCBG). Notably, this final synthesis reaction of the 7-MorBDCBG also produces an ethyl-linked N7-substituted morpholino DCBG, the N7-morpholinoethyl-DCBG (7-MorEDCBG), also referred to as ibezapolstat (IBZ; ACX-362E). Following the SAR development of N2,N7-substituted guanines for improved potency and selectivity, one compound become a lead compound for the treatment of a gastrointestinal disease, *Clostridioides difficile* infection (CDI), that required a narrow-spectrumm antibitoic: ibezapolstat (IBZ).[487]

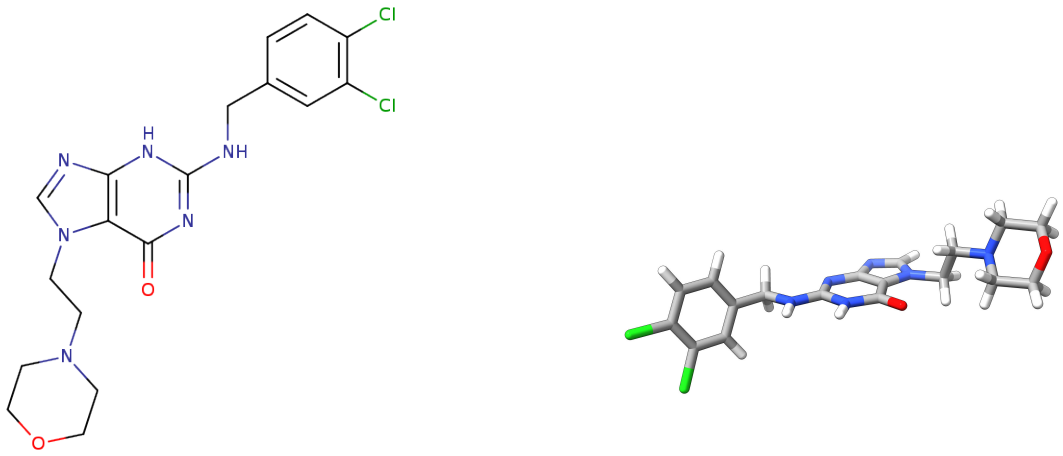

Figure 1.2: Ibezapolstat (IBZ). IUPAC name: 2-[(3,4-dichlorophenyl)methylamino]-7-(2-morpholin-4-ylethyl)-1H-purin-6-one. Image obtained August 12, 2025 from DrugBank[602] by CC BY-NC 4.0. Identifiers: DrugBank DB16189; NCBI PubChem 136022209; EMBL-EBI ChEMBL4571518; IUPHAR/BPS Ligand 11030.

Ibezapolstat (IBZ, PubChem 136022209; Figure 1.2), is a moderately lipophilic (2.23 consensus $LogP_{O/W}$[603]) poorly water soluble (0.216 mg/mL, -3.3 $AlogP_S$)[604] small molecule with a molecular weight of 423.3 grams/mol (computed by PubChem 2.2[605,606]) 6 rotatable bonds, 2 hydrogen bond donor groups, 7 hydrogen bond acceptor groups, and a topological polar surface area of 88.07 Å.[607] The chemical synthesis of IBZ has been previously described.[487] Essentially, the dichlorobenzylguanines (DCBGs) possess a guanine purine central moiety, an N2-substituted 3,4-dichlorophenyl and an N7-substituted morpholino group that increased the *in vitro* potency of inhibition of the $polC^+$ *B. subtilis* without significant alterations to that of $polC^-$ *E. coli*.[487] While the membrane permeability of IBZ across Caco-2 monolayers has not yet been explicitly studied,[608] these data suggest IBZ would be either a Class II or Class IV drug

according the biopharmaceutical classification system (BCS) of drugs.

Pharmacologically, the *in vitro* and *in vivo* efficacy of IBZ against *C. difficile* infection (CDI) has been shown across a couple notable studies. Using the Lineweaver-Burke method[609] of Michaelis-Menten kinetics,[610,611] Torti *et al* showed IBZ competitively inhibits dGTP binding to *C. difficile* PolC with an estimated *in vitro* potency of 325 nM.[612,613] Furthermore, *in vitro* studies suggest even sub-inhibitory concentrations of IBZ growth can slow the cellular replication with a presumed slowed or stalling of DNA replisomes.[614] Despite these tremendous efforts, an improved understanding of dynamics of DNA replisome stalling or stopping in response to PolC inhibitors would further aid GPSS antibiotic drug development.

Microbiologically, Dvoskin *et al* showed the *in vitro* activity against 22 clinical isolates and one ATCC strains was further evaluated according to the 2007 *ed.* of CLSI M11-A7 for the determination of antibiotic susceptibility in anaerobes, per the investigators. They reported the $MIC_{50/90}$ of these 23 strains were 2/4 μg/mL, respectively. They comment the reduced MIC_{90} susceptibilities of these isolates to vancomycin (VAN) and metronidazole (MTZ), 4 and 4 μg/mL, respectively, did not confer cross-resistance to IBZ. Murray *et al* also reported the $MIC_{50/90}$ of 2/4 μg/mL across a moderately sized collection of 104 clinical *C. difficile*.[615] Van Eijk *et al.* reported the IBZ $MIC_{50/90}$ of 2/4 μg/mL across a genetically diverse collection of 363 clinical *C. difficile* isolates from Europe.[614] Schwartz *et al* also reported IBZ $MIC_{50/90}$ of 4/4 μg/mL across their collection of clinical isolates from the eastern Mediterranean region.[616] Critically, Basseres *et al* showed IBZ is microbiologically active *in vitro* against clinical *C. difficile* isolates with high levels of resistance to VAN and fidaxomicin (FDX).[617] In summary, several lines of *in vitro* evidence suggest IBZ is microbiologically active against globally circulating *C. difficile*.

Torti *et al* also showed IBZ inhibits four strains of *C. difficile*, including ATCC 70057 with a minimum inhibitory concentration (MIC) range of 0.5 – 2 μg/mL, whereas that of *polC*$^{-}$ *Bifidobacterium brevi* ATCC 3967, *Eggerthella lenta* ATCC 1274 [p_Actinomycetota], and *Bacteroides fragilis* ATCC 0123 [p_Bacteroidota] were > 32 μg/mL. How might this increased selectivity for the PolC of *Bacillota* over the DnaE of *Bacteroidota* and *Actinomycetota* translate to a reduced impact on the human gut microbiome for the treatment of *C. difficile* infection (CDI)? To answer this question, Dvoskin *et al* also evaluated the *in vivo* efficacy of IBZ in an animal model of CDI compared to VAN.[618] They showed orally

Table 1.1: Commonly Discussed Bacteria.

Species	Family	Comment
Bacillus subtilis	*Bacillaceae*	Model Gram-**positive**
Escherichia coli	*Enterobacteriaceae*	Model Gram-**negative**
Clostridioides difficile	*Peptostreptococcaceae*	Pathobiont
Staphylococcus aureus	*Staphylococcaceae*	Pathobiont
Enterococcus spp.	*Enterococcaceae*	Pathobiont
Clostridium leptum	***Oscillospiraceae***	Clostridium Cluster **IV**
Blautia coccoides	***Lachnospiraceae***	Clostridium Cluster **XIVa**
Thomasclavelia ramosa	*Coprobacillaceae*	Clostridium Cluster **XI**

Table 1.2: Commonly Discussed Antibiotics.

Drug	Acronym	Target	Target Process	Reference
Ibezapolstat	IBZ	PolC	DNA Replication	[487]
Vancomycin	VAN	D-Ala-D-Ala	Cell-wall biosynthesis	[435]
Fidaxomicin	FDX	RpoB	RNA Transcription	[480]

administered IBZ was protective against mortality in an animal model of CDI (subcutaneous clindamycin, 15 mg/kg once; oral 0.5 $\times 10^7$CFU *C. difficile* strain ATCC 43255) with comparable efficacy to that of 50 mg/kg orally administered VAN. More specifically, how does the metagenomic composition of the microbiome change in response to IBZ *in vivo*? This question was answered by Wolfe *et al* using a microbiome-humanized animal model of CDI treated with IBZ versus VAN, FDX, and MTZ. Using 16S rRNA metagenomics, they showed IBZ and FDX have reduced impacts on the metagenomic diversity than MTZ or VAN; increased relative abundance in *polC*$^-$ Bacteroidota and Actinomycetota were observed in the IBZ-treated animals.[619] In summary, these data support the hypothesis that IBZ is a narrow-spectrum antibiotic for the treatment of CDI with reduced collateral impact on the metagenomic diversity of the human gut microbiome.

HOW DOES IBEZAPOLSTAT, A POLC INHIBITOR, ALTER THE HUMAN GUT MICROBIOME?

One sometimes finds what one is not looking for.

– Alexander Fleming

2

Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-Recurrence Effects in Clostridioides difficile Infection

Introduction

Clostridioides difficile infection (CDI) is the most common cause of infectious gastroenteritis in hospitalized patients and the most common cause of death due to gastroenteritis in the United States of America.[3] The pathophysiology of CDI includes disruption of the healthy gut microbiome, usually with high-risk antibiotics.[381] Oral vancomycin, the antibiotic most commonly used to treat CDI, is effective at killing vegetative C. difficile but disrupts the microbiota, leading to a high rate of recurrence after the end of antibiotic therapy.[408] A key change in the microbiome that increases the risk of CDI and recurrent CDI is decreasing the abundance and diversity of microbiota, including key bacterial species responsible for conversion of primary bile acids to secondary bile acids, in the gut. This dysbiosis allows the germination

of C. difficile spores, which are ubiquitous in the environment, to cause disease.[620] Ideally, a new drug in development would display similarly potent activity against C. difficile but would not have activity against key host microbiota preventing dysbiosis and would not allow for further germination and infection by C. difficile once therapy is completed.[621] Two recent Phase III clinical trials highlight the importance of understanding the pathophysiology of C. difficile recurrence and antibiotic pharmacology earlier in the drug development process.[622–627]

Cadazolid, a novel, nonabsorbable antibiotic primarily targeting Gram-positive Firmicute or Actinomycetota phyla, has a minimal effect on Bacteroidota,[622] and thus has a narrower spectrum than vancomycin. Positive Phase II clinical trials led to a large Phase III trial, in which a sustained clinical cure was not observed.[623] Surotomycin, a cyclic lipopeptide, had a similar spectrum of activity as cadazolid and similar positive Phase II clinical trial results.[622,625] However, a sustained clinical response difference was not observed in the Phase III clinical trial.[626,627] Although each of these two antibiotics had a minimal effect on host microbiota, in particular, the phylum Bacteroidota, more advanced microbiome evaluations were not performed during the clinical trial drug development process. Ibezapolstat is a Gram-positive selective spectrum (GPSS) PolC-type DNA Polymerase III inhibitor currently in the clinical trial drug development process, having completed Phase I healthy volunteer studies.[591] The design for the Phase I study included a comparator arm with vancomycin and daily stool samples collected for microbiome analysis. This provided a unique opportunity to develop an approach to assess the possible anti-recurrence effect of ibezapolstat using the known pathophysiology of C. difficile recurrence. The goals of this study were to assess the microbiome (taxa, alpha, and beta diversity) changes as well as the bile acid changes associated with ibezapolstat compared to those associated with vancomycin by using samples obtained from the Phase I healthy volunteer study.

Methods

Materials

Standards for primary bile acids cholate (CA) and chenodeoxycholate (CDCA), conjugated primary bile acids glycocholate (GCA), taurocholate (TCA), glycochenodeoxycholate (GCDCA), and taurochenodeoxycholate (TCDCA), secondary bile acids lithocholate (LCA), deoxycholate (DCA), ursodeoxycholate (UDCA), and hyodeoxycholate (HDCA), and conjugated secondary bile acids glycolithocholate (GLCA), taurolithocholate (TLCA), glycodeoxycholate (GDCA), and taurodeoxycholate (TDCA) were purchased from Sigma. Description of clinical trial. Stool samples were collected daily as part of a recent Phase I healthy volunteer study from the multiday, ascending dose arm that included ibezapolstat (300 or 450 mg, given twice daily) with a vancomycin comparator arm (125 mg four times daily) and a placebo, as described.[591] Institutional Review Board approval was obtained (Midlands Institutional Review Board IRB no. 222220170383), and all volunteers signed an informed consent form prior to performing any study procedures. For this analysis, stool samples were collected daily for Days 0 (baseline) to 13, along with a Day 30 follow-up, if available. Stool samples were immediately frozen at –80C prior to shipping to the University of Houston on dry ice for analysis. Stool DNA extraction and Shotgun Metagenomic Sequencing. Stool DNA was extracted using a DNeasy Power Soil Pro Kit (Qiagen, catalog number 1288-100) in a QiaCube automated DNA extraction system, as previously described.[591] Shotgun metagenomic sequencing was carried out at the University of Houston Sequencing and Gene Editing Core (Houston, TX, USA) using a Nextera DNA Flex Library Prep Kit for DNA library preparation and an Illumina NextSeq 500 platform for sequencing. CLC Genomic Workbench version 12 (Qiagen) was used for the metagenomic assembly and the creation of the abundance table. Specifically, the tutorial "Taxonomic profiling of whole shotgun metagenomic data" was used to remove host DNA and perform quality control checks. (https://resources.qiagenbioinformatics.com/tutorials/Taxonomic_Profiling.pdf, accessed Mar 28, 2022).

Extraction of bile acids from stool samples

Stool samples were aliquoted and weighed (ranging from approximately 10 to 150 mg). Each aliquot was mixed well with 1 mL of 100% methanol containing the internal standards (LCA-d5 and CA-d5, 200 μg/L) by vortexing and ultrasonication. The mixture was placed overnight at 4°C and was centrifuged for 3 min at 10,000 g. The supernatant was transferred into a new tube and diluted 10-fold with pure water. Subsequently, the diluted supernatant was applied to the preconditioned Sep-Pak C18 Classic Cartridge or Waters Corp. Oasis HLB 96-well Plate (Waters, USA). After being washed with 5% methanol, the bile-acid fraction was eluted with 100% methanol. The elution was dried under nitrogen, resuspended in 2 mL of methanol/water (1:1, vol/vol), and stored at −20°C until further analysis was to be completed.

Bile acid analysis

Bile acids were quantified using a targeted liquid chromatography mass spectrometry (LC-MS) analysis performed on a QTRAP 5500 mass spectrometer (Sciex, Framingham, MA, USA) adapted from a previously described method.[628] Briefly, chromatographic separation between bile acids of similar mass and chemical structures was conducted on a C18 column (Phenomenex, Torrance, CA, USA) via a gradient method using two mobile phases (Solvent A: methanol-water [1:1, vol/vol] with 10 mM ammonium acetate and 0.1% [wt/vol] ammonium hydroxide [pH 9]; Solvent B: methanol with 10 mM ammonium acetate and 0.1% [wt/vol] ammonium hydroxide [pH 9]). Quantification of each type of bile acid was calculated from the standard curves generated using unlabeled and stable isotope-labeled standards of bile acids. Bile acid concentrations were normalized by the corresponding sample weights.

Bile acid-inducible (bai) gene abundance

A previously published species-specific quantitative polymerase chain reaction (qPCR), which detected the bai gene abundance present in Clostridium scindens and Clostridium hylemonae (baiCD), was adapted.[629] The baiCD analysis was performed using the QuantStudio 5 Real Time PCR System (Applied

Biosystems). Also, baiCD gene cluster-specific primers were used, including the forward primer baiCD-F (5 -CAGCCCRCAGATGTTCTTTG -3) and the reverse primer baiCD-R (5 -GCATGGAATTCHACTGCRTC-3). The DNA quantity was assessed, and qPCR was performed on each sample in triplicate in a final volume of 20 μL containing 25 ng DNA template, primers at 0.5 μM, and QuantiTech SYBR green Mixes (Qiagen). Threshold cycle values were converted to copies per ng of DNA using a standard curve. Standards were prepared by genomic DNA related to the copy number of Clostridium scindens and a series of serial 10-fold dilutions of the organism DNA. A range of 10-fold serially diluted standard DNA (3×10^6 to 30 copies) was run on each qPCR plate in triplicate. Standard curve R2 values were calculated for the standards. Copies per gram of stool were calculated, accounting for initial sample DNA concentrations and stool weights.

Statistical Analysis

Subject specific and summary changes in bacterial taxa and alpha diversity were generated using the R software package. Linear regression models were built to assess proportional taxa differences at the phylum, class, order, and family levels over time for subjects given vancomycin or ibezapolstat, normalizing to taxa present in at least five percent of the total samples. Linear regression models were also built to assess daily changes in alpha diversity measures (Shannon, Simpson, and Pielous) over time for subjects given vancomycin or ibezapolstat. The LEfSe algorithm was used to visualize and identify significant differences in microbiota composition between baseline samples and Day 10 samples.[630] Linear regression models were also built to assess primary and secondary bile acid changes over time as well as the ratio of primary:secondary bile acids over time from subjects given vancomycin or ibezapolstat. All linear regression models used placebo results as baseline values and controlled for subject age, weight, and sex. SAS version 9.4 (SAS Institute, Cary, NC) or R were used for all statistical analyses. The correlation between microbiota and bile acid changes were evaluated at the family taxa for primary and secondary bile acid amounts. To account for multiple analyses per aim and limit the false detection rate, a reduced P value of $P < 0.005$ was considered to be indicative of statistical significance (unless otherwise stated).[631]

Data availability

All data associated with this study are available in the main text or in the supplemental material. The Illumina paired-end FASTQ files have been deposited in NCBI under BioProject ID PRJNA847068.

Results

Description of clinical trial

Twenty-two subjects (female: 33%) aged 30 ± 8 years were enrolled. Six patients each were given either vancomycin, ibezapolstat 300 mg, or ibezapolstat 450 mg, and an additional four were given a placebo. A full description of the Phase I study, including safety, food effects, pharmacokinetics, and initial metagenomic analyses, has been described previously.[591]

Metagenomic analysis

Microbiota were not different at baseline (Day 0 samples) between study groups. The daily changes of individual phyla and Shannon's index alpha diversity for subjects given ibezapolstat, vancomycin, or the placebo are shown in Figure 2.1. Interindividual phylum differences were evident. However, the proportion of Pseudomonadota or Fusobacteria increased in subjects given vancomycin, while the proportion of Actinomycetota increased consistently in subjects given ibezapolstat. In general, alpha diversity decreased on therapy for individual subjects who received either ibezapolstat or vancomycin compared to those who received the placebo. A statistical analysis of the changes in alpha diversity over time is shown in Table 2.1. Using three separate alpha diversity indices (Shannon, Simpson, and Pielous), ibezapolstat 450 mg and vancomycin showed statistically significant changes in alpha diversity over time compared to the placebo. Ibezapolstat 300 mg did not demonstrate statistically significant changes compared to the placebo. Summary measures for alpha diversity changes (Shannon) over time by treatment group is shown in Figure 2.2.

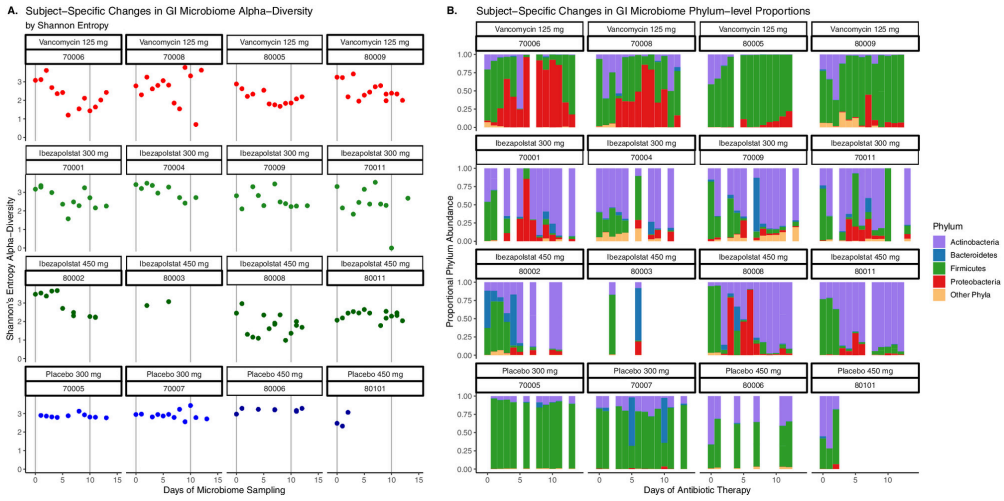

Figure 2.1: Participant-Specific Changes in Intestinal Alpha-Diversity and Metagenomic COmposition. (A) (B) From: Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-Recurrence Effects in Clostridioides difficile Infection.[588]

Beta diversity changes confirmed that microbiota were significantly different between study groups (Figure 2.2). A principle coordinate analysis revealed that baseline samples were similar in all study groups, while distinct ellipses representing 95% confidence bounds for each cluster were significantly different for the vancomycin-treated subjects compared to subjects treated with either dosage of ibezapolstat or the placebo. Cladograms at baseline compared to end of therapy, generated by the Linear Effect Size (LEfSe) algorithm, are shown in Figure 2.3. Vancomycin had a more wide-ranging effect on the microbiome, including significantly lower proportions of most taxa, except for an increased proportion of Gammaproteobacteria. Ibezapolstat demonstrated a decreased proportion of Eubacteriales and increased proportions of Actinomycetota including certain species of Bifidobacteriaceae. Bacterial taxa changes at the phylum, class, order, and family levels are shown in Table 2.2.

Bile acids

Seventeen baseline samples were available for bile acid analysis along with 17 samples from Day 5 and 14 samples from Day 10. Concentrations of bile acids for each drug and time period are shown in Figure 2.4. Baseline samples were similar for all study groups and were comprised primarily (>95%) of secondary bile acids. Primary bile acids increased and secondary bile acids decreased with exposure to all

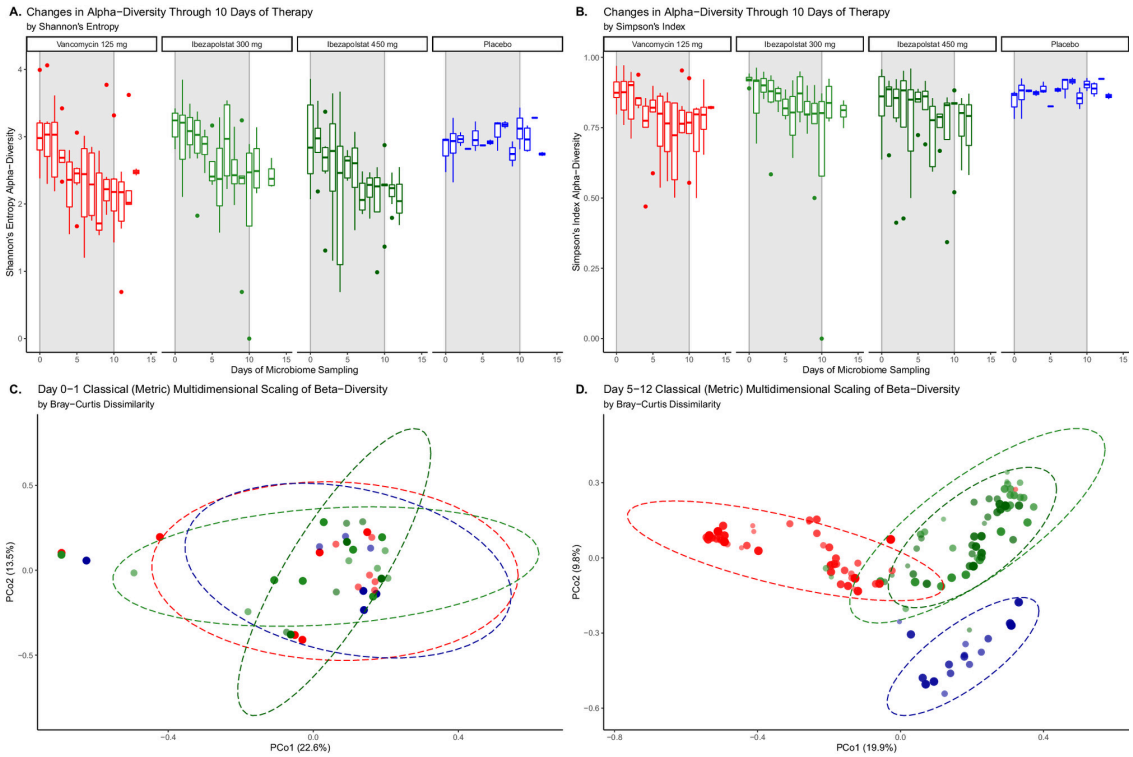

Figure 2.2: Intestinal Alpha- and Beta-Diversity in Healthy Adults Administered Oral Ibezapolstat or Vancomycin. Microbiomes of participants that received oral vancomycin (red), ibezapolstat (green), or placebo (blue) were characterized by (A) Shannon's Entropy and (B) Simpson's Index. Beta diversity was calculated using Bray–Curtis dissimilarity and visualized with classical (metric) multidimensional scaling (MDS) for participants at (C) baseline (Day 0 – 1) and (D) by end of therapy (scaled: smaller/lighter = day 5; larger/darker = day 12). From: Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-Recurrence Effects in Clostridioides difficile Infection.[588]

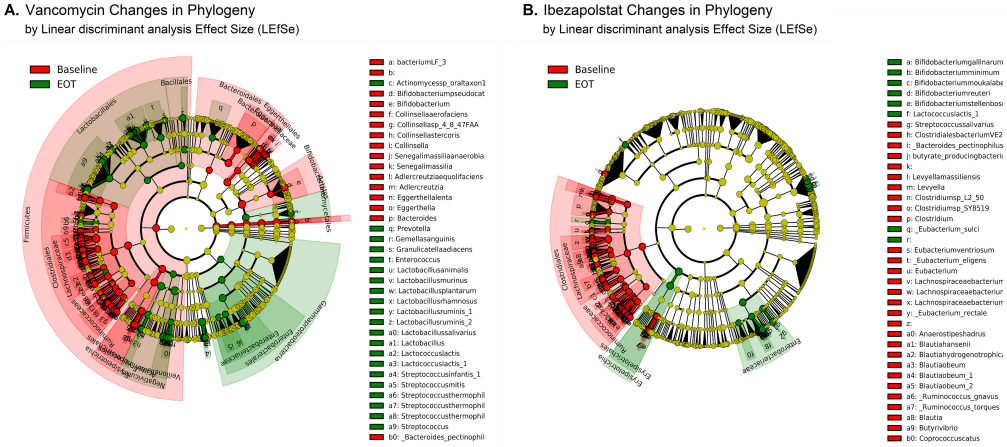

Figure 2.3: Healthy Adult LeFSe with Oral Ibezapolstat or Vancomycin. Statistically significant changes in bacterial taxa in the oral (A) vancomycin or (B) ibezapolstat groups by Linear discriminant analysis effect size (LEfSe) found taxa enriched at baseline (red), end of therapy (EOT; green), or had no statistically significant difference (no color). From: Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-Recurrence Effects in Clostridioides difficile Infection.[588]

Table 2.1: Comparison of daily alpha-diversity and bile acid changes during therapy for ibezapolstat versus oral vancomycin [a]

Analysis	Ibezapolstat		Vancomycin
	300 mg (*)	450 mg (*)	125 mg (*)
A. Alpha-diversity			
Shannon	-0.12 ± 0.12 (0.31)	-0.45 ± 0.12 (0.0001)	-0.36 ± 0.11 (0.0014)
Simpson's	-0.013 ± 0.023 (0.59)	-0.072 ± 0.022 (0.0019)	-0.070 ± 0.023 (0.0020)
Pielous	-0.0040 ± 0.024 (0.87)	-0.051 ± 0.024 (0.031)	-0.073 ± 0.024 (0.0016)
B. Bile acids			
1° bile acids, μg/L	-3.7 ± 172 (0.98)	307 ± 161 (0.061)	963 ± 146 (<0.001)
2° bile acids, μg/L	-913 ± 675 (0.18)	-971 ± 629 (0.13)	$-1,266 \pm 570$ (0.030)
1°:2° bile acid ratio	-1.3 ± 4.1 (0.75)	6.2 ± 3.8 (0.11)	19 ± 3.5 (<0.0001)

[a]Numbers represent average change $\pm$ standard deviation over the study time period. A negative ($-$) number represents decreased (A) diversity or (B) bile acid concentration. 1°: primary; 2°: secondary; *, *P* value versus placebo, controlling for patient age, weight, and sex.

study drugs, but more pronounced findings were observed with vancomycin (Figure 2.5). Using a linear regression analysis and after controlling for subject demographics, vancomycin was associated with significant increases in primary bile acids as well as primary:secondary bile acid ratios. Although similar effects were noted with ibezapolstat 450 mg, these results were not statistically significant (Table 2.1).

Correlation between microbiota and bile acid changes

Correlations between family taxa and primary and secondary bile acid concentrations are shown in Table 2.3.4. Enterobacteriaceae were most highly correlated with primary bile acid concentrations (r = 0.63; P < 0.0001) while Oscillospiraceae were negatively correlated with primary bile acid concentrations (r = −0.37; P = 0.0025). Also, Oscillospiraceae were positively correlated with secondary bile acid concentrations (r = 0.44; P = 0.0002), and Pseudomonadaceae were positively correlated with secondary bile acid concentrations (r = 0.38; P = 0.0017).

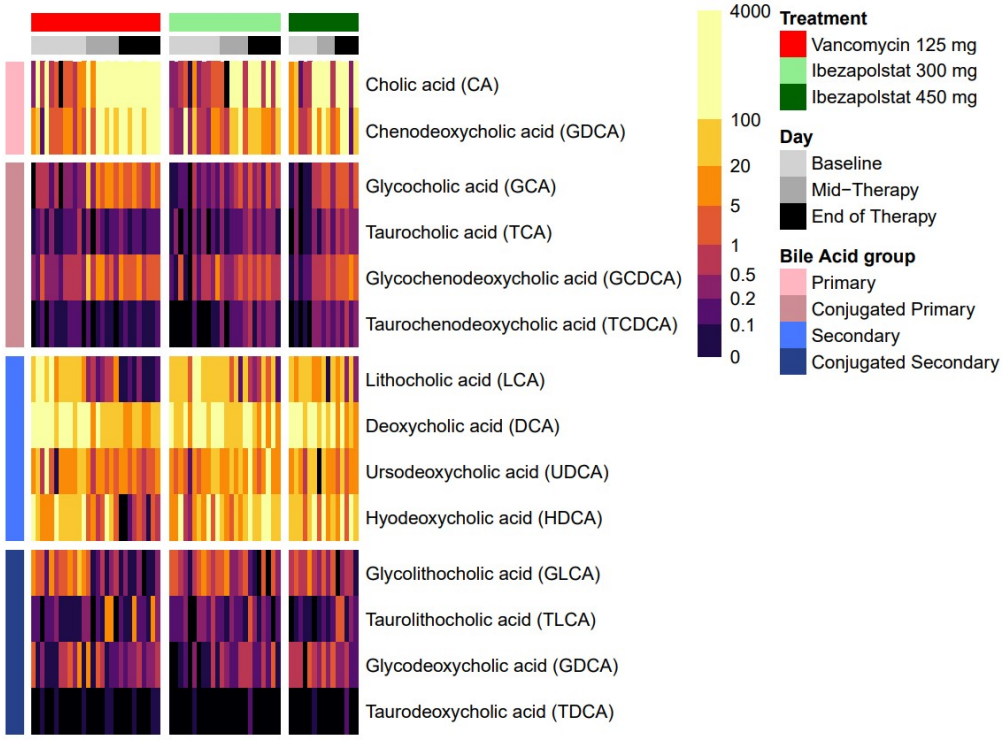

Figure 2.4: Bile Acid Changes In Healthy Adults Administered Oral Ibezapolstat or Vancomycin. Quantile-scaled heatmap of bile acids (ng/mL) grouped by (pink) primary and (blue) secondary, or unconjugated (light pink/blue) versus conjugated (dark pink/blue) in the vancomycin (red) or ibezapolstat (green) treatment groups through time (baseline = light gray; mid-therapy = darker gray; end of therapy = black). From: Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-Recurrence Effects in Clostridioides difficile Infection.[588]

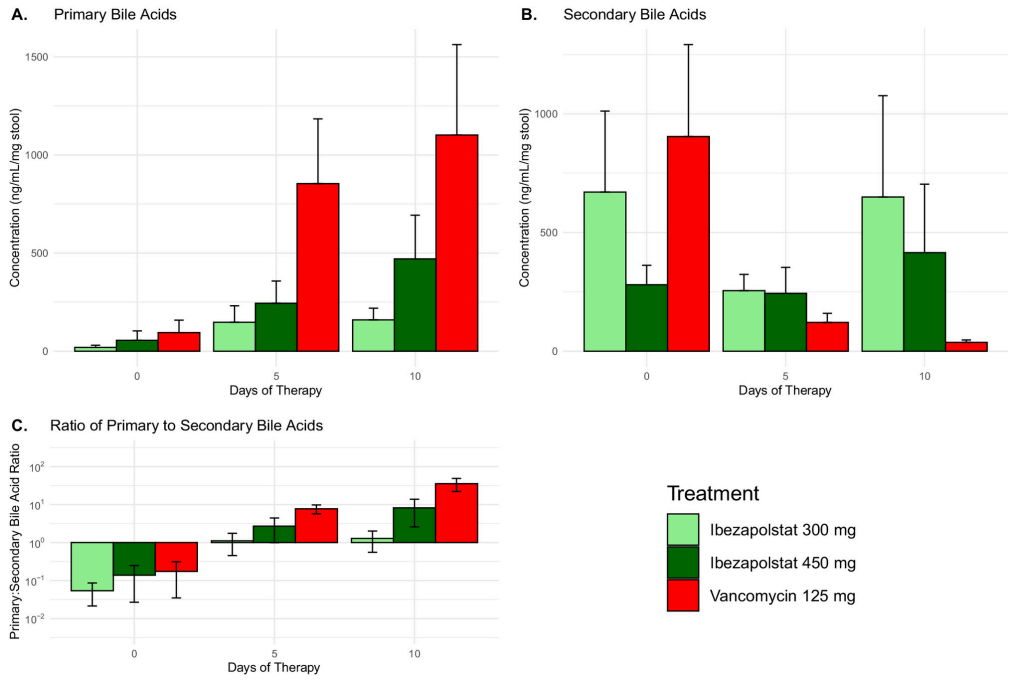

Figure 2.5: Ratios of Bile Acid Pools in Healthy Adults Administered Oral Ibezapolstat or Vancomycin. Concentration of (A) Primary and (B) secondary bile acids in stool; (C) ratio of primary-to-secondary bile acids in stool From: Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-Recurrence Effects in Clostridioides difficile Infection.[588]

Table 2.2: Proportional changes in taxa in healthy subjects given vancomycin or one of two doses of ibezapolstat[a]

Taxa	Vancomycin 125 mg		Ibezapolstat 300 mg		Ibezapolstat 450 mg	
	Change (mean ± SE)	*P*	**Change (mean ± SE)**	*P*	**Change (mean ± SE)**	*P*
***p*_Bacteroidota**	−0.034 ± 0.024	0.16	−0.0055 ± 0.025	0.83	−0.013 ± 0.025	0.61
***p*_Bacillota**	−0.14 ± 0.058	0.014	−0.47 ± 0.060	< 0.0001	−0.50 ± 0.06	< 0.0001
*c*_Clostridia	−0.50 ± 0.052	< 0.0001	−0.49 ± 0.054	< 0.0001	−0.52 ± 0.054	< 0.0001
*o*_Clostridiales	−0.50 ± 0.052	< 0.0001	−0.49 ± 0.054	< 0.0001	−0.52 ± 0.054	< 0.0001
*f*_Lachnospiraceae	−0.24 ± 0.024	< 0.0001	−0.22 ± 0.025	< 0.0001	−0.26 ± 0.025	< 0.0001
*f*_Oscillospiraceae	−0.25 ± 0.033	< 0.0001	−0.27 ± 0.034	< 0.0001	−0.25 ± 0.035	< 0.0001
*c*_Bacilli	0.30 ± 0.043	< 0.0001	0.016 ± 0.044	0.72	0.017 ± 0.045	0.39
*o*_Lactobacillales	0.30 ± 0.043	< 0.0001	0.016 ± 0.044	0.7117	0.017 ± 0.045	0.6972
*f*_Lactobacillaceae	0.28 ± 0.041	< 0.0001	0.024 ± 0.042	0.5755	0.015 ± 0.043	0.7307
***p*_Actinomycetota**	−0.11 ± 0.05	0.032	0.31 ± 0.053	< 0.0001	0.31 ± 0.054	< 0.0001
*c*_Actinobacteria	−0.074 ± 0.051	0.14	0.27 ± 0.052	< 0.0001	0.29 ± 0.053	< 0.0001
*o*_Bifidobacteriales	−0.080 ± 0.051	0.1201	0.27 ± 0.053	< 0.0001	0.29 ± 0.053	< 0.0001
*f*_Bifidobacteriaceae	−0.078 ± 0.051	0.1293	0.27 ± 0.053	< 0.0001	0.29 ± 0.053	< 0.0001
*c*_Coriobacteriia	−0.038 ± 0.015	0.0145	0.036 ± 0.016	0.0221	0.024 ± 0.016	0.1431
*o*_Coriobacteriales	−0.031 ± 0.015	0.0375	0.035 ± 0.016	0.0264	0.026 ± 0.016	0.1013
*f*_Coriobacteriaceae	−0.032 ± 0.015	0.0338	0.034 ± 0.016	0.0298	0.025 ± 0.016	0.1122
***p*_Pseudomonadota**	0.23 ± 0.045	< 0.0001	0.12 ± 0.05	0.0094	0.09 ± 0.05	0.053
*c*_Gammaproteobacteria	0.21 ± 0.045	< 0.0001	0.12 ± 0.046	0.0094	0.092 ± 0.046	0.0478
*o*_Enterobacterales	0.17 ± 0.042	< 0.0001	0.11 ± 0.043	0.0099	0.094 ± 0.044	0.0336
*f*_Enterobacteriaceae	0.17 ± 0.041	< 0.0001	0.11 ± 0.042	0.0082	0.087 ± 0.043	0.043
***p*_Fusobacteriota**	0.036 ± 0.015	0.0165	0.0011 ± 0.015	0.9414	0.00046 ± 0.015	0.9762

[a]c: class; o: order; f: family. Dark gray shading indicates at least a 10% increase in relative proportion compared to baseline, and light gray shading represents a 10% decrease in relative proportion compared to baseline (only variables with a $P < 0.005$ significance colored).

Table 2.3: Correlation of microbiota with bile acids[a] (sorted by *P* for Secondary bile acids)

Family	Primary bile acids	*P*	Secondary bile acids	*P*
Oscillospiraceae	**–0.36391**	**0.0025**	**0.44424**	**0.0002**
Pseudomonadaceae	**0.27146**	**0.0263**	**0.37721**	**0.0060**
Lachnospiraceae	**–0.33184**	**0.0061**	0.01017	0.9349
Enterobacteriaceae	**0.62888**	**< 0.0001**	–0.16676	0.1774
Lactobacillaceae	**0.26868**	**0.0279**	–0.09527	0.4432
Coriobacteriaceae	–0.23574	0.0548	–0.02838	0.8197
Erysipelotrichaceae	–0.12744	0.3041	–0.03216	0.7962
Fusobacteriaceae	–0.0662	0.5946	–0.04921	0.6925
Bacteroidaceae	–0.20096	0.103	–0.10486	0.3984
Bifidobacteriaceae	–0.07082	0.569	–0.03019	0.8084
Methanobacteriaceae	0.00194	0.9876	–0.01041	0.9333

[a]Boldface entries indicate analyses that were statistically significant.

BaiCD gene abundance analysis

Baseline and follow-up stool samples were available for five patients who received vancomycin and ibezapolstat 450 mg and for four patients that received ibezapolstat 300 mg. The baiCD gene positivity rate was similar between subjects, irrespective of the type of therapy given (80 to 90%). The proportion positive and quantity of baiCD genes decreased during all three types of therapy (Figure 2.6).

Conclusion

The pathophysiology of CDI involves disruption of the human gut microbiota, usually with high-risk antibiotics, and can lead to a dysbiosis that enables *C. difficile* spores to germinate and cause active disease.[381] Thus, ideal characteristics for a new drug directed toward CDI include potent activity against *C. difficile* and minimal further disruption of host microbiota.[621] Laboratory and animal models are generally able to identify small molecules with potent in vitro activity against *C. difficile* isolates. However, due to the complex nature of the gut microbiome, identification of a potential ability to reduce the likelihood of CDI recurrence is generally not possible until large Phase III studies are undertaken. This leads to costly and unfortunate mistakes in Phase III clinical studies of novel CDI-directed antibiotics, despite the fact

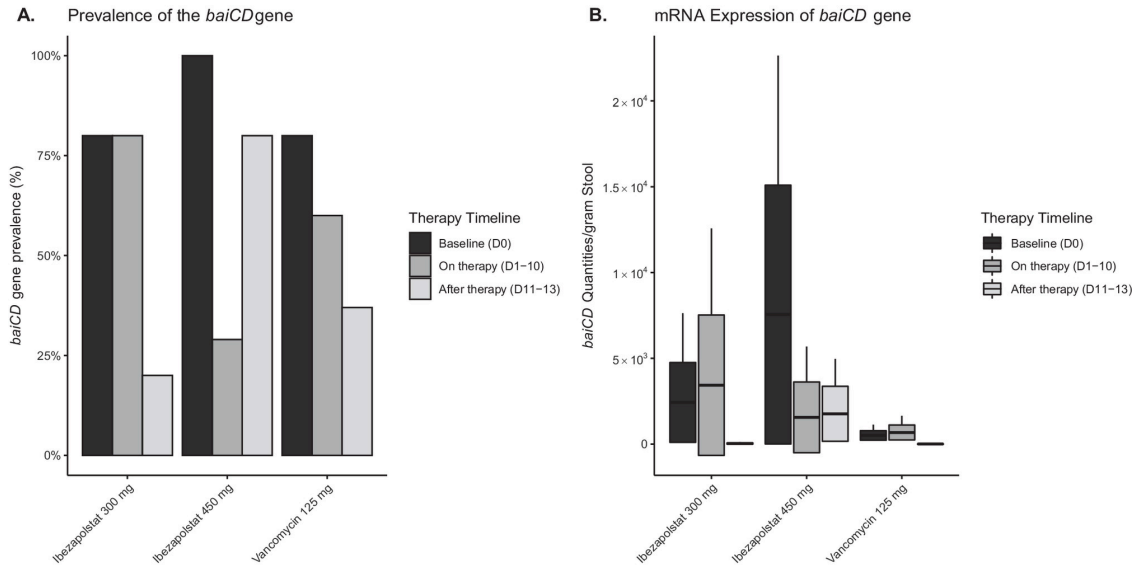

Figure 2.6: Alterations in *baiCD* Expression in Healthy Adults Administered Oral Ibezapolstat or Vancomycin. From: Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-Recurrence Effects in Clostridioides difficile Infection.[588]

that these antibiotics have different effects on the microbiota than comparator antibiotics do.[623,627] Thus, a method to identify possible anti-recurrence effects earlier in the clinical trial development process is urgently needed. In this study, we used stool samples from the Phase 1 healthy volunteer study to compare ibezapolstat, a PolC-type DNA Polymerase III inhibitor, to vancomycin, a glycopeptide antibiotic and the most commonly used antibiotic to treat CDI, to a placebo. PolC-type DNA Polymerase III inhibitors target low G+C bacteria, namely, *Bacillota*, but have no effect on other Gram-positives (*Actinomycetota*) or Gram-negatives (*Bacteroidota*, *Pseudomonadota*).[487] Alternatively, vancomycin has broad spectrum activity against all of these phyla and would be expected to have a larger effect on the microbiome.[632] Using metagenomic sequencing, we confirmed this pharmacology and demonstrated that both antibiotics affected the human microbiome, though they did so in completely distinct manners and produced two distinct microbiome profiles. Using mass spectrometry, we then demonstrated that this change in the microbiome was associated with a reduced effect on the ratio of primary to secondary bile acids in the gut for ibezapolstat compared to that of vancomycin. Family taxa differences observed in subjects given vancomycin or ibezapolstat were highly correlated with concentrations of primary or secondary bile acids. These results are highly suggestive of a possible anti-recurrence effect for ibezapolstat compared to the gold standard,

vancomycin. This represents the most thorough evaluation of functional metagenomic changes ever undertaken during Phase I clinical trials for CDI-directed antibiotics. This extends and strengthens the ongoing functional metagenomic work being performed during the Phase II clinical trials of ridinilazole.[633,634] Taken together, these analyses could become a new standard in the drug development process for CDI-directed antibiotics and, in general, could be understood alongside other systemic antibiotics to evaluate their likelihood to increase the risk of CDI with use. Important advances in the understanding of the pathophysiology of CDI and the mechanisms underlying colonization resistance to C. difficile have transformed our understanding of how certain microbial taxa reduce the likelihood of CDI and recurrent CDI.[635] Key metagenomic findings in this study include a consistent decrease in the Clostridia class with both antibiotics, an expansion of the Actinomycetota class in ibezapolstat-treated subjects, and an expansion of the Gammaproteobacteria class, the Enterobacterales order, and the Enterobacteriaceae family in vancomycin-treated subjects. Within the Firmicute phylum, vancomycin was also associated with an increased proportion of Bacilli class taxa.

Most metagenomic studies with C. difficile have focused on recurrent CDI and the effect of fecal microbiota transplantation (FMT).[636] An expansion of the Enterobacteriaceae family has been previously identified as a significant risk factor for recurrent CDI.[637,638] FMT studies have also shown that the resolution of CDI recurrence was associated with the restoration of secondary bile acids. An increasing amount of laboratory evidence has helped to further elucidate the importance of bile acids in the pathophysiology of CDI.[317,401] These include findings that the presence of secondary bile acids prevent the germination of C. difficile spores, while primary bile acids increase sporulation. Primary bile acids are metabolized by key taxa in the human gut microbiota by the 7-alpha dehydroxylation pathway, and murine studies have shown that antibiotic treatment leads to a loss of secondary bile acids.[366] Prior to conversion to secondary bile acids, primary bile acids are deconjugated by commensal bacteria that possess bile salt hydrolase genes. These genes are present in widely distributed taxa. Thus, it is not surprising that no differences were observed in the proportion of conjugated versus non-conjugated bile acids in our study, despite differences in the microbiome profiles. On the other hand, the 7-alpha dehydroxylation pathways are encoded in the bile acid-inducible (bai) operon. Only a unique set of key species, most commonly Clostridium

scindens, Clostridium sordelli, and a small subset of other Bacillota, are known to possess the full gene for the bai operon.[400,639] Proportions of all of these Eubacteriales would be expected to be reduced following vancomycin or ibezapolstat, as demonstrated by changes in baiCD gene abundance during therapy. However, the preservation of secondary bile acids in our study is supported by the Phase II ridinilazole clinical study, in which a similar preservation of secondary bile acids was observed, despite in vitro activity of ridinilazole to C. scindens and C. sordelli.[633] This suggests that other bacterial taxa also contribute to primary bile acid metabolism[640] or that a group of bacteria that have a subset of the bai pathways could collaboratively synthesize secondary bile acids from conjugated primary bile acids.[641] This is a future area of research, but these results suggest that the findings from this study will be applicable to future CDI clinical trials with ibezapolstat. Our plans are to validate and expand these findings in upcoming Phase II studies.

This study has certain limitations. We recruited young, healthy patients into the Phase I clinical trial. The Actinomycetota phylum is more prevalent in younger adults and is replaced by Bacteroidota with age.[642] CDI is more prevalent in older patients, and thus, the baseline microbiota would not be indicative of a healthy microbiome of an elderly patient. However, the Bacteroidota phylum was present in the majority of our samples and thus was represented as a minority phylum in our study. Whether an expansion of Actinomycetota can be observed in elderly patients with CDI will require further study. If not, an intriguing possibility for a future clinical trial would be to add a probiotic that contains the Actinomycetota phylum to promote Actinomycetota expansion. Likewise, the microbiome of CDI patients may already be characterized by an expansion of Pseudomonadota.[638] Whether ibezapolstat would be able to reduce this phylum via the expansion of Actinomycetota will require further mechanistic and clinical studies. A common limitation of all human gut microbiome studies is the dependence on daily bowel movements for daily sample collection. As this was not the case for all subjects, samples were not available for each study day for all patients. Lastly, we plan to explore whether these types of analyses could be performed in preclinical, mini-bioreactor models in the future.[643]

Using data from the Phase 1 healthy volunteer trials and a novel analysis technique, beneficial changes suggestive of a lower risk of CDI recurrence were associated with ibezapolstat compared to van-

comycin. This novel functional metagenomics approach may enable the better and earlier prediction of anti-CDI recurrence effects for antibiotics in the clinical development pipeline.

HOW DOES IBZ IMPACT THE MICROBIOME OF ADULTS WITH CDI?

...I have strayed onto paths where the gold was still lying by the wayside. It takes a little luck to be able to distinguish gold from dross...

– Robert Koch

3

Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial

Introduction

Clostridioides difficile infection (CDI) is the most common cause of healthcare-associated infections in the United States,[4] where it is responsible for almost 500 000 incident infections and 29 000 deaths.[3] Current treatment guidelines recommend only 2 antibiotics for initial treatment of CDI, namely, oral vancomycin or fidaxomicin.[408] Vancomycin is associated with unacceptably high recurrence rates and both antibiotics are associated with the emergence of antimicrobial resistance.[469,643,644] CDI recurrence is due to the continued perturbation of the gut microbiome, most commonly characterized by decreased proportions of Bacillota, Bacteroidota, and Actinomycetota phyla with subsequent overgrowth of Pseudomonadota.[645] These taxa changes reduce colonization resistance to C. difficile by eliminating the taxa respon-

sible for bile acid metabolism, leading to a higher concentration of primary bile acids which support spore germination and onset of CDI or recurrent CDI.[620] Ideally, new drugs in clinical development should have a unique mechanism of action with similarly potent activity against C. difficile but without activity against key host microbiota.[621] This spectrum of activity is crucial to prevent the treatment-associated dysbiosis that allows for further germination and infection by C. difficile after therapy completion.

Ibezapolstat (formerly ACX-362E) represents a unique class of gram-positive selective spectrum antimicrobials that bind to and inhibit bacterial PolC-type DNA Polymerase III.[487] The PolC-type DNA Polymerase III enzyme is essential for replication of low–G + C content (fewer G and C DNA bases than A and T bases) in gram-positive bacteria, including Bacillota such as C. difficile, yet it is absent in Actinomycetota and gram-negative host microbiota, including Bacteroidota species. Ibezapolstat was minimally absorbed in a hamster model, leading to high colonic and low systemic concentrations and was shown to be effective for CDI.[614] A phase 1 healthy volunteer study demonstrated a similarly advantageous pharmacokinetic (PK) profile and a favorable safety profile.[591] In contrast to vancomycin, ibezapolstat did not cause overgrowth of Pseudomonadota and preserved a favorable ratio of secondary-to-primary bile acids that would be predictive of an anti-CDI recurrence effect.

This phase 2a study was conducted as the first human validation of the PolC-type DNA Polymerase III target in a diseased population of patients with CDI. The primary objectives of this study were to assess CDI clinical cure rates 2 days after the end of treatment (EOT) and the safety/tolerability of ibezapolstat given to adult patients with CDI. The secondary objectives were to evaluate plasma and fecal PK characteristics, microbiologic eradication, quantitative microbiome changes in relevant fecal bacterial communities and microbial diversity, bile acid effects, and sustained clinical cure (SCC) associated with ibezapolstat in patients with CDI.

Methods

Study Design

This was a single-arm, open-label, phase 2a segment of a multicenter phase 2 trial, and enrolled adults at 4 centers in the United States in 2019–2020 (protocol no. ACX-362E-201; ClinicalTrials.gov identifier NCT04247542). Patients received three 150-mg ibezapolstat capsules (total dose, 450 mg) orally every 12 hours for 10 days. After the EOT, patients were followed up for an additional 28 days to evaluate clinical response, adverse events (AEs), and status of the fecal microbiome. The study was conducted in accordance with the Declaration of Helsinki. The study protocol and amendments were approved by an institutional review board at each study site, and written informed consent was obtained for each enrolled subject before the study commenced.

Patients

Eligible participants included adults aged 18–90 years with CDI defined as >3 watery bowel movements in the 24 hours before enrollment and classified as nonsevere CDI, as defined by Infectious Diseases Society of America/Society for Healthcare Epidemiology of America guidelines (white blood cell count ≤15 000/mL and serum creatinine level <1.5 mg/dL).[4,32] Enrolled patients must have had CDI diagnosed using a positive free toxin–based fecal test (C. DIFF QUIK CHEK COMPLETE [TechLab] or Immunocard Toxin A&B [Meridian Bioscience]). Patients were excluded if they had >24 hours of other CDI-directed antibiotics at the time of enrollment, probiotic or laxative receipt, >3 episodes of CDI in the previous 12 months or >1 prior episode in the last 3 months, immunocompromising conditions or medications, inflammatory bowel disease, pregnancy or lactation, active gastroenteritis due to another microorganism, major gastrointestinal surgery within 3 months of enrollment (appendectomy or cholecystectomy permitted), or elevated liver function values (defined as >2 times the upper limit of normal for alanine aminotransferase or aspartate aminotransferase).

Safety Assessments

Safety evaluations included AE assessment, physical examination, vital signs, clinical laboratory tests (chemistry, hematology, and urinalysis), and electrocardiography. Safety end points for all subjects were recorded including nature, frequency, and severity of AEs. AEs were assessed at each visit beginning from the time of enrollment and classified according to the Medical Dictionary for Regulatory Activities (MedDRA; version 15.0). AE severity (mild, moderate, or severe) and causality (unrelated, possibly related, or probably related to the study medication) were assessed by the investigator at each site.

Pharmacokinetic Evaluations

Plasma samples were obtained 2 and 4 hours after the first daily ibezapolstat administration on days 1, 5, and 10. Fecal samples were collected at baseline and daily during days 1–10 of ibezapolstat receipt. Plasma and fecal concentrations were assayed by AltaSciences (Laval), and PK analyses were performed by Learn and Confirm.

Microbiology

Stool samples were cultured for C. difficile growth on a selective cycloserine-cefoxitin fructose agar at 37°C under anaerobic conditions for 48 hours.[643] Isolates were identified as C. difficile based on growth and morphology and confirmed by PCR for C. difficile toxin and tpi genes. C. difficile was strain typed using a PCR-based ribotyping method, as described elsewhere [14].[646] Minimum inhibitory concentrations were determined for ibezapolstat by broth microdilution in 0.1% sodium taurocholate brain heart infusion media.[647]

Microbiome and Bile Acid Evaluations

Fecal samples for microbiome analysis were collected daily during ibezapolstat dosing and on days 2, 10, 20, and 28 after the EOT. Stool DNA extraction was performed using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen; catalog no. 12888-100) in a QIAcube automated DNA extraction system (Qiagen) according to the manufacturer's instructions. Microbiome characterization was performed by sequencing the V1–V3 region of the 16S ribosomal RNA gene, using the MiSeq system (Illumina) [16, 17].[648,649] Each sample was amplified using a barcoded primer, which yielded a unique sequence identifier tagged onto each individual sample library. Genomic DNA was normalized before polymerase chain reaction (PCR) analysis, and PCR products were normalized before pooling. Illumina-based sequencing yielded >15 000 reads per sample. Bile acids were quantified using targeted liquid chromatography mass spectrometric analysis performed on a QTRAP 5500 mass spectrometer (Sciex), adapted from a previously described method.[628] Bile acid levels were normalized by the corresponding stool sample weight.

Efficacy Assessments

The primary efficacy outcome measure was initial clinical cure at the EOT, defined as resolution of diarrhea in the 24-hour period before the EOT and maintained for ≥48 hours after the EOT. SCC was defined as clinical cure with no recurrence of CDI within 28 (±2) days after the EOT.

Statistical Analysis

An intent-to-treat analysis of patients receiving ≥1 dose of ibezapolstat was conducted. Descriptive statistics were calculated for efficacy, safety/tolerability, and PK data generated using SAS version 9.4 software (SAS Institute). Results are expressed as means with standard deviations (SDs) unless otherwise stated. Microbiome summary plots and data visualization was prepared using R software, version 4.1.1 (R Core Team 2021).[650] Alpha diversity for each sample was assessed with the VeganR package version 2.4-2, using the Shannon diversity index and the inverse Simpson index. Differences in alpha diversity (with

both indexes) and bile acids between baseline and during or after therapy were determined using linear regression models. Proportional changes of bacterial taxa over the 10-day dosing interval were calculated using linear regression models for taxa with a ≥1% proportional change during the study time period. Differences were considered significant at $P < .05$.

Results

Patients

Ten patients aged were enrolled, with a mean (SD) age of 49 (15) years (50% female; 100% white race; 80% Hispanic or Latino ethnicity). All 10 patients received ibezapolstat and completed the study (Supplementary Figure 3.1). The median number of unformed bowel movements in the 24 hours before the start of therapy was 4 (range, 3–10). Two of 10 patients received <24 hours of antibiotics, either metronidazole or vancomycin, before starting ibezapolstat. No patients were hospitalized before or after enrollment.

Table 3.1: Phase IIa Adverse Events in Adults with CDI Receiving Ibezapolstat. Adapted from: Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial.[589]

Adverse Event	Frequency	Severity	Relationship	Treatment Required	Outcome
Headache	Intermittent	Mild	Unrelated	No	Resolved
Headache	Intermittent	Mild	Unrelated	No	Resolved
Intertriginous Candidiasis	Continuous	Moderate	Unrelated	Yes	Resolved
Migraine Headache	Continuous	Severe	Unrelated	Yes	Resolved
Nausea	Intermittent	Moderate	Probable	No	Resolved
Nausea	Intermittent	Moderate	Probable	No	Resolved

Safety

A summary of the AEs is provided in Supplementary Table 3.1. Seven AEs were reported in 4 of the 10 patients, with 4 occurring in a single subject. None of the AEs were serious AEs. The severity of AEs was mild (n = 2), moderate (n = 4), and severe (n = 1; drug-unrelated migraine headache). The

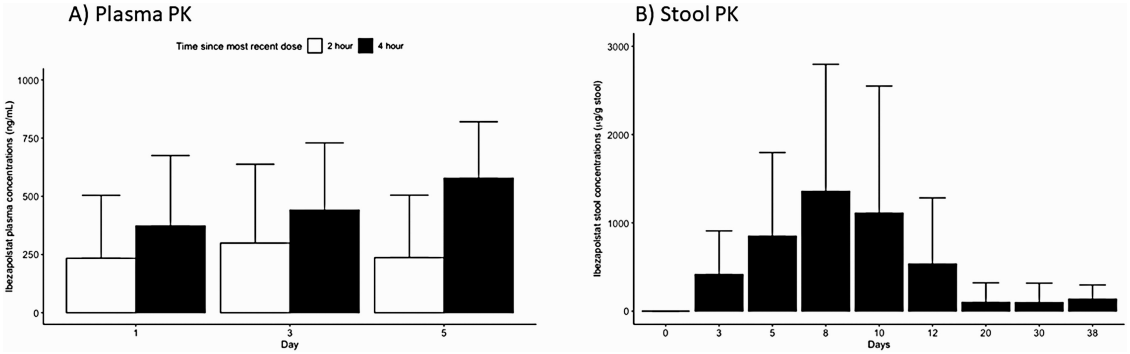

Figure 3.1: Ibezapolstat pharmacokinetics in plasma (A) and stool (B) samples. From:Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial[589]

most common AEs were headache (n = 2) or nausea (n = 2); both episodes of nausea were regarded by the investigator as "probably related" to the study drug. No treatment was required for these AEs, and no AE required a change to the study drug schedule or withdrawal of dosing. All AEs were resolved by the end of the study. No significant clinical laboratory test abnormalities were detected

Pharmacokinetic Results

The 2–4-hour postdose ibezapolstat plasma levels ranged from 233 to 578 ng/mL, with higher concentrations observed at 4 hours (range, 373–578 ng/mL) than at 2 hours (234–299 ng/mL). The mean ibezapolstat stool concentration (SD) was 416 (494) µg/g stool by day 3 of therapy, >1000 µg/g stool by days 8–10, and 535 (748) µg/g stool 2 days after the EOT. Three of 4 stool samples collected on day 38 continued to have detectable stool concentrations of ibezapolstat (mean [SD], 136 [161] µg/g stool). Baseline stool and plasma concentrations (before drug administration) were undetectable. Full stool and plasma PK data are shown in Figure 3.1.

Microbiology Results

Toxigenic C. difficile grew in 6 of 7 baseline stool samples (86%) available for microbiology studies but not in stool samples from any other sampling day (range, 7–9 samples per day). Identified ribotypes

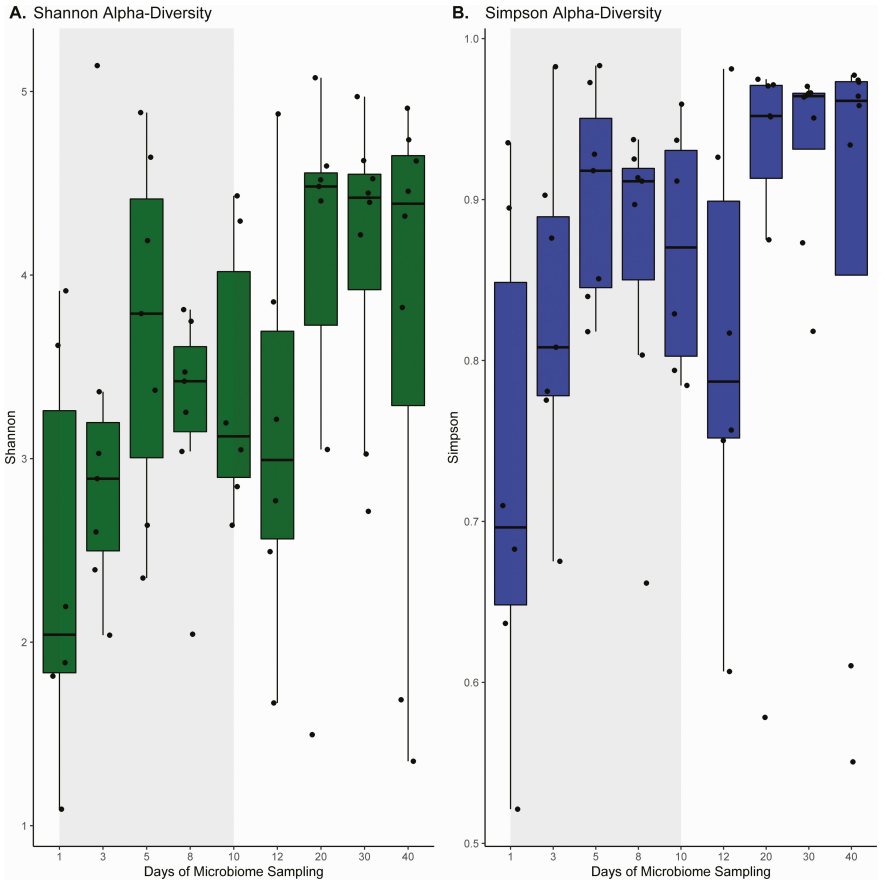

Figure 3.2: Summary estimates of changes in alpha-diversity over time with the Shannon (A) and inverse Simpson (B) [1 - Dominance] diversity indeces. From:Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial[589]

included F078–126 (n = 2), F014–020 (n = 2), F106 (n = 1), and FP435 (n = 1). The minimum inhibitory concentrations of ibezapolstat were 0.25 (n = 1), 0.5 (n = 3), or 1.0 (n = 1) ug/mL.

Microbiome and Bile Acid Results

Eight participants provided stool samples for microbiome and bile acid analysis. Although interindividual changes were noted, a rapid increase in alpha diversity was noted from baseline samples using both the inverse Simpson and Shannon diversity indexes (Figure 3.2). Compared with baseline, inverse Simpson index diversity increased by a mean (SD) of 0.14 (0.06) points during ibezapolstat therapy (P = .02) and by 0.22 (0.10) points after the EOT (P = .003).

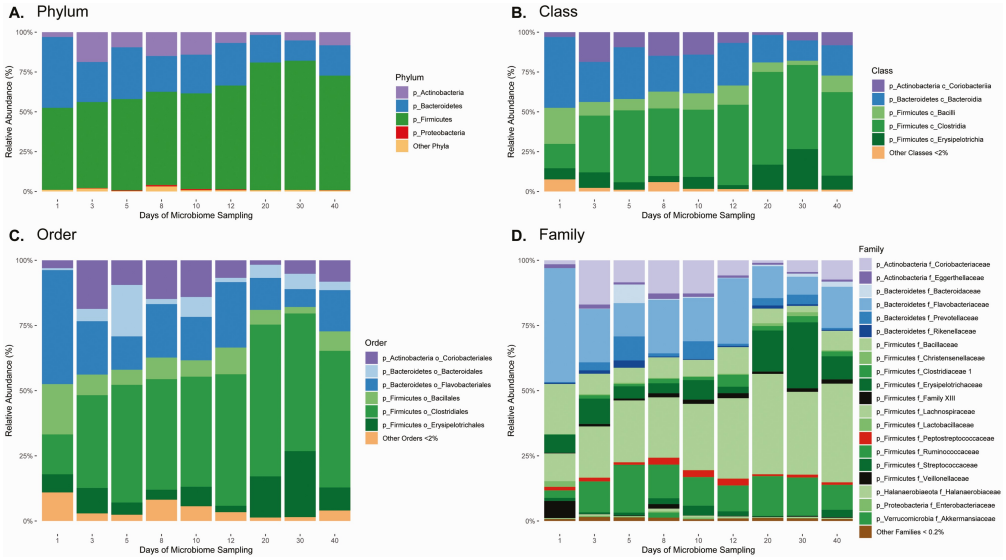

Figure 3.3: Effects of ibezapolstat on relative abundance of taxa by phylum (A), class (B), order (C), and family (D). From: Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial.[589]

Similar results were observed using the Shannon diversity index; diversity increased by a mean (SD) of 0.98 (0.48) points during ibezapolstat therapy (P = .049) and by 1.7 (0.87) points after the EOT (P = .04), compared with baseline. Taxa changes during and after ibezapolstat therapy are shown in Figure 3.3. A proportional decrease in Bacteroidota phylum was observed (mean change [SD], −10.0% [4.8%]; P = .04), most commonly owing to a decreased proportion of Bacteroidia class taxa (−10.0% [4.8%]) and Flavobacteriaceae family taxa (−8.8% [4.8%]). An increased proportion of Bacillota phylum was observed (mean change [SD], +14.7% [5.4%]; P = .009), most commonly owing to an increased proportion of *Lachnospiraceae* (+12.7% [6.0%]) and Oscillospiraceae (+2.8% [2.7%]). Other Bacillota had decreased proportions, most notably Bacillales (mean change [SD], −4.4% [2.3%]) and Lactobacillales (−3.7% [2.2%]) order taxa. Abundance tables for individual patients are shown in Supplementary Figure 3.4.

Results of the bile acid analysis are shown in Figure 3.5. Compared with baseline, total primary acid concentrations in stool samples decreased by a mean (SD) of 40.1 (9.6) ng/mg stool during therapy (P < .001) and 40.5 (14.1) ng/mg stool after the EOT (P = .007). Compared with baseline, total secondary bile acid concentrations increased by a mean (SD) of 65.6 (146.7) ng/mg stool during therapy (P = .66) and 97.5 (215.4) ng/mg stool after the EOT (P = .65).

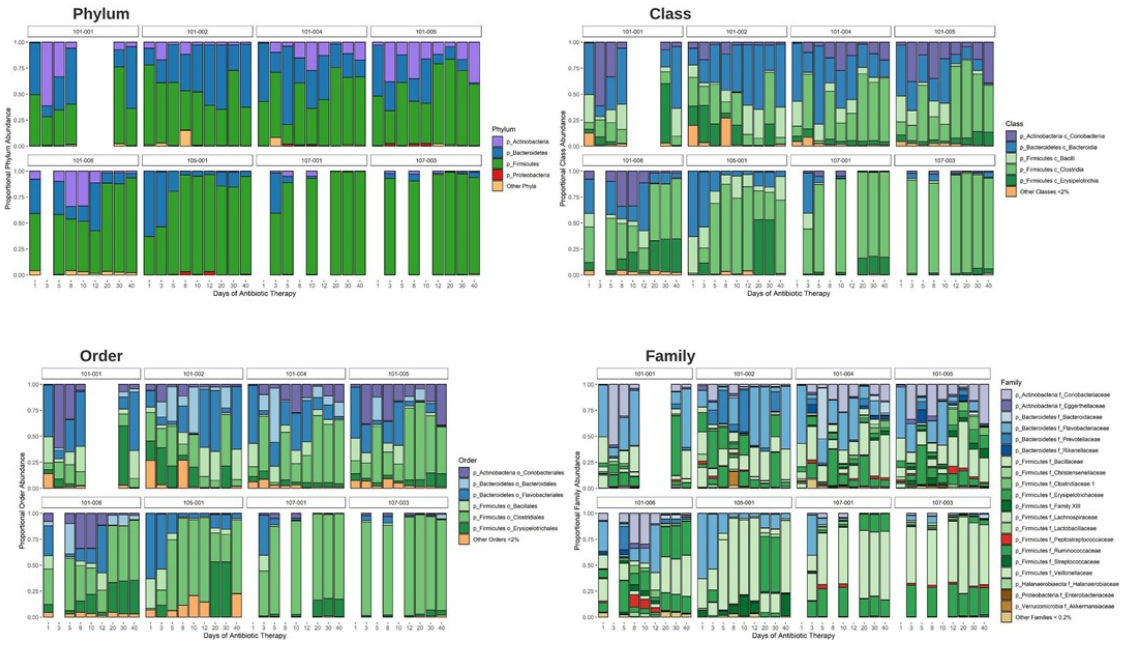

Figure 3.4: Participant-level view of ibezapolstat's impact on microbiota. The panels are different taxonomic levels of the same dataset, such that bacterial phyla (top-left), class (top-right), order (bottom-left), and family (bottom-right) show the participant-specific changes in *Actinomycetota* (purple); *Bacteroidota* (blue); *Bacillota* (green); *Peptostreptococcaceae* (red) through time. From:Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial [589]

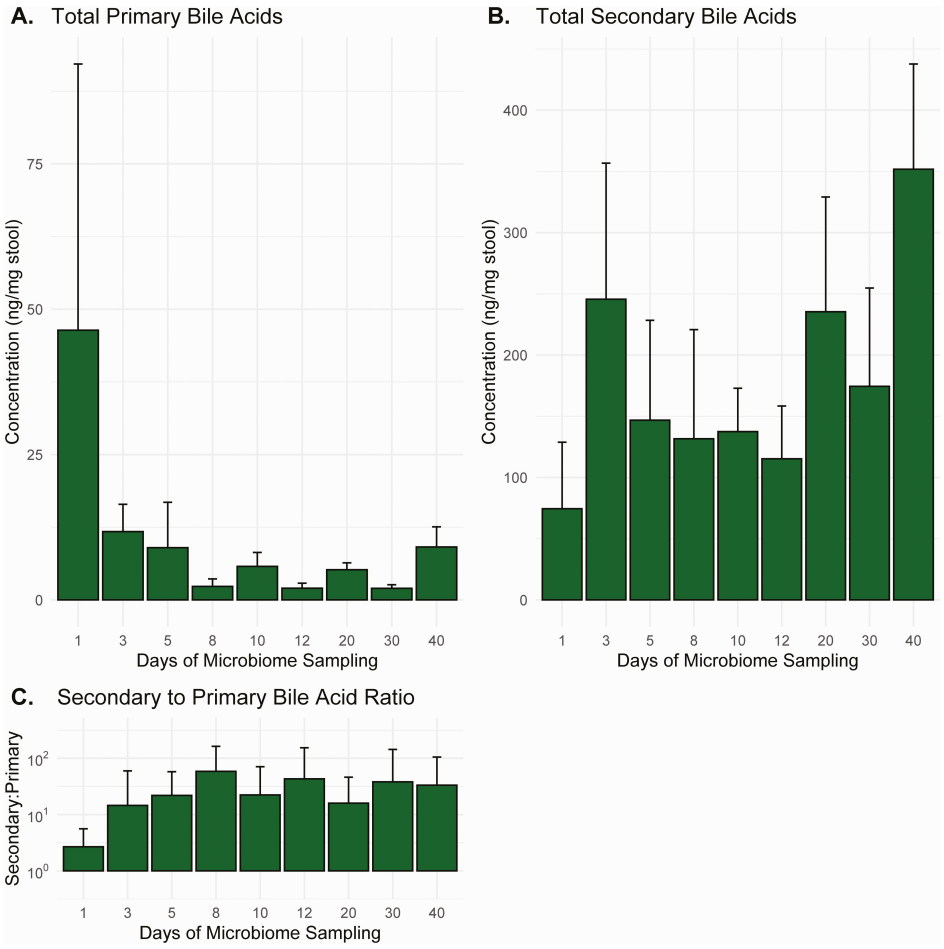

Figure 3.5: Bile acid changes over time. Changes in primary (A) and secondary (B) bile acid concentrations and the ratio of secondary to primary bile acid concentrations (C). Values represent means with standard errors. From:Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial [589]

Efficacy Outcomes

The initial clinical cure rate at the EOT was 100% (10 of 10 patients). The mean time to resolution of diarrhea was 5 days (range, 3–7 days). The SCC rate at 28 ± 2 days after the EOT was also 100% (10 of 10 patients).

Conclusion

In this open-label, phase 2a study, ibezapolstat was well tolerated and had a safety profile consistent with results from the phase I study.[591] PK findings were also similar to those seen in the healthy volunteer study. Ibezapolstat achieved high stool concentrations and plasma concentrations that did not exceed 1 ug/mL. Favorable changes to the microbiome were observed, most notably C. difficile eradication by day 3 and an increased proportion of healthy microbiota, including Eubacteriales order taxa known to metabolize primary bile acids to secondary bile acids via the 7α-dehydroxylation pathway.[651] These proportional changes were associated with bile acid changes, including a reduction in primary and an increase in secondary bile acids during ibezapolstat therapy, predictive biomarkers of a lower chance of CDI recurrence. Finally, clinical efficacy evaluations demonstrated that 100% of the 10 patients experienced initial clinical cure and SCC evaluated at 28 days.

There are currently only 2 Food and Drug Administration–indicated antibiotics for the treatment of CDI: vancomycin and fidaxomicin.[408] As resistance to both these antibiotics has been noted, new CDI-directed antibiotics are urgently needed.[469,643] The first-in-class gram-positive selective-spectrum antimicrobial ibezapolstat is a novel PolC-type DNA Polymerase III inhibitor with a unique mechanism of action that targets low–G + C content gram-positive bacteria.[652]

The microbiome studies in this phase 2a study provide additional insight into the effect of ibezapolstat on a mixed bacterial community such as the gut microbiome. Because ibezapolstat has no direct activity on gram-negative organisms, the decrease in Bacteroidota phylum was perhaps a secondary result of ibezapolstat's effect on other targeted gram-positive bacteria. Likewise, the increased proportion of the

favorable Eubacteriales order taxa demonstrates ibezapolstat selectivity within low–G + C content bacteria. PolC-type DNA Polymerase III is thought to be present in most Bacillota, and the reasons for this selectivity will require further study.

Although the current study is underpowered to statistically evaluate secondary bile acid changes, an increased concentration of secondary bile acids was observed during and after ibezapolstat therapy, which is known to be correlated with colonization resistance against C. difficile.[403] In addition, the decrease in primary bile acids and the favorable increase in the ratio of secondary to primary bile acids suggest that ibezapolstat may reduce the likelihood of CDI recurrence compared with vancomycin. Phase 2b and 3 studies will allow further investigations of these mechanistic findings. Finally, although favorable efficacy results were demonstrated, these will need to be validated in a larger population using a double-blind study design.

In conclusion, in the current study, ibezapolstat was well tolerated in adults with CDI and demonstrated a PK profile ideal for a CDI antibiotic with low systemic absorption and high colonic concentrations. Advantageous microbiome abundance and bile acids changes coupled with successful efficacy data support the continued development of ibezapolstat for use in the adult CDI population.

It is not difficult to make microbes resistant to penicillin in the laboratory... and the same thing has occasionally happened in the body.

– Alexander Fleming

4

The microbiome-restorative potential of ibezapolstat for the treatment of *Clostridioides difficile* infection is predicted through variant PolC-type DNA polymerase III in Lachnospiraceae and Oscillospiraceae

Introduction

Clostridioides difficile is the most common healthcare-associated pathogen in the United States and causes C. difficile infection (CDI) affecting approximately 500,000 patients per year.[7] The pathogenesis of CDI involves disruption of a healthy gut microbiome leading to a dysbiotic environment enabling C. difficile spores to germinate and cause disease. Antibiotics used to treat CDI can also further disrupt the microbiome contributing to high rates of disease recurrence. Of the two guideline-recommended antibiotics for the treatment of CDI, the RNA polymerase II inhibitor, fidaxomicin (FDX) is a more narrow-

spectrum antibiotic on healthy gut commensal organisms than vancomycin (VAN), a glycopeptide antibiotic that inhibits D-ala-D-ala cell-wall synthesis. In head-to-head comparison, FDX was shown to be superior to VAN in the prevention of recurrent CDI (rCDI).[451,454,653] This supports drug discovery efforts to identify drug targets that kill C. difficile without affecting the healthy gut commensal organisms.

Ibezapolstat (IBZ; formerly ACX-362E) is a first-in-class antibiotic that targets the PolC-type DNA polymerase III alpha-subunit (PolC) found in *Bacillota* and not in other important human gut microbiota phyla including *Actinomycetota*, *Bacteroidota*, or *Pseudomonadota*. IBZ has completed phase 2 clinical trials for the treatment of CDI. Data from the phase 1–2 clinical trials showed IBZ minimally disrupted certain *Bacillota*, specifically Lachnospiraceae, *Oscillospiraceae* (formerly *Oscillospiraceae*), and *Coprobacillaceae* within *Erysipelotrichales* despite also having the PolC.[588,589,591] The reason for this unexpected IBZ sparing of select commensal *Bacillota* is unknown. We hypothesized that polymorphic differences in PolC among different G + C species would influence IBZ spectrum of activity. In silico studies have discovered antibiotic mechanism of action for targeted antibiotics for the fatty acid synthesis protein enoyl-ACP reductase II (FabK).[654] In this regard, we utilized in silico methods to better understand this narrower than expected spectrum of activity of IBZ for *Lachnospiraceae*, *Oscillospiraceae*, and *Erysipelotrichaceae*/*Coprobacillaceae* during therapy.

Methods

Phylogenetics of PolC Variations

Protein Sequence Acquisition and Phylogenetic Tree Construction

Genomic analyses were performed in the CLC Genomics Workbench version 24.0 (Qiagen). A custom microbial database was built comprising 620 RefSeq-deposited reference and representative complete genome assemblies within the phylum *Bacillota*. From each genome, the polC gene was extracted using their automated homology annotations, resulting in 1,113 gene sequences. These 1,113 genes were trans-

lated to their respective protein sequences using their coding sequence (CDS) track annotations, resulting in 1,158 protein sequences. These 1,158 protein sequences were further annotated via HMMER (v.3.1b1; May 2013) with Pfam (v.35.0) functional domains to identify the PolC-defining RNaseT (PF00929.27) inserted within the Polymerase and Histidinol Phosphatase (PHP) domain (PF02811.22). Manual screening for protein sequences for this RNaseT insertion within the PHP domain resulted in 620 final PolC sequences (9–11).[655–657] These 620 PolC sequences were aligned with the CLC Genomics Workbench "Create Alignment" tool (v.1.02) using the very accurate multiple sequence alignment (MSA) algorithm, a gap open cost = 10.0, gap extension cost = 1.0, end gap cost = "as any other," re-do alignments = "no," and use fixpoints = "no." The resulting MSA served as input for the "Create Tree" tool in CLC Genomics Workbench using the Neighbor-Joining algorithm, the Jukes-Cantor distance measure, and 100 replicates of Bootstrapping. The resulting phylogenetic tree was subsequently visualized as a circular phylogram with color-coded to taxonomic family, and node annotations of clinically relevant families. Leaves were manually annotated with general susceptibility to IBZ as either generally IBZ non-susceptible (red), or generally IBZ susceptible (green) based on the results of our metagenomic studies.[588,589]

Protein Structure Prediction

In the absence of clinically relevant three-dimensional protein structure data, AlphaFold2[658] was used to predict the structure of the PolC-type DNA polymerase III (protein ID = CBE03476.1) from the polC gene (gene = dnaF; locus tag = CDR20291_1146) of the C. difficile strain R20291 (NCBI accession = NC_013316.1; GenBank = FN545816.1; RefSeq Assembly = GCF_000027105.1). Using C. difficile strain R20291 PolC (CdiPolC) protein sequence, the three-dimensional structure was predicted via a ColabFold (Google) colab notebook.[659] Relevant ColabFold parameters include MSA_method = MMseqs2,[660] pair_mode = "unpaired," num_relax = 0, use_ptm = "True," rank_by = "pLDDT" (predicted local distance difference test), num_models = 5, num_samples = 1, num_ensemble = 1, max_recycles = 3, is_training = "False," and use_templates = "False." Output quality metrics of prediction accuracy include the MSA coverage, predicted contacts, predicted distograms, predicted alignment error (PAE), the predicted local distance difference test (pLDDT), and a settings log file.

Molecular Docking

The top-ranked AlphaFold2 CdiPolC structure (model 3, pLDDT: 88.5, and pTMscore: 0.7481) served as the input for parallel structure- and template-blind molecular docking of IBZ (PubChem Conformer3D_CID_136022209). The best binding pose was detected using structure- and template-based docking via the CB-Dock2[549] server that combines CurPocket[661] curvature-based cavity detection with AutoDock-Vina[546,662] blind docking of the three-dimensional IBZ conformer. The five top-ranked binding poses in CdiPolC cavities were produced, ranked by Vina Score and cavity volume. Visual inspection of the CdiPolC•IBZ complexes was performed in (Schrodinger) Maestro[663,664] and UCSF ChimeraX.[547]

Binding Pocket Homology Modeling

Using prior knowledge of Bacillus subtilis azp12 strain resistant to IBZ-predecessor compounds,[551] the third rank docked complex was modeled homology modeling of the IBZ binding site near this same active site. The CdiPolC residues that mediated good contacts with IBZ were visually identified using Schrodinger Maestro, and further confirmed by the protein-ligand interaction profiler.[665] Following the identification of contact residues, conservation analysis of these residues across two MSAs was performed using CLC Genomics (Qiagen). First, the conservation per residue was analyzed across the same 620 PolC amino acid sequence used above. Second, the conservation of these residues was modeled across 16 representative PolC from 16 clinically relevant species. Data were presented using sequence logos generated via WebLogo.[666] Figures were made using BioRender.

Results

Phylogenetic Relatedness of Bacillota PolC

An amino acid phylogenetic tree was constructed for the PolC from 620 representative *Bacillota* species (Figure 4.1). The tree was annotated with color-coded taxonomic families from the NCBI (col-

ored branches, nodes, outer ring, and name). The intra-phylum phylogenetic relatedness of PolC coincided with the established 16S rRNA evolutionary determinants of taxonomy. Visualization of the tree identified a section of one PolC clade that largely consisted of the *Eubacteriales* (formerly Clostridiales; coined Clade 3) that contained the IBZ-sparing *Lachnospiraceae* , *Oscillospiraceae*, and *Erysipelotrichales*. Another section of Clade 3 PolC with a root taxonomic family of *Thermoactinomycetaceae* (cerise magenta) contained *Clostridiaceae* (*C. butyricum*, *H. histolytica*, *C. septicum*, and *C. sporogenes*) and *Peptostreptoccaceae* (*C. difficile* and *Paeniclostridium* sordellii) that were generally killed during IBZ-therapy in the phase 2 clinical trial.

Molecular Structure Analysis

To further understand the predicted pharmacological affinity of IBZ, in silico template-based cavity detection and structure-based molecular docking via CB-Dock2 were used to identify the binding site pocket and best pose of PolC for C. difficile, *Lachnospiraceae* , *Oscillospiraceae*, and *Erysipelotrichales*. A three-dimensional predicted protein structure of the PolC from C. difficile strain R20291 (CdiPolC) was generated using a ColabFold notebook running python 3.10 on a Google Cloud A10 GPU using the MMseqs2 sequence alignment algorithm.(Figure 4.2) The AF2_CdiPolC quality metric of the pLDDT showed a drop in model confidence around positions 180–200, corresponding to a 20-residue stretch of residues preceding the Exo domain. Otherwise, the majority of the AF2_CdiPolC had a high level of model confidence (average pLDDT 88.5), including the oligonucleotide binding (OB) domain, the duplex binding (DB) domain, and the polymerase palm, thumb, index, and middle fingers. Given the global confidence of AlphaFold2 in the predicted AF2_CdiPolC, the top-ranked model was docked to IBZ using CB-Dock2 using parallel CurPocket for cavity detection and AutoDock-Vina for virtual docking. Upon visual inspection of the top five docked complexes, one complex whose binding pocket was close to the enzymatic active site of the Polymerase palm where oligonucleotide extension occurs was used for further study. This complex was chosen for further study due to the proximity to the enzymatic active site and prior evidence that the B. subtilis azp12 mutant PolC identified a single-amino acid change near this site that confers resistance to the azopyrimidine predecessor compound to IBZ, 6-(p-hydroxyphenylazo)-uracil (HPUra) (22, 25).[551,667]

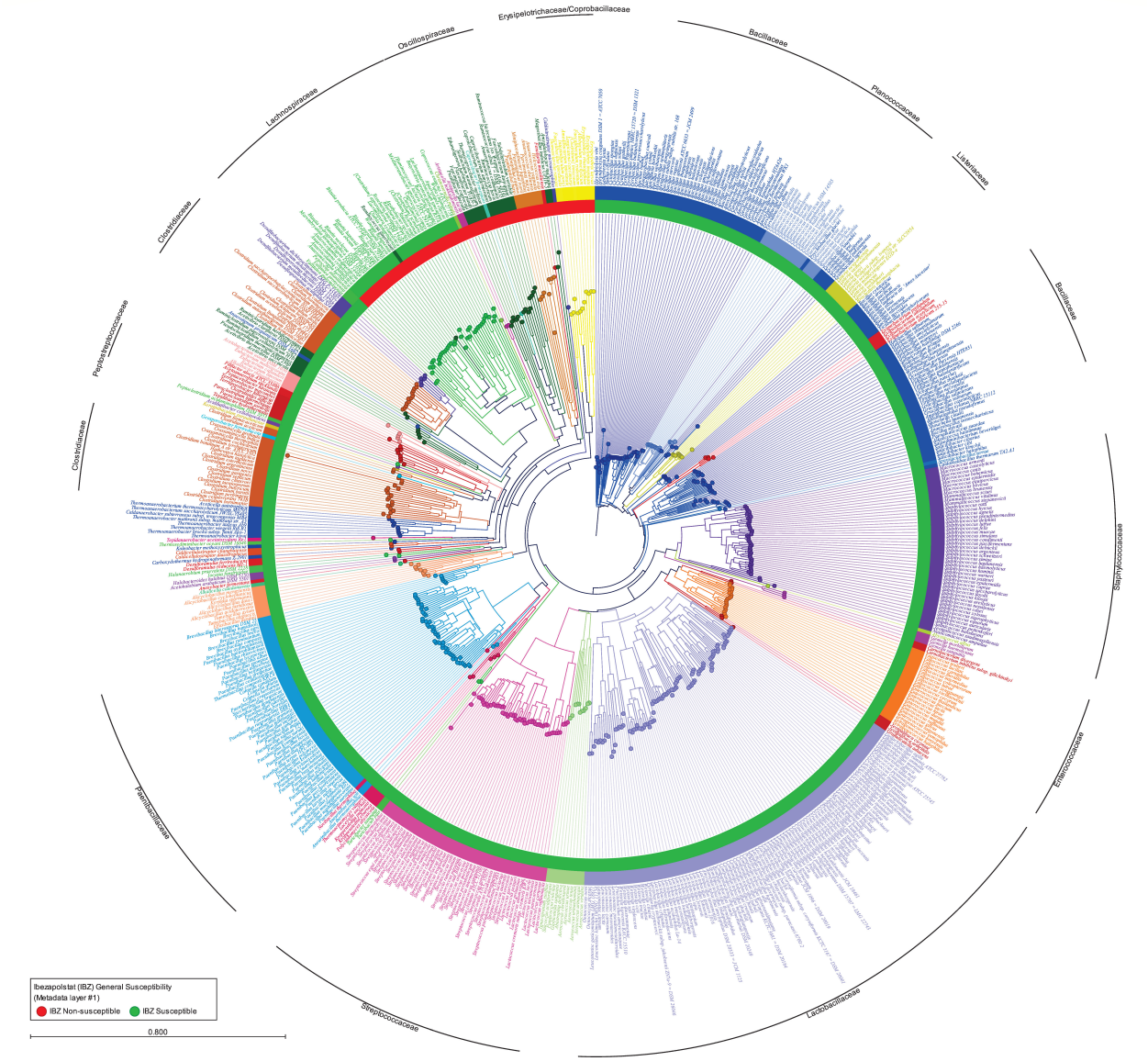

Figure 4.1: Phylogenetic Conservation of the PolC across the *Bacillota* phylum. From: The microbiome-restorative potential of ibezapolstat for the treatment of Clostridioides difficile infection is predicted through variant PolC-type DNA polymerase III in *Lachnospiraceae* and *Oscillospiraceae*.[590]

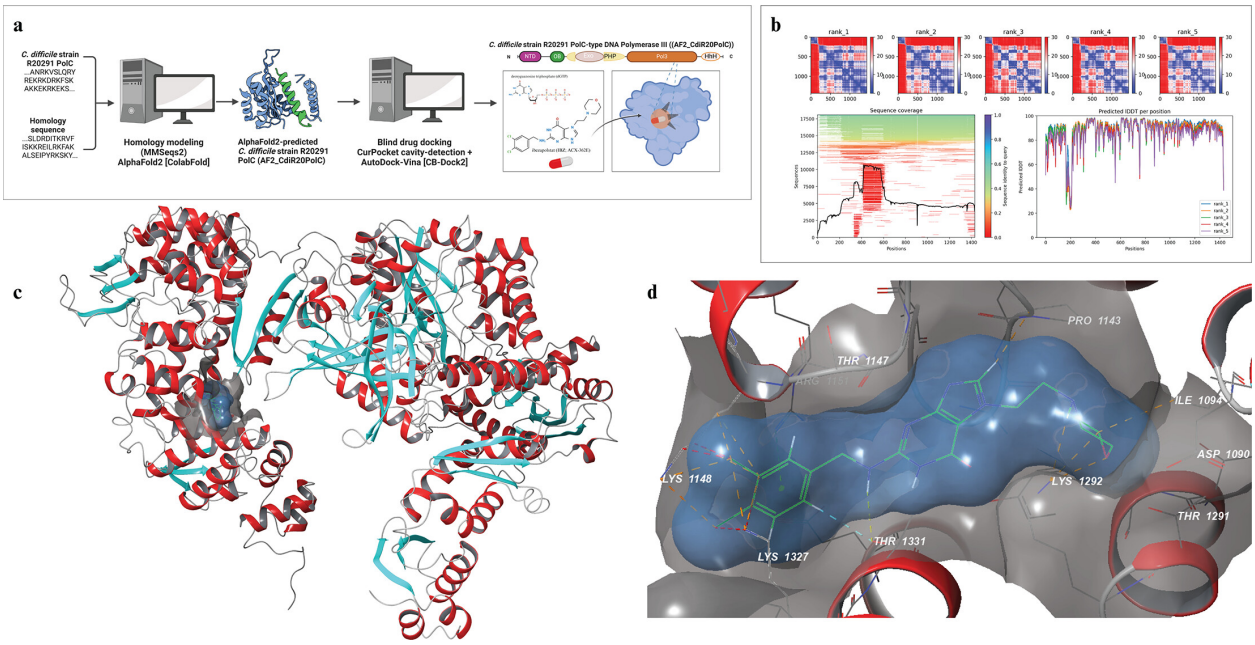

Figure 4.2: AlphaFold2-based docking with AutoDock-Vina finds the binding pocket and pose of ibezapolstat to the C. difficile PolC. From: The microbiome-restorative potential of ibezapolstat for the treatment of Clostridioides difficile infection is predicted through variant PolC-type DNA polymerase III in *Lachnospiraceae* and *Oscillospiraceae*.[590]

Homology Modeling of the PolC Binding Pocket of IBZ

Visual inspection of the IBZ•AF2_CdiPolC complex using Maestro (Schrodinger) identified PolC residues within 5 Å of IBZ (Figure 4.3). Notably, several residues mediated the binding pocket, but only a fraction provided a phylogenetically conserved explanation for the observed IBZ PolC narrower spectrum of activity. First, upon visual inspection, two distantly encoded but closely positioned lysine residues, CdiPolCLys1148 and CdiPolCLys1327 were identified near the N2-substituted ((3,4-dichlorophenyl)methyl)amino functional group of IBZ. This would allow electrostatic interaction between the negatively charged chlorines and positively charged nitrogen to lock the two lysine residues in a rare (<10%) rotamer conformation that could bind and hold IBZ. These two lysine "gates" across 16 representative species PolC demonstrated that CdiPolCLys1327 was a highly conserved residue throughout representative PolC species, except Blautia coccoides and C. scindens (*Lachnospiraceae*), *C. leptum* (*Oscillospiraceae*), and *Thomasclavelia ramosa* (*Coprobacillaceae*). The second lysine "gate," CdiPolCLys1148, was also a highly conserved residue that followed an evolutionary selection of positively charged arginine or lysine across the *Bacil-*

lota phylum except for the *Lachnospiraceae* (negatively charged aspartate), the *Oscillospiraceae* (histidine residue), and the *Coprobacillaceae* (methionine residue). The CdiPolCThr1327 predicted binding to the polar hydrogens of the IBZ central guanine moiety and via the formation of a hydrogen bond network may have an IBZ "anchoring" interaction. This residue is conserved throughout the 16 representative species except for the *Lachnospiraceae* , *Oscillospiraceae*, and *Erysipelotrichales*. Finally, the N7-substituted (2-(4-morpholinyl)ethyl) IBZ functional group was in proximity to a handful of potentially interacting residues including CdiPolCThr1291, CdiPolCLys1292, CdiPolCIle1094, and CdiPolCAsp1090. Possibly, the morpholino group of IBZ may interact with CdiPolCThr1291 in a C. difficile-nearly specific manner that also locks the residue in a rare rotamer conformation. Phylogenetically, CdiPolCThr1291 aligned across the 16 representative species reveals a phylum-conserved preference for aliphatic residues valine or isoleucine, including B. coccoides (*Lachnospiraceae*) and C. leptum (*Oscillospiraceae*). This residue, CdiPolCThr1291, may be a C. difficile-specific sensitizer residue specifically or perhaps for other *Peptostreptococcaceae*. T. ramosa also has threonine at this relative position, which may balance its sensitivity to moderately de-sensitized to IBZ, second to *Lachnospiraceae* and *Oscillospiraceae*. Finally, aspartates are considered the catalytic residues of DNA synthesis reactions, the proximity of IBZ to CdiPolCAsp1090 may explain the competitive inhibition observed in prior studies of steady-state C. difficile PolC kinetics (26).[613] Taken together, the homology modeling of the binding pocket of PolC predicted several variant residues that confer IBZ non-susceptibility in *Lachnospiraceae* , *Oscillospiraceae*, and *Erysipelotrichaceae*/*Coprobacillaceae*.

Conclusion

IBZ is a PolC-type DNA polymerase III alpha-subunit (PolC) inhibitor currently in clinical development for the treatment of CDI. During clinical trials, a narrower than expected spectrum of activity was observed that included increased proportion of certain key microbiota of the *Bacillota* phylum known to confer health benefits, specifically *Lachnospiraceae* , *Oscillospiraceae* (formerly *Oscillospiraceae*), and *Coprobacillaceae* within *Erysipelotrichales*. The purpose of this study was to use in silico techniques

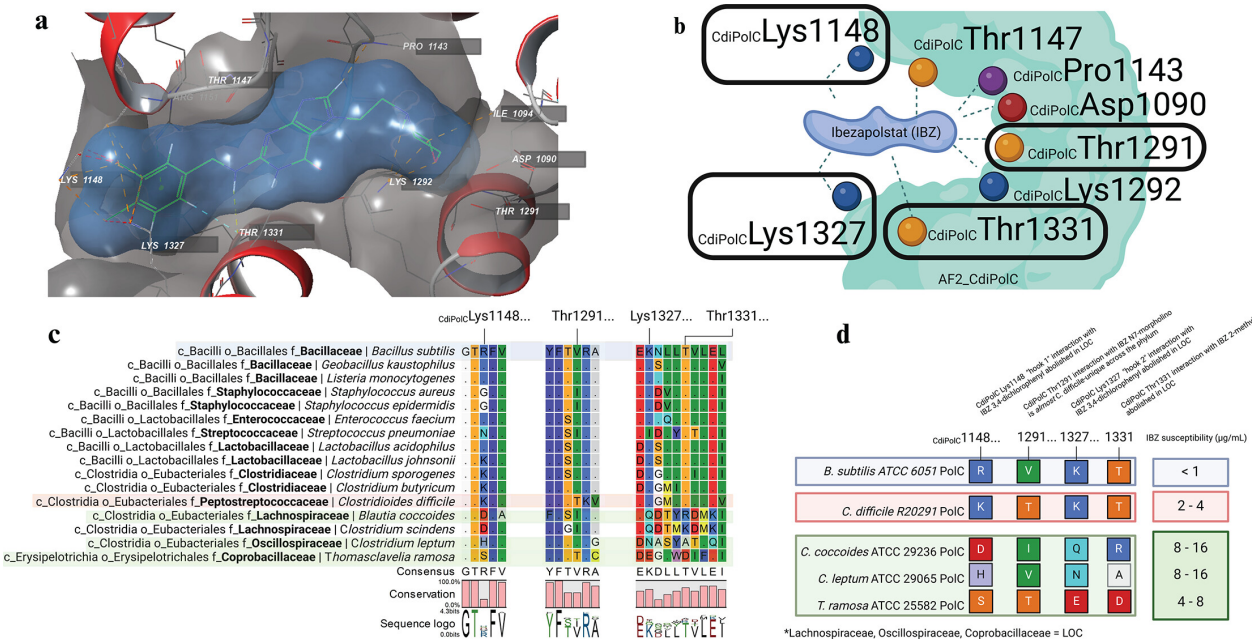

Figure 4.3: Binding Pocket Differences Associated with Reduced Ibezapolstat Susceptibility. From: The microbiome-restorative potential of ibezapolstat for the treatment of Clostridioides difficile infection is predicted through variant PolC-type DNA polymerase III in *Lachnospiraceae* and *Oscillospiraceae*.[590]

to hypothesize the mechanism underlying this finding. Recent evidence combining structural biology and phylogenetics of the fidaxomicin-RNAP II interaction identified a single polymorphic "sensitizer residue" at the RNAP β (K84) sufficiently confers a more narrow-spectrum of activity to only two of the four phyla of the human gut microbiota (27).[368] We hypothesized that a similar phenomenon could be responsible for the narrow-spectrum activity of IBZ within the *Bacillota* phylum. The major finding of this study was that the predicted pharmacophore ensemble of interactions between IBZ and PolC (PolC•IBZ) is conserved across the majority of the *Bacillota* phylum except for *Lachnospiraceae* and *Oscillospiraceae*, and *Erysipelotrichales* (including Erysipelotrichaceae and *Coprobacillaceae*), taxa that were not killed or regrown in IBZ-treated subjects while on therapy. Within this taxa, residues that were predicted to be phylogenetic variants that may ablate key PolC•IBZ interactions were: two lysine "gates" (CdiPolCLys1148 and CdiPolCLys1327) that are predicted to "latch" onto the compound; an "anchoring" interaction (CdiPolCThr1331) to the central moiety; and a stabilized set of C. difficile sensitizer residues (CdiPolCThr1291 and CdiPolCLys1292) that may result in the prolonged inhibition of a catalytic residue (CdiPolCAsp1090). While these results will need to be confirmed in experimentally determined structures

and molecular genetic approaches, they provide a working hypothesis for the selective narrow spectrum of activity of IBZ.

This study has certain limitations. We used AlphaFold2 with AutoDock-Vina to in silico predict key binding site residues IBZ. Despite the accuracy of AlphaFold2 to predict structures from millions of structures from the primary amino acid structures, there are still limitations of the tool. There are only three deposited PolC structures from one species (PDB 3F2B, 3F2C, and 3F2D, *Geobacillus kaustophilus*) for model training. IBZ MICs were higher for commensal bacteria than C. difficile; however, these results will need to be confirmed in larger studies using clinical isolates. These results need to be confirmed structurally using the C. difficile PolC and in enzymatic, molecular interaction, and cellular genetic assays. Finally, whether the regrowth of these *Lachnospiraceae* and *Oscillospiraceae* in IBZ treatment subjects confers a health benefit will require further study.

In conclusion, our in silico model predicts that the, in vivo, observed IBZ sparing of *Lachnospiraceae* , *Oscillospiraceae*, and *Erysipelotrichaceae*/*Coprobacillaceae* is due to phylogenetically variant PolC•IBZ binding pocket residues. Further in vitro studies that confirm a PolC structural basis for the IBZ narrower than expected activity needed to confirm these in silico findings.

I told my three sons stories about germs more than fifty years ago as fanciful bedtime tales

– Arthur Kornberg

5

Globally circulating Clostridioides difficile are Devoid of Mutations Associated with PolC Inhibitor Resistance

Introduction

Understanding how the human gut microbiome influences human health is an incredibly exciting and challenging field of biomedical science. The ecological diversity of bacteria of the human gut microbiome (microbiota) provide a vast breadth, depth and diversity of host-microbe and microbe-microbe interactions. For example, how oral antibiotics destroy the microbiota, predisposing a host to pathobiont and pathogenic infections, is an area of active research often called 'colonization resistance'. One leading human gut pathobiont, *Clostridioides difficile*, is the quintessential dysbiosis infectious disease. CDI pathogenesis often occurs by loss of microbiota diversity upon receipt of oral broad-spectrum antibiotics that reduces host colonization resistance, *C. difficile* toxin-mediated damage of host intestinal epithelium, and resulting life-threatening infectious diarrhea that spurs the fecal-oral route of transmission. The limited therapeutic options for the treatment of CDI is a public health threat that warrants the development of narrow-

spectrum, *C. difficile*-active oral antibiotics that preserve the bacteria of microbiome, or microbiota, in the human gut. Currently available guideline recommended antibiotics for the treatment of CDI include broad- and narrow-spectrum agents, vancomycin and fidaxomicin, respectively. To develop microbiota-sparing antibiotics, the ideal druggable target would be present in a fraction of microbiota, providing a phylogenetically restricted basis for narrow-spectrum activity. One such target, the PolC-type DNA polymerase III (PolC), is phylogenetically found in the bacterial phylum *Bacillota* and not other common gut bacterial phyla, including Pseudomonadota, Bacteroidota, and Actinomycetota. Biologically, the PolC is the essential catalytic subunit of the twelve-subunit DNA replisome that conducts template-directed DNA synthesis during DNA replication. Pharmacologically, the PolC has been studied for its role as a potential drug target for the development of nucleotide analogs as Gram-positive selective spectrum (GPSS) antibiotics for the treatment of common Gram-positive bacterial infections. Recent evidence suggests the *C. difficile* PolC is inhibited by PolC inhibitor nucleotide analogs, such as the dichlorobenzylguanine (DCBG) ibezapolstat (IBZ) through a competitive mode of inhibition (Torti 2011). Microbiological studies found IBZ inhibits the *C. difficile in vitro* with relatively low minimum inhibitory concentration (MIC) values in the range of 2 - 4 μg/mL, including 67 strains of multi-drug resistant C. difficile. Ibezapolstat (IBZ) is a semisynthetic small-molecule inhibitor of the PolC-type DNA Polymerase III (PolC) that belongs to the chemical class of dichlorobenzylguanine (DCBG) analogs. The chemical structure of IBZ includes a central guanine moiety with an N7-morpholino-ethyl and an N2-dichlorbenyzl (Figure 5.1). Clinically, dose finding studies have shown IBZ has a low bioavailability *per oral* administration with average plasma concentrations of less than 1 μg/mL and average stool concentrations of 2,000 μg/g (Garey 2020). Metagenomic studies performed on healthy human subjects and CDI patients treated with *per oral* IBZ have shown a preservation of key commensal microbiota responsible for coordinating host colonization resistance to *C. difficile*, including *Lachnospiraceae* and *Oscillospiraceae* (formerly *Oscillospiraceae*) (McPherson 2022; Garey 2022). Comparative modeling studies suggest of the *Bacillota* PolC have shown family-level differences in the PolC active site and proposed IBZ binding site may be correlated with reduced susceptibility in *Lachnospiraceae* and *Oscillospiraceae* (formerly *Oscillospiraceae*) at the PolC lysine 1327, lysine 1148, and threonine 1331 associated IBZ resistance (McPherson 2025). Taken together, these studies suggest the IBZ inhibition of the phylogenetically restricted PolC will not only treat CDI but also promote host

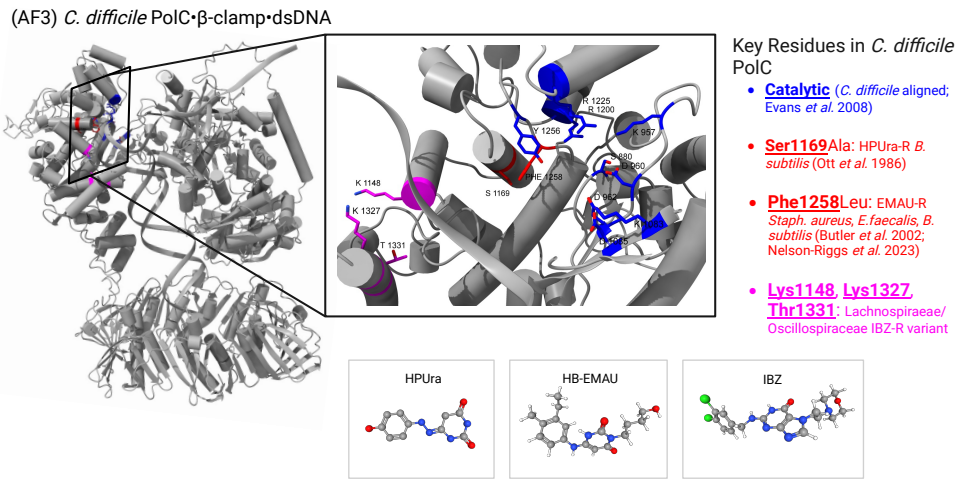

Figure 5.1: Currently Known or Suspected Determinants of PolC Inhibitor Resistance.

colonization resistance through sparing and promotion of the commensal microbiota. These findings in narrow-spectrum antibiotic development for the treatment of CDI are encouraging, however little is known about the susceptibility of globally circulating *C. difficile* to IBZ. This observation poses the question, how might IBZ resistance arise or already be present in *C. difficile*? How widespread is IBZ resistance in *C. difficile*? To address these clinically relevant questions, we applied previously reported findings of resistance to related PolC inhibitor resistance in laboratory strains of related *Bacillota*. With these data, we used AlphaFold3 homology models and publicly available genomic data to characterize the conservation and phylogeography of the *polC* from globally circulating *C. difficile*.

Methods

Genomic sequence acquisition, annotation, and alignment of the key polC residues associated with PolC inhibitor resistance to that of *C. difficile* was performed in the commercial bioinformatics software, CLC Genomics Workbench (Qiagen). We validated the changes in the HPUra-resistant *B. subtilis* strain azp12 (parent strain 168) partial polC [NCBI accession M33543.1] and complete polC [NCBI M22996.1] against the polC [locus BSU_16580] from the *Bacillus subtilis* parent strain 168 complete genome [NCBI accession NC_000964.3]. Consistent with the prior experimental findings, the EMAU-resistant polC [lo-

cus CFC57_RS06555] previously identified in *Staphylococcus aureus* ATCC 13709 'Smith' strain [NCBI accession CP029751.1] (Panthee et al 2020) was translated and manually annotated to match the serine 1261 leucine substitution previously reported (Barnes 2002; Nelson-Rigg 2023). To unify these findings, EMAU-resistant *Staph. aureus* PolC serine 1261 was aligned with to *B. subtilis* strain 168 PolC phenylalanine 1264. Third, the amino acid differences associated with IBZ-resistant *polC* from *Blautia coccoides* ATCC 29236 (representative *Lachnospiraceae*) [NCBI accession CP136422.1, locus BLCOC_RS06105] and *Clostridium leptum* DSM 753 (representative *Oscillospiraceae*; formerly *Oscillospiraceae*) [NCBI accession ABCB02000020.1, locus CLOLEP_02966]. Finally, we aligned these positions to the PolC from *Clostrdioides difficile* epidemic ribotype NAP1/B1/F027 strain R20291 [NCBI accession NC_013316.1, locus CDR20291_RS06355].

DNA sequence acquisition, alignment and translation of the amino acid changes in focus from that of *B. subtilis* strain 168 (model Gram-positive bacterium), *C. difficile* R20291 strain (pathobiont of interest), *Blautia coccoides* ATCC 29236 (a representative *Lachnospiraceae*) and *Clostridium leptum* ATCC 29065 (a representative Oscillospiraceae) were performed using CLC Genomics Workbench (CLC) version 25 (Qiagen). AlphaFold3 (DeepMind AlphaFoldServer) predicted homology models of the PolC•β-clamp•dsDNA macromolecular complex from the above four species were used to estimate the general proximity of these five important residues. UCSF ChimeraX was used to superimpose and inspect the relative positions of residues associated with PolC inhibitor resistance.

We analyzed 32,938 *Clostridioides difficile* (NCBI txid1496) nucleotide sequences comprising approximately 22,000 genomes of any level of assembly. From these nucleotide sequences, we extracted 1,482 *polC* coding sequences (CDS) using the 'extract annotated regions' tool for annotations including the annotation 'polC' from automated homology annotations via the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Of note, the above 218 *polC* sequences from 'circular' genomes are included among these 1,482 sequences, for their inclusion in further conservation analysis. Of these 1,482 *polC* sequences, 1,258 *polC* sequences with 4,299 nucleotide length and the *polC*-distinguishing insertion of an exonuclease into the polymerase and histidinol phosphatase domain; 14 *polC* sequences were of length 4,297, 4,298 or 4,300 nucleotide length were excluded. These 1,258 *polC* sequences of length 4299 nucleotide and their

translations were included for conservation analysis. These sequences were aligned using the algorithm described above and their conservation data was visualized using R (R Core Team).

Bioinformatic sequence retrieval, typing, extraction, annotation, alignment, and tree building was performed in CLC. From the 32,938 nucleotide sequences include 240 'circular' genomes with complete assembly and previously annotated by automated sequence homology via the PGAP as Clostridioides difficile (NCBI txid1496). From these complete sequences, 226 'polC' coding sequences (CDS) of 4,297 to 4,300 nucleotide length were extracted using the tool 'extract annotated regions'. These 226 CDS were further annotated with 'annotated CDS with PFAM domains' with PFAM A-v35. Manual inspection of these 226 annotated CDS led to the removal of 8 of 226 CDS that are annotated as 'polC' but were not only much shorter (average nucleotide length of 500 versus the consensus 4299 nucleotide length) but also lacked the PolC-distinguishing feature of an exonuclease domain [[PF00929]] inserted into the polymerase and histidinol phosphatase domain [CD07435]. We included one CDS of 4,297 nucleotide length (locus RHN71_06505 CP133824) and two CDS of 4,300 nucleotide length (RHN80_06735 CP133827; *polC* CP026597) for their consistency with these parameters; these three non-4,299 CDS were also included in the fourteen CDS analyzed for insertion/deletion codon analysis. These 218 *polC* sequences were aligned using a Smith-Waterman-based algorithm with a gap open cost of 10.0, gap extension cost of 1.0, and end gap cost as any other. Manual dataset cleaning of these sequences for country, year and host-organism of sample collection was performed. Phylogenetic tree construction used Neighbor Joining and Jukes-Cantor nucleotide distance with 100 replicates of bootstrapping.

Results

Current knowledge of PolC inhibitor resistance is limited to target biding pocket changes conferred by *polC* non-synonymous mutations. Prior findings in laboratory strains of related *Bacillota* have shown the *B. subtilis* PolC serine 1175 is associated with HPUra resistance (*C. difficile* PolC serine 1169), *Staph. aureus* phenylalanine 1261 associated with EMAU resistance (*C. difficile* PolC phenylalanine 1258), and the *C. difficile* PolC lysine 1327, lysine 1148, and threonine 1331 associated with *Lachnospiraceae* and Oscil-

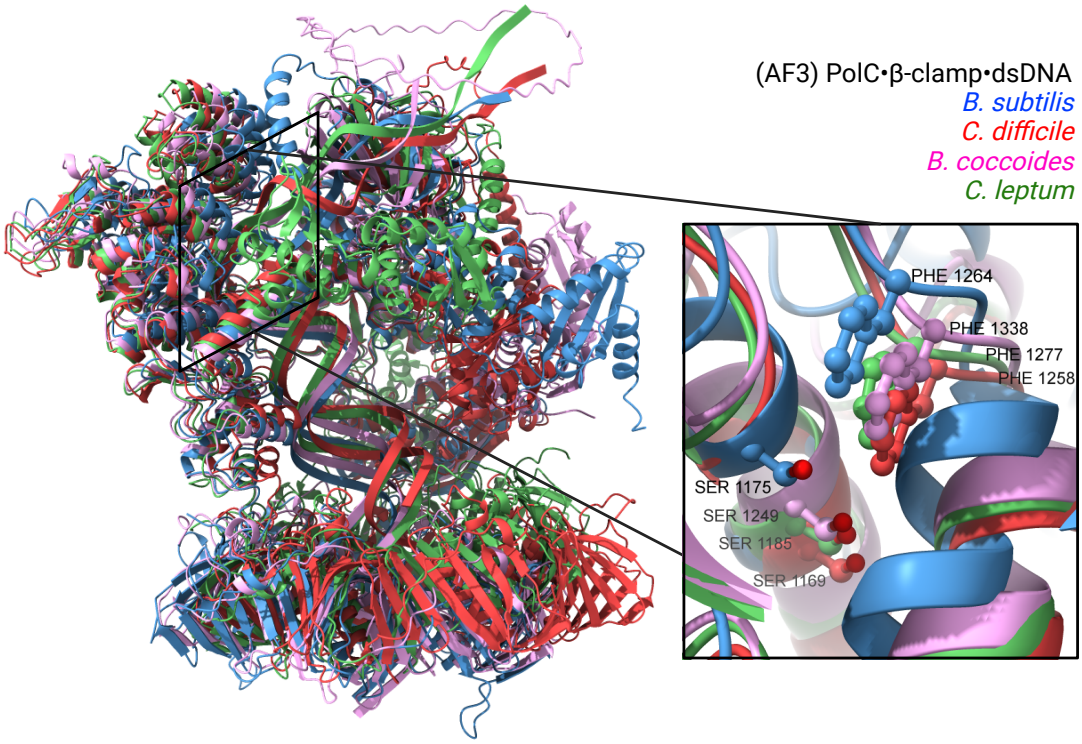

Figure 5.2: Superimposed AlphaFold3 PolC of Determinants of Resistance to PolC Inhibitors.

lospiraceae IBZ resistance (McPherson et al 2025), are in general proximity to one another, as well as the 3' OH of the primer DNA near the enzyme active site, consistent with the proposed competitive slow-off mode of action of PolC inhibitors (Figure 5.1). To better understand the proximity of these residues associated with PolC inhibitor resistance in a single binding pocket, we predicted the homology model of the PolC•β-clamp•dsDNA using AlphaFold3 (Figure 5.2). This progressive approach describes the ability of AlphaFold3 to accurately model not only three-dimensional protein structures but also protein-protein and protein-nucleic acid macromolecular complexes.

Next, we curated 32,938 complete and partial *C. difficile* genomes with any level of assembly, including 240 complete genomes. From dataset we curated a larger dataset of 1,258 distinct *C. difficile polC* sequences; of note, these 1,258 sequences also includes the 215 of the 218 sequences from complete genomes of *C. difficile* that are used in further phylogeographic analysis (Figure 5.3). The conservation of these 1,258 *C. difficile polC* sequences of 4,299 nucleotide length extracted from were analyzed at the

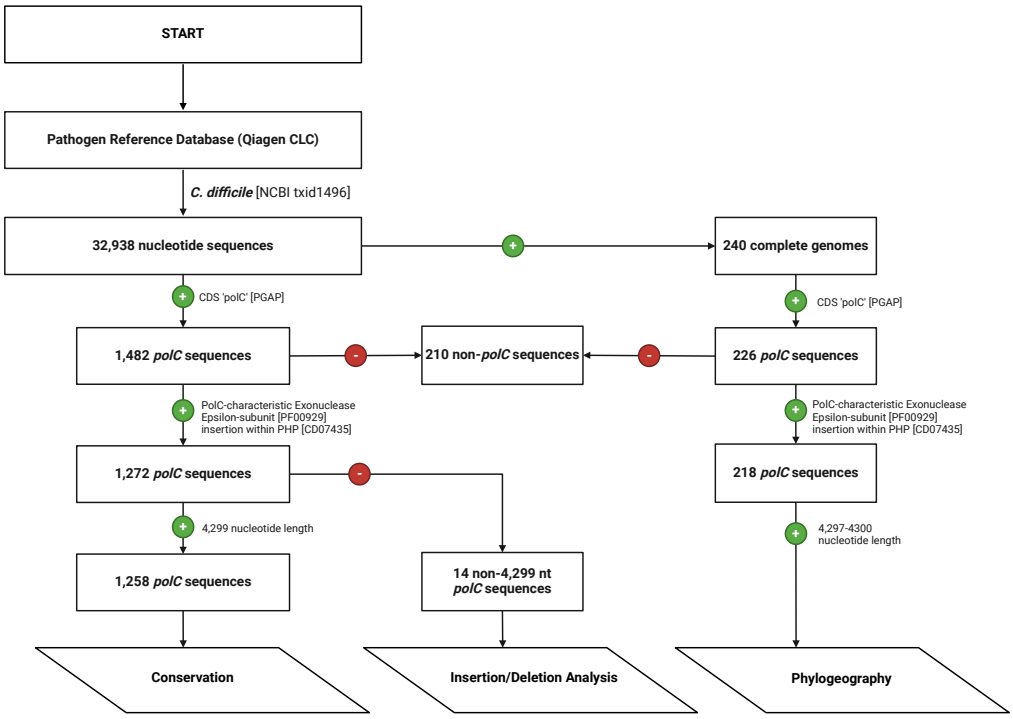

Figure 5.3: Data Acquisition Flowchart of Publicly Available *C. difficile* Genomes.

nucleotide and translated amino acid levels (Figure 5.4A). At a high-level view, we found the *C. difficile polC* is highly conserved, with only seven single nucleotide polymorphisms (SNPs) that fall below 75% conservation: *polC* SNP C/T 612, A/G/T 1245, A/G 1701, C/T 2143, A/G 2871, C/T 3651 and C/T 3669.

Interestingly, we find that the conservation of these seven SNPs below 75% (herein an arbitrary cutoff) are the result of synonymous codon mutations the *C. difficile polC* (Figure 5.4B). Codon analysis found SNP C/T 612 results in synonymous codons AGT, AGC encoding PolC serine 204; *polC* SNP A/G/T 1245 results in synonymous codons TCA, TCG, and TCT encoding PolC serine 415; SNP A/G 1701 results in synonymous glutamic acid codons GAA, GAG encoding PolC glutamic acid 567; SNP C/T 2143 results in synonymous codons CTA and TTA encoding PolC leucine 714; SNP A/G 2871 results in synonymous codons AAA and AAG encoding PolC lysine 957; SNP C/T 3651 results in synonymous codons AGC and AGT encoding PolC serine 1217; SNP C/T 3669 results in synonymous codons AGC and AGT encoding PolC serine 1223. Consistent with these findings, we find remarkable conservation at the

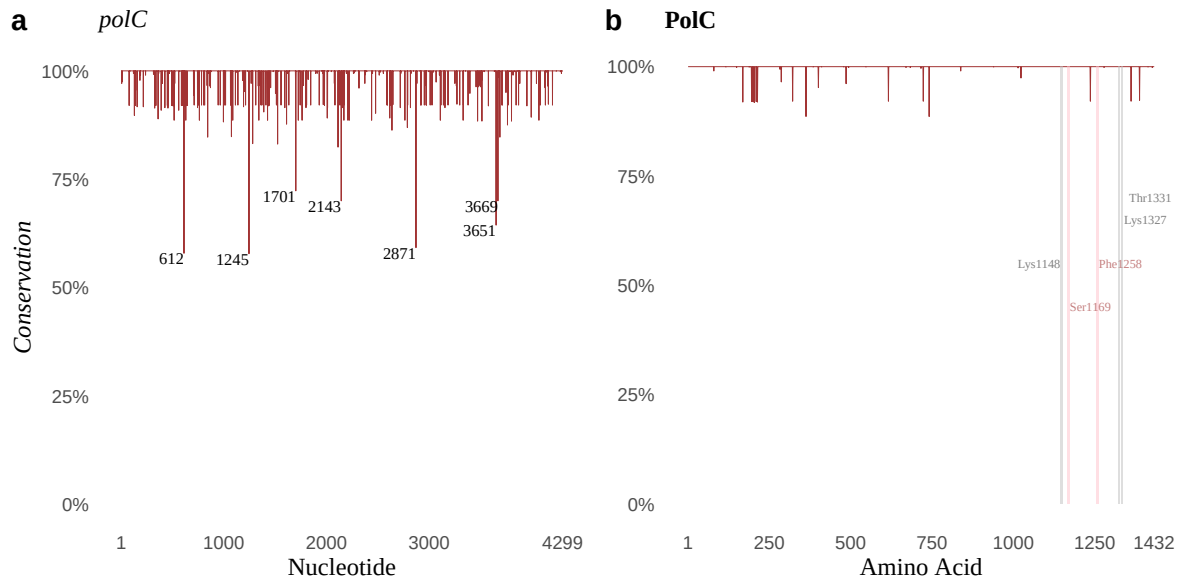

Figure 5.4: Conservation Analysis of Publicly Available *C. difficile polC*/PolC Sequences.

translated amino acid level of the *C. difficile* PolC. We found no residue across the 1,432 amino acids of these 1,258 *C. difficile* PolC sequences below 75% in conservation, and most notably – the five residues in question aligned to the *C. difficile* PolC phenylalanine 1258 (associated with EMAU resistance in *B. subtilis*, *Staph. aureus*, and *E. faecalis*), serine 1169 (associated with HPUra resistance in *B. subtilis*), lysine 1327, lysine 1148, and threonine 1331 (associated with relative abundance of *B. coccoides* and *C. leptum* in humans and CDI patients treated with oral IBZ), are 100.0% conserved in these *C. difficile* PolC (Figure 5.4B).

Furthermore, we analyzed fourteen *polC* sequences that contained the characteristic exonuclease insertion within the PHP domain, but were non-4,299 nucleotide length. Notably, nine of these fourteen *C. difficile* contain internal stop codons, resulting in truncated translations. Among the five sequences without internal stop codons that still resulted in full-length translations, we found the aligned serine 1169 (5/5), phenylalanine 1258 (5/5), lysine 1327 (5/5), lysine 1148 (5/5), and threonine 1331 (5/5) were conserved in the translated protein sequences of these five non-4,299 nucleotide *polC*.

Last, we sought to curate and characterize the phylogeographic distribution of the *polC* from complete genomeic sequences of *C. difficile* (Figure 5.5). Using CLC Genomics Workbench (Qiagen), we man-

ually cleaned the associated metadata of these 218 *C. difficile polC* for sample collection country, year and presumed host species. The collection source of these 240 *C. difficile* complete genomes resulting in 218 *polC* from *C. difficile* of length 4,297 to 4,300 were geographically isolated from the countries of the United States, Canada, Australia, New Zealand, South Korea, Japan, Belgium, Germany, Ireland, the Netherlands, Switzerland, the United Kingdom, Ghana, China, Taiwan, Thailand and Indonesia between the years 1982 and 2023. The documented sources of these *C. difficile polC* include Homo sapiens (humans, 135/218), unknown (54/218), Canis lupus familiaris (the domestic dog, 22/218), Equus caballus (the domestic horse, 3/218), Sus domesticus (the domestic pig, 2/218), Sarcophilus harrisii (the Tasmanian devil, 1/218), and Tiliqua rugosa (the shingleback lizard, 1/218). In our resultant phylogeographic analysis of *polC* from complete genomes of *C. difficile* , we generated a circular phylogram that focuses on the country of isolation. We found that the *C. difficile polC* is rooted from the United States and South Korea.

Conclusion

The development of PolC-selective inhibitors was instrumental to the discovery of PolC, beginning with cytosine arabinoside (ara-C), followed by 6-(p-hydroxyphenylazo)-uracil (HPUra), leading to the ethyl-methyl-anilouracils (EMAU) and dichlorobenzylguanine (DCBG) analogs with increased selectivity towards the PolC of *Bacillus subtilis* and not DnaE of *Escherichia coli*. Today, the prototypical DCBG, ibezapolstat (IBZ), is in clinical development for the treatment of *Clostridioides difficile* infection (CDI) for its proposed narrow-spectrum activity. However, the major hurdle to IBZ effectiveness would be PolC inhibitor resistance in globally circulating *C. difficile*. Our knowledge of PolC inhibitor resistance is currently limited to target gene mutations in laboratory strains, such as the *Staph. aureus* PolC Ser1261Leu and *B. subtilis* Azp12 PolC Ser1175Ala. Here we sought to characterize the predicted susceptibility profile of globally circulating *C. difficile* via knowledge of *polC* mutations associated with resistance to HPUra, EMAU, and DCBGs in laboratory strains of related *Bacillota*. Our approach uses a combination of three-dimensional protein structure modeling and large genomic databases to predict the likelihood of globally circulating IBZ-resistance before the introduction of IBZ to the clinic.

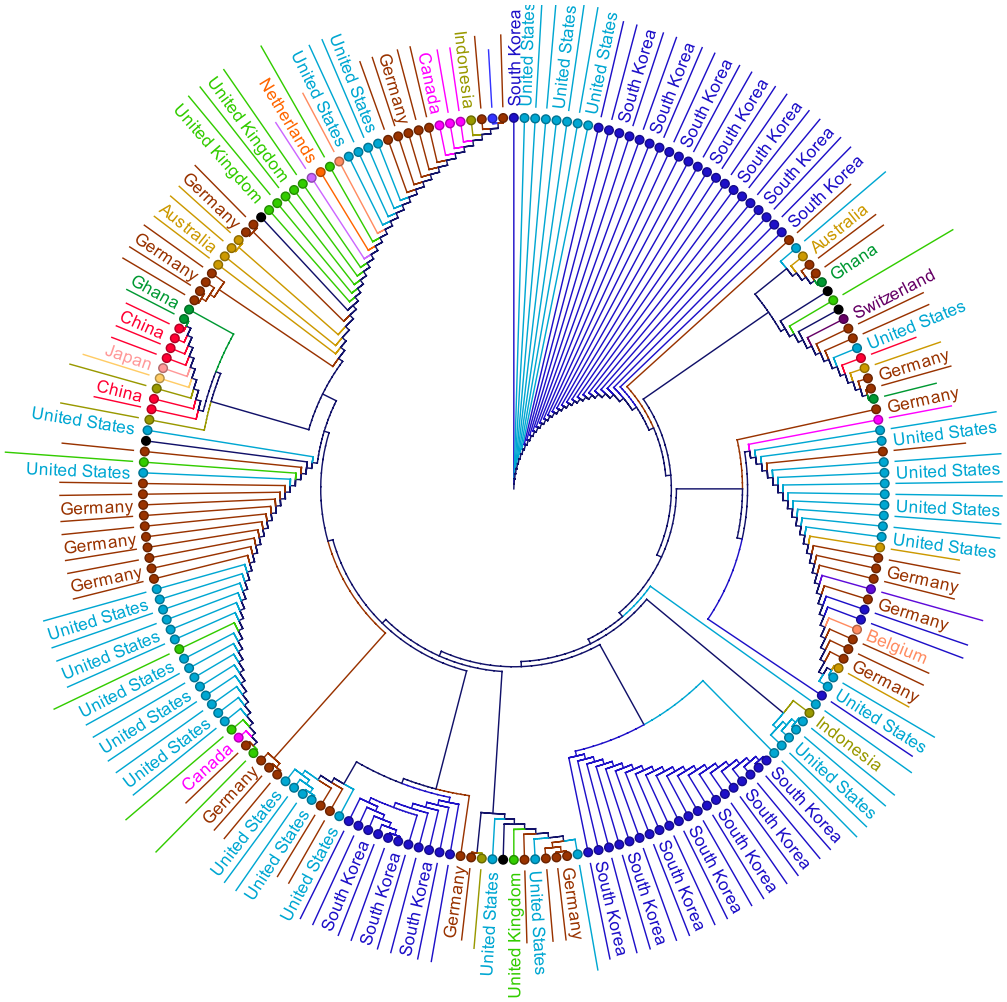

Figure 5.5: Phylogeography of *polC* from Complete Genomes of *C. difficile*.

To scale these extrapolations to the real-world for inference of globally circulating IBZ-resistant CDI. Consistent with other studies, most of these *C. difficile polC* sequences originated from countries with genomic sequencing capacity and a relatively higher burden of CDI, such as North America, Europe and East Asia. After scaling up our scope in data from a couple hundred complete genomes to over thirty-thousand complete and partial genomes, we found the *C. difficile polC* is highly conserved, with single-nucleotide polymorphisms (SNPs) in the *polC* leading to synonymous codons. Correspondingly, we found the globally circulating *C. difficile* PolC is highly conserved. However, the most striking feature we found was the 100.0% conservation of the five *C. difficile* PolC-aligned residues associated with PolC inhibitor resistance in related *Bacillota*: the phenylalanine 1258 (extrapolated from *Staph. aureus* and *E. faecalis* resistance to EMAUs), serine 1169 (extrapolated from *B. subtilis* resistance to HPUra), lysine 1327, lysine 1148, and threonine 1331 (extrapolated from *Lachnospiraceae* and Oscillospiraceae resistance to IBZ). We find these data support the lack of predicted PolC inhibitor resistance in globally circulating *C. difficile*.

This study has several strengths and weaknesses to be considered. First, our progressive approach to model the positions of PolC inhibitor associated residues *in silico* with AlphaFold3 generates highly informative insights but not experimental truth. We find these models highly informative in the limited amount of structural data on the PolC, currently limited to *Geobacillus kaustophilus*. It remains unclear how the bias in data availability of the *E. coli* DnaE-type DNA Polymerase III influences the AlphaFold homology models of the more sparsely available PolC. Furthermore, although we now have the power to generate an informative model of macromolecular structures, recent efforts suggest molecular docking compounds via AutoDock-Vina to AlphaFold2 structures *in silico* does not correlate with experimental results in vitro. Although this progressive approach needs further improvement and validation, we find these *in silico* algorithms and large amounts of genomic data will overall empower us in the ever-evolving landscape of antimicrobial drug development and resistance.

A second limitation of this study is our extrapolation of PolC inhibitor cross-resistance from not only different chemical classes but also species of *polC+ Bacillota*. To the best of our knowledge at the time of this writing, the HPUra-resistant *B. subtilis* azp12 strain (PolC Ser1175Ala) has not been well characterized in detail for its cross-resistance to either of the prominent EMAUs (HB-EMAU, ME-EMAU),

IBZ or other DCBGs; likewise, the EMAU-resistant *Staph. aureus* PolC Phe1261Leu, *E. faecalis* PolC Phe1264Leu, or *B. subtilis* POLC27 strain Phe1264Ser, have not been characterized in detail for their resistance to HPUra or IBZ (or other DCBGs). Despite the lack of data on PolC inhibitor cross-resistance, we find the proximity of amino acid changes to the enzyme active site across different species and chemical classes generally points toward the likelihood of PolC inhibitor cross-resistance. Taken together, these extrapolations of PolC inhibitor cross-resistance need further validation in vitro.

Third, a limitation of this paper is the narrow definition of PolC inhibitor resistance as those *polC* single nucleotide polymorphisms encoding PolC amino acid changes, falling under the larger mechanism of 'target modification' antimicrobial resistance. We do not currently know how PolC inhibitors penetrate the cell membrane and peptidoglycan wall, or whether it is extruded by efflux. Additionally, it is possible that other mechanisms of PolC inhibitor resistance may yet exist, such as those emerging mechanisms of resistance in related *Bacillota* of significant clinical importance, such as *E. faecium* and *Staph. aureus*. An improved understanding of the bacterial cell influx and efflux of PolC inhibitors would give insight into the diverse possibilities of PolC inhibitor resistance.

Finally, we find a major strength of this paper is the scale by which publicly available genomic data can aid inference to guide research on antibiotic resistance across space and time. For many years, the amount of publicly available *C. difficile* genomic data has grown as a product of increased genomic sequencing capacity and decreased costs. To ensure high reliability of curated sequencing datasets, we used professional bioinformatic software to curate, inspect and annotate these datasets. In this study, we collected more than 32,000 complete and partially assembled genomes, most of which being very small scaffolds and contigs, and extracted more than 1,000 full-length *polC* that specifically belong to *C. difficile*. These sequences are automatically annotated in NCBI by protein homology models and identify these sequences as belonging to *C. difficile*. Furthermore, these sequences are annotated by their original depositors for their origin, including host species, country and year. Despite these advantages, we find a limitation of this approach is the bias of these data to academic teaching hospitals and research microbiology labs in wealthy countries. The genomics and epidemiology of *C. difficile* in less affluent regions of the world are less clear, but no less important to the development of *C. difficile*-active antibiotics.

In conclusion, we found the *C. difficile polC*/PolC is remarkably conserved within the species across publicly available genomic data. Furthermore, we found these globally circulating *C. difficile polC*/PolC are devoid of genetically encoded amino acid changes near the PolC enzyme active site associated with HPUra, EMAU and IBZ PolC inhibitor resistance. Notwithstanding additional mechanisms of resistance, these data suggest the future effectiveness of IBZ for the treatment of CDI. Further studies are needed to better understand the mechanisms of PolC inhibitor resistance at the genetic, protein structure, protein function and epidemiological levels.

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6

Conclusion

In conclusion, toxigenic *Clostridioides difficile* infection (CDI)[1] represents one of the most Urgent Threat antimicrobial resistant (AMR) bacterial infectious diseases that should also be classified as a Bacterial Priority Pathogen.[75,184] The agent, *C. difficile*, is a sporulating, toxigenic, biofilm-producing, Gram-positive pathobiont of the human gut microbiome.[1] Broad-spectrum antibiotics, such as fluoroquinolones and carbapenems, are considered high-risk antibiotics to the epidemiology of CDI due to their damage to the human gut microbiome that is essential to establishing colonization resistance via bile acid biotransformation, and short-chain fatty acid (SCFA) fermentation.[44,314] The current guideline-recommended antibiotics used to treat CDI, vancomycin and fidaxomicin, have significant limitations, including but not limited to diminished sustained clinical cure, microbiological cure, cost of treatment, and increasing rates of resistance. Fecal microbiota transplantation (FMT) was a treatment modality that showed superiority for the prevention of recurrent CDI (rCDI) by re-introduction of bacteria that confer colonization resistance,[369] however safety concerns[372] has spurred the advent of defined consortia live biotherapeutic products (LBPs).[378] Despite the promise of LBPs, their administration and strain engraftment is limited by their own susceptibility to vancomycin and fidaxomicin.[379] Hence, a narrow-spectrum antibiotic with reduced activity against these taxa, *Lachnospiraceae* and *Oscillospiraceae*, but high activity against toxigenic *C. difficile* is sorely and urgently needed.

To address this unmet medical need, the PolC-type DNA Polymerase III alpha-subunit (PolC) is

the exciting drug target for narrow-spectrum antibiotic drug development.[486,487] The PolC is the essential replicative subunit of the bacterial DNA replisome.[510,543] The gene *polC* has a unique genetic origin that limits its presence to the genomes of only one of the top bacterial phyla of the human gut microbiome, *Bacillota*, and are devoid from the genomes of Actinomycetota, Bacteroidota, and Pseudomonadota.[489] However, metagenomic studies of a lead PolC inhibitor, ibezapolstat (IBZ), has found a surprisingly more narrow-spectru, microbiome-sparing activity than originally anticipated that may suggest reduced susceptibility in commensal bacteria, *Lachnospiraceae* and *Oscillospiraceae*.[588–591] This was a moment of positive serendipity because not only are these taxa the previously unculturable microbiota[587] not previously tested for their antibiotic susceptibility to antibiotics, like IBZ, but also because these taxa play a critical role in establishing colonization resistance to CDI through bile acid biotransformation and SCFA fermentation.[366,400]

WHAT NEW KNOWLEDGE WAS GENERATED?

We initially set out on this journey with the specific aims to capture and compare the structure and function of the PolC-type DNA polymerase III (PolC) from *C. difficile*, *B. subtilis*, *B. coccoides* (representative species of *Lachnospiraceae*), and *C. leptum* (representative species of Oscillospiraceae). There have been several very inspiring works that study the structure, function, and pharmacology of receptors and enzymes using cryo-EM with functional assays of target inhibition.

Here I attempt to show early *in silico* efforts to predict the mechanism by which IBZ potentially ”restores” the human gut microbiome occurs through phylogenetic differences between the PolC-IBZ pharmacophore of *Lachnospiraceae* /Oscillospiraceae and *C. difficile*. Critically, the differences in PolC structure are an important feature for PolC inhibitor development for infections caused by *Staphylococcus aureus*,[525,550,577,578] *Enterococcus faecium*,[526,668] and *C. difficile*. While these early investigations require more in-depth follow-up with confirmatory studies, this has been a worthwhile investigation into the im-

portance of antibiotic resistance in commensal and symbiotic bacteria for the restoration of colonization resistance.

WHAT DID I LEARN ABOUT SCIENCE?

While these data have their own limitations, the computational approaches utilized herein represent the potential applications of an analyst approach to biological data to more efficiently and effectively *predict* outcomes of biological significance. Even though I experienced my own learning curve through these efforts, I have benefited from learning (R) statistics, data-science, (python) bioinformatics, artificial intelligence, (Ubuntu; AWS) server management, (GitHub) version-control, and (LaTeX) typesetting for academic writing. My expectation is the future application of these approaches can empower biomedical scientists to better answer questions and discover newfound knowledge regarding the human gut microbiome, antimicrobial resistance, and transmission dynamics of infectious diseases.

A

Calculations

Ibezapolstat Molarity

Example Basis: 1,000 ng/mL, or 1 $\mu g/mL$ Ibezapolstat

Step 1: Convert to g/L

$$= 1{,}000\,\text{ng/mL} \times 10^{-9}\,\text{g/mL}$$

$$= 1 \times 10^{-6}\,\text{g/mL} \times 1{,}000\,\text{mL/L}$$

$$= 0.001\,\text{g/L}$$

Step 2: Convert to mol/L

$$= 0.001\,\text{g/L} \times \frac{1\ \text{mol}}{423.3\ \text{g}}$$

$$\approx 2.36 \times 10^{-6}\,\text{mol/L}$$

$$= 2.36\,\mu\text{M}$$

Alpha (α)–Diversity

Shannon's Entropy (H)[669]

$$H' = -\sum_{i=1}^{S} p_i \ln(p_i)$$

Simpson's Diversity (1 - Dominance)[670]

$$1 - D = 1 - \sum_{i=1}^{S} p_i^2$$

Beta (β)–Diversity

Bray–Curtis Dissimilarity[671]

$$\mathrm{BC}(\mathbf{x}, \mathbf{y}) = \frac{\sum_{i=1}^{S} |x_i - y_i|}{\sum_{i=1}^{S} (x_i + y_i)} \in [0, 1].$$

B

Ibezapolstat: Additional Clinical Trials

Table B.1: Summary of dosing and group design for the randomized, placebo-controlled study. From: A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects[591]

Study Period	**Period 1**	**Period 2**	**Period 3**
Design	Single ascending dose (SAD)	Food effect crossover (FEC)	Multiple ascending dose (MAD)
Regimen (PO)	once	once	twice-daily × 10 days
Dose (mg)	150, 300, 600, 900	300	300, 450
Comparator (n)	Placebo (2)	—	Placebo (2), Vancomycin (6)
Cohort Size (N)	6	8	6
Purpose	Safety	Safety	Safety
PK	Blood, Stool	Blood, Stool	Blood, Stool
MGX	—	—	Stool
MBX	—	—	Stool

Note: PO = per os; PK = Pharmacokinetics; MGX = Metagenomics; MBX = Metabolomics.

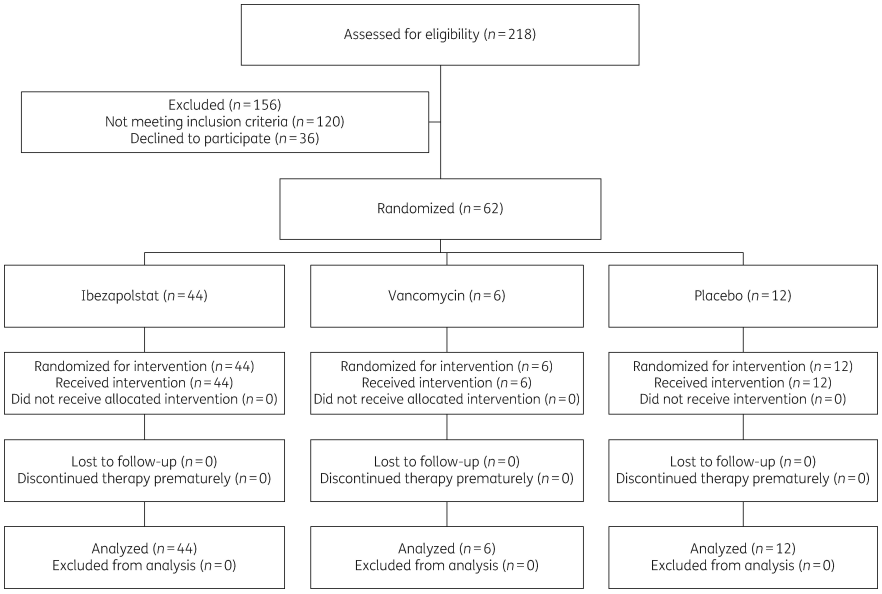

Figure B.1: Phase I Trial Profile. From: A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects [591]

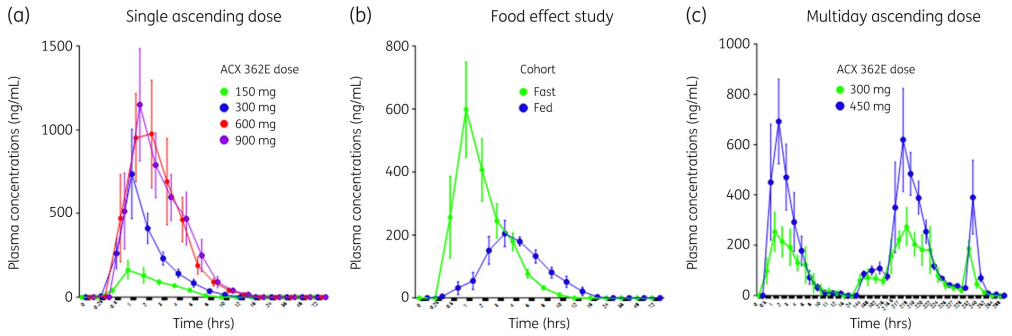

Figure B.2: Absorption of Oral Ibezapolstat. Concentrations in plasma (ng/mL) from (a) single ascending dose (SAD) study period; (b) food effect crossover (FEC) study period (300 mg PO once); (c) multiple ascending dose (MAD) study period (PO twice daily $\times$ 10 days); From: A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects. [591]

Figure B.3: Fecal Excretion of Oral Ibezapolstat. Concentrations in stool (µg/g) from (a) single ascending dose (SAD) study period; (b) food effect crossover (FEC) study period (300 mg PO once); (c) multiple ascending dose (MAD) stud period (PO twice daily × 10 days); From: A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects.[591]

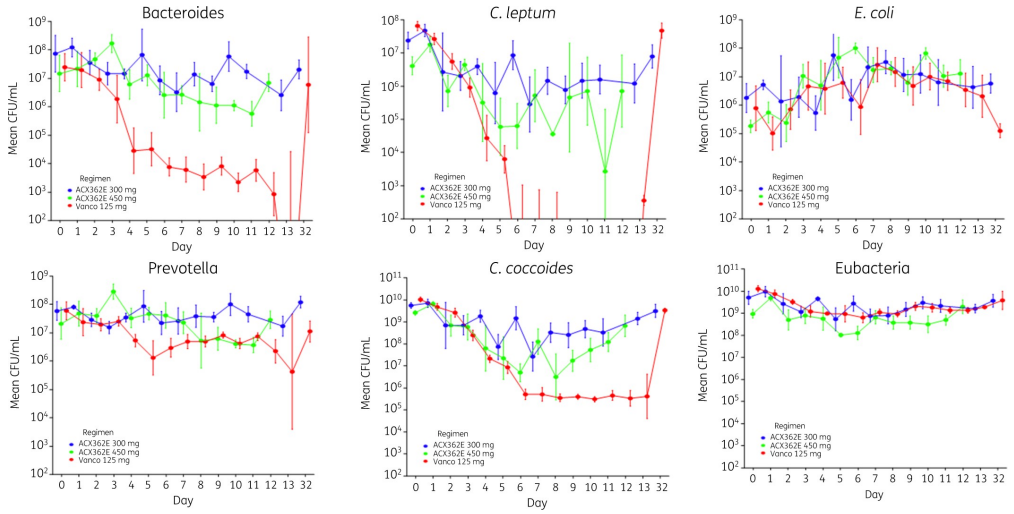

Figure B.4: Effects of ibezapolstat on relative abundance of taxa. The specific taxa of bacteria quantified include two genera, [top-left] *Bacteroides* and [bottom-left] *Prevotella*, of the *Bacteroidota* phylum (formerly *Bacteroidetes*); the families, [top-center] *Oscillospiraceae* (formerly *Oscillospiraceae*, *C. leptum* Group, or Clostridium Cluster IV) and [bottom-center] *Lachnospiraceae* (formerly *C. coccoides* Group, or Clostridium Cluster XIVa), of the *Bacillota* phylum (formerly *Firmicutes*); the species, [top-right] *E. coli*, of the phylum *Pseudomonadota* (formerly *Proteobacteria*); and the [bottom-right] domain Bacteria (formerly *Eubacteria*). From: A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects.[591]

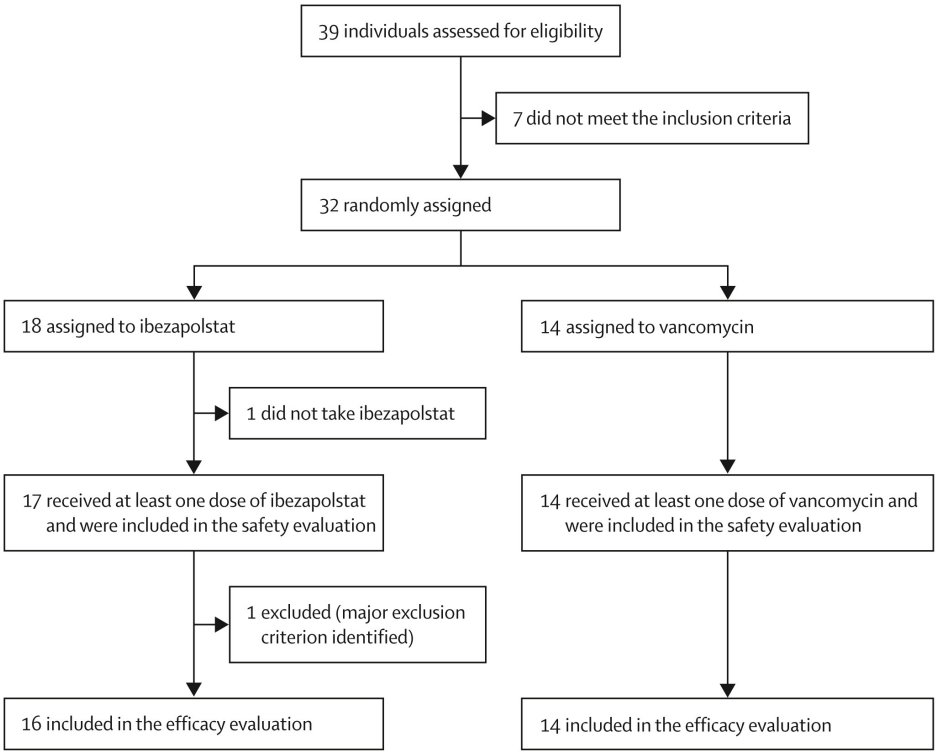

Figure B.5: Phase IIb Trial Profile.
From: Efficacy, safety, pharmacokinetics, and associated microbiome changes of ibezapolstat compared with vancomycin in adults with Clostridioides difficile infection: a phase 2b, randomised, double-blind, active-controlled, multicentre study.[592]

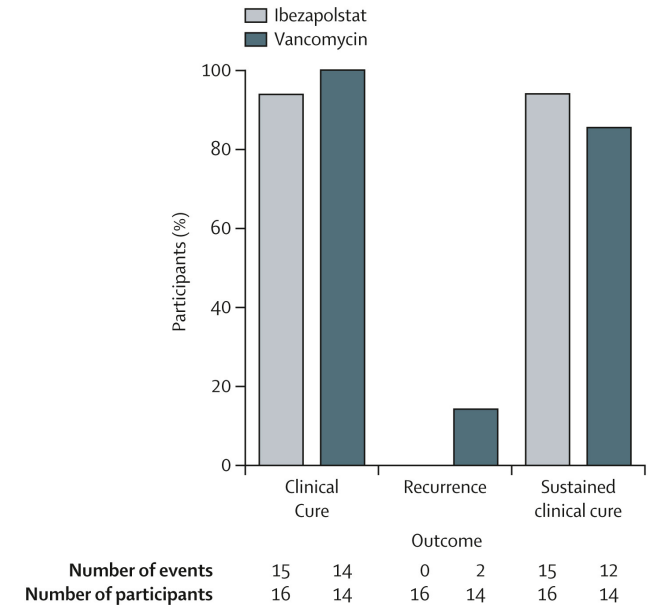

Figure B.6: Efficacy in the per-protocol population. From: Efficacy, safety, pharmacokinetics, and associated microbiome changes of ibezapolstat compared with vancomycin in adults with Clostridioides difficile infection: a phase 2b, randomised, double-blind, active-controlled, multicentre study.[592]

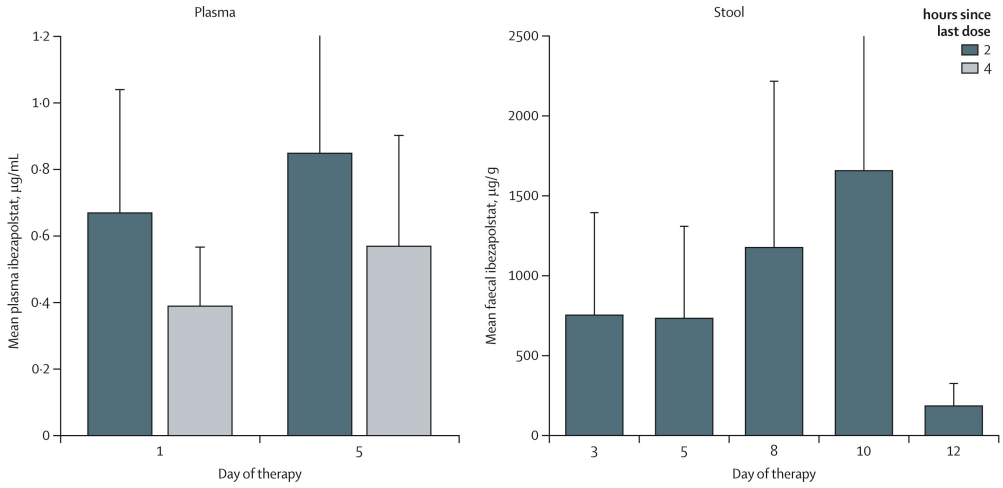

Figure B.7: Ibezapolstat concentrations in plasma and stool of the per-protocol population. Adapted from: Efficacy, safety, pharmacokinetics, and associated microbiome changes of ibezapolstat compared with vancomycin in adults with Clostridioides difficile infection: a phase 2b, randomised, double-blind, active-controlled, multicentre study.[592]

Table B.2: Drug-related Adverse Events in Healthy Adults Administered Ibezapolstat in Multiple Ascending Dose [*]. From: A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects.[591]

Dose (mg), PO BID × 10 days	Adverse Event	Number of Events	CTCAE[672] Severity
300	COUGH	1	Mild (Grade 1)
	CYSTITIS–NON INFECTIVE	1	Mild (Grade 1)
	DIZZINESS	1	Mild (Grade 1)
	EPIGASTRIC PAIN[*]	1	Mild (Grade 1)
	HEADACHE[*]	3	Mild (Grade 1)
	HEADACHE	1	Moderate (Grade 2)[*]
	NASAL CONGESTION	1	Mild (Grade 1)
	TWITCHING SENSATION	1	MILD (Grade 1)
450	DYSPEPSIA[*]	1	Mild (Grade 1)
	NAUSEA[*]	1	Mild (Grade 1)
	PROLONGED PR INTERVAL[*]	1	Mild (Grade 1)
	SHORTNESS OF BREATH[*]	1	Mild (Grade 1)
	TACHYCARDIA[*]	1	Mild (Grade 1)

[*]Possibly or probably related; PO = *per os*; BID = twice daily; AEs in placebo group (n=2): Headache, rash, left hand ecchymosis;
No AE required a change to intervention.

Table B.3: Drug-Related Adverse Events in Healthy Adults Administered Oral Ibezapolstat. From: A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects.[591]

	Single Ascending Dose (SAD)		Food Effect Crossover (FEC)	Multiple Ascending Dose (MAD)	
Dose (mg)	*IBZ (n=6)*	*Placebo (n=2)*	*IBZ (n=8)*	*IBZ (n=6)*	*Placebo (n=2)*
300	0%	50%	37.50%[##]	33%	50%
450	X	X	X	0%	0%
600	33%	50%	X	X	X
900	33%	50%	X	X	X
Total AEs	5	5	3	5	1
in No. of Participants	5	4	2	2	1

[##]Fed: n=2; Fast: n=1; X: not tested

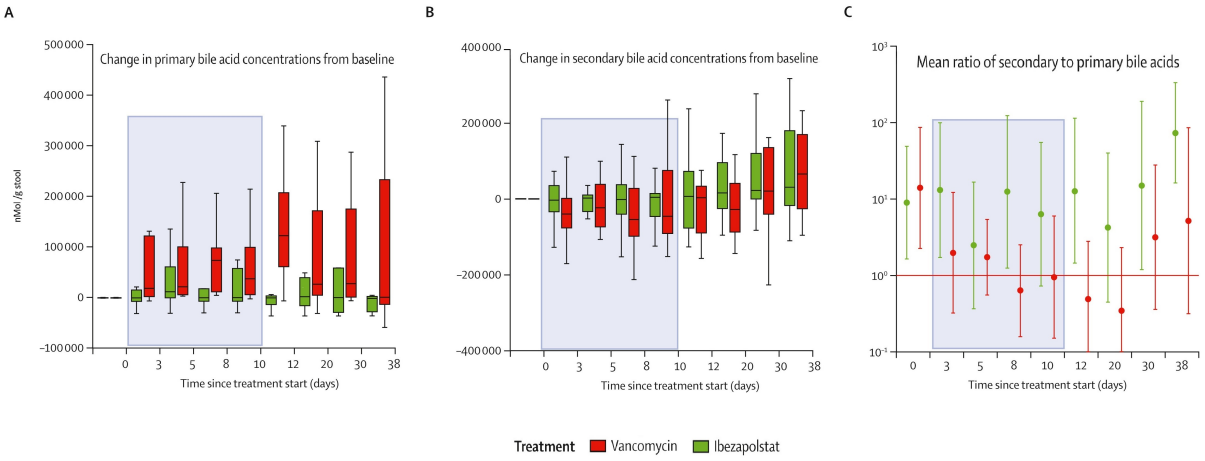

Figure B.8: Bile acid changes in the per-protocol population. Adapted from: Efficacy, safety, pharmacokinetics, and associated microbiome changes of ibezapolstat compared with vancomycin in adults with Clostridioides difficile infection: a phase 2b, randomised, double-blind, active-controlled, multicentre study.[592]

Table B.4: Demographics and baseline characteristics in the per-protocol population.
From: Efficacy, safety, pharmacokinetics, and associated microbiome changes of ibezapolstat compared with vancomycin in adults with Clostridioides difficile infection: a phase 2b, randomised, double-blind, active-controlled, multicentre study.[592]

	Ibezapolstat (n=16)	Vancomycin (n=14)
Age		
>65 years	8 (50 %)	5 (36 %)
>75 years	5 (31 %)	2 (14 %)
Sex		
Female	13 (81 %)	11 (79 %)
Male	3 (19 %)	3 (21 %)
Race		
White	16 (100 %)	13 (93 %)
Black	0	1 (7 %)
Ethnicity		
Hispanic or Latino	11 (69 %)	11 (79 %)
Other	5 (31 %)	3 (21 %)
Charlson Comorbidity Index	2 (1–4)	2 (1–4)
Prior antibiotic use (≥3 doses VAN)	2 (13%)	2 (14%)
C. difficile test for initial diagnosis*	13 (81%)	11 (79%)
Unformed bowel movements at baseline	6 (4–7)	6 (4–8)
Ribotypes of *C. difficile* Isolated†		
014–020	0	3 (27 %)
027	1 (9 %)	2 (18 %)
106	3 (27 %)	1 (9 %)
002	1 (9 %)	1 (9 %)
116	0	1 (9 %)
Other	6 (55 %)	3 (27 %)

Data are n (%) or median (IQR); VAN = vancomycin
* All participants diagnosed using a *C. difficile* free toxin test within 24 h before treatment (C. difficile Quik Chek Complete, Techlab®).
† Not all patients had *C. difficile* growth at baseline.

Table B.5: Drug-related adverse events.
Adapted from: Efficacy, safety, pharmacokinetics, and associated microbiome changes of ibezapolstat compared with vancomycin in adults with Clostridioides difficile infection: a phase 2b, randomised, double-blind, active-controlled, multicentre study.[592]

Drug-Related Adverse Events (AEs)	**Ibezapolstat (n=17)**	**Vancomycin (n=14)**
Mild [#]	3 (18%)[†]	0
Moderate [#]	0	1 (7%)[*]
Serious	0	0
Treatment Withdrawal	0	0

Intention-to-Treat (ITT) population evaluated for safety.
[#]Possibly drug-related; [*]headache; [†]two (12%) gastro-oesophageal reflux disease and one (6%) nausea.

C

Clostridioides difficile infection: an Overview

Toxigenic *Clostridioides difficile* Infection

Clostridioides difficile was identified by John G. Bartlett in 1978 as the causative agent of antibiotic-associated pseudo-membranous colitis[42,43] later referred to as *C. difficile* infection (CDI),[1,13,66–69,314] a life-threatening gastrointestinal infectious disease. The disease, CDI, is also sometimes referred to in the literature as clindamycin-associated colitis,[70,365] or *C. difficile*-associated disease (CDAD).[71,72] CDI has a rich history as an ongoing global epidemic[230] with limited therapeutic options,[73] increasing resistance,[466] and substantial burden of cost,[74] morbidity and mortality.[3,7] Yet, while *C. difficile* is a top-ranked **Urgent Threat** according to the CDC Antibiotic Resistance Threats Report,[75] it is not listed among the WHO Bacterial Priority Pathogens list.[184]

The origin of CDI as a global epidemic of major concern is generally attributed to fluoroquinolone use for other indications of bacterial infections in the 1990s, Canada, where clinicians reported a significant increase in the incidence and mortality CDI cases in a single center between 1991 and 2003.[76] Around this time period, twelve more hospitals in the region reported an increased incidence and mortality due to single strain of *C. difficile* containing *gyrA* mutation conferring GyrA Thr82Ile conferring fluoroquinolone-resistance, and a partial deletion in the *tcdC* repressor of the pathogenicity locus (PaLoc) leading to consti-

tutive toxin de-repression.[45] Preceding oral administration of fluoroquinolones for other infectious indications were strongly associated with CDI incidence, presumably through giving fluoroquinolone-resistant *C. difficile* a fitness advantage in the human gut microbiomes of patients that received oral fluoroquinolones.

Not long thereafter, this strain, also known as the pulsed-field gel electrophoresis North American Pulsotype 1 (NAP1), or the fluorescence PCR ribotype 027 (F027) (commonly referred to in the literature as NAP1/F027), was identified around the world in CDI patients across the United States,[77,229] Europe,[78] Australia,[79,80] and Japan,[81], indicating widespread geographic dispersal. Notably, subsequent phylogenomic analyses identified the amino acid change at GyrA Thr82Ile was twice separately selected for in circulating clones of North American *C. difficile* strain NAP1/F027, leading to the separate lineages of FQR1 and FQR2.[230] To minimize further global spread, a risk assessment framework was proposed giving attention to international travel, livestock trade, and antibiotic use - such as clindamycin and fluoroquinolones.[82] Together, the history of the origin and global dispersal of an antibiotic-resistant, hyper-virulent strain of *C. difficile* highlights the the need for integrated surveillance systems with operational genomic and microbiologic capabilities, and an appreciation for the within-host evolution of bacterial pathobionts of the human gut microbiome upon exposure to highly potent selective pressures, such as antibiotics.

WHAT ARE RISK FACTORS FOR *C. DIFFICILE* INFECTION

Epidemiology

Clostridioides difficile infection (CDI) is a major cause of morbidity, mortality and cost in the United States.[2–4] While efforts to vaccinate the public against toxigenic *C. difficile* is ongoing,[5] the epidemiological identification of modifiable risk factors assocaited with CDI have led to the fruitful implementation of antibiotic stewardship practices and reduction in CDI.[6,7] Hence, the epidemiological risk factors associated with CDI have been extensively reviewed.[8–13] The nosocomial transmission of CDI was

recognized early through environmental studies that revealed frequent contamination of hospital rooms, patients, and the hands of healthcare workers.[14] In response, infection control practices for managing CDI have emphasized the use of soap and water over alcohol-based hand sanitizers,[15] along with enhanced environmental cleaning using hypochlorite disinfectants.[16] Despite these targeted infection control strategies, whole-genome sequencing studies have shown that a substantial proportion of CDI cases arise from genetically diverse strains,[17] consistent with the One Health nature of CDI.[18] Prominent reservoirs of toxigenic *C. difficile* include but are not limited to animals[19] our shoes[20–22] community[23–25] and healthcare settings.[16,26–30]

However, specific hospital environments such as intensive care units (ICUs) remain high-risk due to intense antibiotic use and elevated colonization pressure.[31] One study demonstrated that ICU patients colonized with toxigenic *C. difficile* were nine times more likely to progress to symptomatic infection.[32] These findings are further supported by genomic evidence confirming CDI transmission within ICUs.[33,34]

Not all antibiotics exert equal effects on the gut microbiome.[35,176,201] Generally, antibiotics administered orally, or *per os* (PO), are associated with an elevated risk of CDI.[36–39,44] Different antibiotic classes have different relative CDI risk categories ranging from low- to high-risk.[40] For example, clindamycin is among the highest-risk antibiotics and was historically linked to "clindamycin-associated colitis." In 1977, John G. Bartlett and colleagues identified the causative agent as toxigenic *C. difficile*.[41] Similarly, fluoroquinolones are considered high-risk due to their role in selecting for the hypervirulent Fo27 ribotype, which carries a fluoroquinolone resistance–conferring mutation in the *gyrA* gene.[45,229] Moreover, the cumulative exposure to oral antibiotics over time further increases the likelihood of developing CDI.[39,46,47] This disruption is linked to multiple CDI-related outcomes, including asymptomatic colonization,[48,49] community-acquired CDI (CA-CDI),[50–52] hospital-onset CDI (HO-CDI),[53–56] and recurrence following treatment (rCDI).[44,57–59]

In addition to antibiotics, several non-antibiotic drugs also disrupt the gut microbiome.[177] Among these, proton pump inhibitors (PPIs) are especially notable for their association with increased CDI risk.[60–65] Although PPIs are indicated for conditions such as gastroesophageal reflux disease and stress ulcer prophylaxis, the precise mechanisms by which they predispose patients to CDI remains incompletely understood.

WHAT IS C. DIFFICILE?

Microbiology

Clostridioides difficile (Cd; Table 1.1; NCBI:txid1496) is an anaerobic,[227]) toxigenic,[249] endosporulating,[304–306] Gram-positive[312,673] bacterium originally identified in 1935 as *Bacillus difficilis* for its rod-shaped cellular morphology and *difficulty* to grow.[674] Shortly thereafter, in 1938, *B. difficilis* was renamed to *Clostridium difficile*, to reflect its re-classification to the polyphyletic (no single recent common ancestor) Clostridium genus on the differentiating basis of anaerobiosis, a "drumstick" distortion upon endosporulation, and the absence of catalase activity.[675] More recently, in 2016, *C. difficile* was re-classified once more to the Clostridioides genus of the Peptostreptococcaceae family on the basis of its phylogenetic distance to the type species, *C. butyricum*, thus changing its name to the current nomenclature of *Clostridioides difficile*.[676] The laboratory conditions for the growth and isolation of *C. difficile* mimics that of an antibiotic-disrupted human gut microbiome, including broad-spectrum antibiotics, primary bile salts, and anoxia.

So why do some bacteria grow at atmospheric levels of oxygen while others do not? Generally speaking, there are five categories of bacteria on the phenotypic basis of aerotolerance: (1) obligate aerobes, (2) obligate (or 'strict') anaerobes, (3) facultative anaerobes, (4) microaerophiles, and (5) aerotolerant bacteria. Advances in anaerobe microbiology paved the way for the study of *C. difficile* and other obligate anaerobes, such as the Hungate Method,[677,678] a simplified glove box[679], and advances in culturomics (the generation of collections of cultures) that allow us to culture the "unculturable" microbiota.[135,680] Together these advances in anaerobe microbiology made possible to grow and characterize *C. difficile*, Lachnospiraceae and Oscillospiraceae from the human gut microbiome.[587,674,675]

Obligate anaerobiosis in bacteria has several mechanisms involved in the protection against oxidative stress.[175] Early investigators suggested the superoxide dismutase (SOD) theory of anaerobiosis,[174]

stating the absence of the enzyme SOD, responsible for the metabolism of toxic radical superoxide anions ($O_2^{\bullet -}$) to less toxic oxygenic molecules, explains the intolerance of anaerobes to atmospheric levels of oxygen.[681] This theory has been refined over the years as more data became evident,[682,683] shifting the focus to additional reactive oxygen species (ROS) capable of intoxicating iron-sulfur (Fe-S) clusters, or inorganic cofactors of critical enzymes involved in reduction-oxidation (redox) reactions of fermentation.

A few mechanisms linked to iron and sulfur homeostasis protect *C. difficile* against oxidative stress. To list a few, investigators have connected cysteine desulfurase enzyme, IscS2,[227] the heme-sensing membrane protein system (HsmRA),[342] and the ferrosome iron homeostasis membrane protein, FezB,[684] to *C. difficile* anaerobiosis. Furthermore, the critical enzyme, pyruvate:ferredoxin oxidoreductase (PFOR), encoded by *nifJ* in *C. difficile*, is responsible for not only the conversion of pyruvate to acetyl-CoA during fermentation, but also the reduction of the anti-anaerobe antibiotic, metronidazole, to its reactive nitroso intermediate.[470,685,686] It's worth noting that while metronidazole is an anti-anaerobe antibiotic introduced decades ago for the treatment of CDI[418,687] it is no longer guideline recommended[408,688] due to inferiority to vancomycin for severe CDI,[423] increasing rates of resistance[689] and decreasing clinical efficacy.[429,690] Curiously, however, heme is was recently shown to be a critical component in the determination of metronidazole resistance in *C. difficile*, further lending weight to the relationship between iron homeostasis and protection against oxidative stress.[691] In summary, an anoxic environment is essential to the growth of *C. difficile*, Lachnospiraceae and Oscillospiraceae, and the theory of oxygen intolerance in bacteria has evolved from the absence of SOD to the ROS intoxication of Fe-S clusters in enzymes responsible for redox reactions during fermentation.

Antibiotics and primary bile salts, similar to the environment of a dysbiotic patient, also aid the laboratory isolation of *C. difficile* similar to the environment of the antibiotic-perturbed (dysbiotic) human gut microbiome. For instance, an optimized protocol typically utilizes an induction of *C. difficile* spores with 0.1 % (w/v) sodium taurocholate (1,860 μM, 537.7 g/mol molecular weight)[692] followed by isolation on the selective media, cycloserine-cefotixin fructose agar (CCFA),[693,694] containing 10.0 % (w/v) cycloserine (100,000 μg/mL; 9,795 μM, 102.09 g/mol) and 1.56 % (w/v) cefoxitin (15,600 μg/mL; 34,700 μM, 449.45 g/mol).[695] At these concentrations, this selective media inhibits the growth of representative

bacterial species of other phyla of the human gut microbiome, such as *Bacteroides fragilis* ATCC 25285 (Bacteroidota), *Escherichia coli* ATCC 25922 (Pseudomonadota), and *Fusobacterium nucleatum* ATCC 25586 (Fusobacteroidota) (Anaerobe Systems, CCFA Product Insert).

Mechanistically, cycloserine inhibits two bacterial enzymes responsible for cell wall biosynthesis, the $_L$-alanine racemase (*alr*) responsible for the conversion of $_D$-alanine to $_L$-alanine,[696] and $_D$-alanyl-$_D$-alanine synthetase (*ddlA*) responsible for incorporation of $_D$-alanyl-$_D$-alanine incorporation into the pentapeptide cross-linkage of peptidoglycan.[697] While data on the *C. difficile* susceptibility to cycloserine are not described in great detail, the standard CCFA medium concentration of 250 μg/mL suggests a mechanism of *C. difficile* resistance to cycloserine.

Cefoxitin, a cephalosporin β-lactam antibiotic, inhibits several penicillin-binding proteins (PBPs) responsible for the removal the terminal $_D$-ala from the peptidoglycan pentapeptide, creating the tetrapeptide necessary for cross-linking peptidoglycan strands in cell wall biosynthesis,[698] such as some noteable PBPs of *E. coli* K12: *dacA*, *dacB*, *dacC*, *pbpG*, *mrcA*, *mrcB*, *ftsI*;[699] and that of *Streptococcus pneumoniae* ATCC BAA-255/R6: *pbpA*, *pbp1b*, *pbp2a*, *pbp3*.[700] Critically, cefoxitin has reduced activity against *C. difficile* as a consequence of genomically encoded class D β-lactamases (CDD) that are intrinsic to the *C. difficile* species, such as *cdd1* and *cdd2*, that have have a high catalytic efficiency against several β-lactams from both the penicillin and cephalosorins chemical classes. Crucially, this cefoxitin non-susceptibility enhances the isolation of *C. difficile* on CCFA.[701,702] In summary, β-lactam antibiotics, one of the most important discoveries to revolutionize modern medicine,[703] not only disrupts the human gut microbiome, but also aids the laboratory isolation of *C. difficile* from human stool samples of CDI patients for further laboratory characterization using CCFA selective media, primary bile salts, and anoxic growth conditions.

Genomics

Whole-genome sequencing of *C. difficile* isolate collections estimates suggests while the species diverged approximately 1.1 - 85 million years ago (mya), virulence mechanisms were independently introduced through horizontal gene transfer (HGT) into distinct lineages of ”virulent” ribotypes through this

highly dynamic, mosaic genome.[704] The *C. difficile* genome is generally regarded as "open" to reflect a dynamic and mosaic genome that is adaptable to distinct environmental conditions.[705] On average, the 4.1 to 4.3 Mbp genome consists of a larger, diverse pan-genome of up to 9,640 coding sequences (CDS) and a much smaller core genome (600 to 3,000 CDS), representing as low as 16% of the pan-genome.

The global clinical use of orally administered antibiotics significantly shapes the evolution of *C. difficile*, including the selection of genetic mechanisms of resistance and virulence. While HGT of antibiotic resistance genes (ARGs) on plasmids are a major global health concern,[706] *C. difficile* genomes are rich (11%) in mobile genetic elements (MGEs), such as conjugative transposons that transmit ARGs or toxin-encoding genes such as those found in the first complete genome of *C. difficile* belonging to strain 630.[707] Some examples of these ARGs on MGEs may include tetracycline resistance on Tn*916*, Tn*5397*, or Tn*B1230*; chloramphenicol resistance on Tn*4453*a/b; macrolide resistance on Tn*5398*; the multidrug-resistance gene, *cfr*, on Tn*6218*. Fluoroquinolones, broad-spectrum antibiotics with high oral bioavailability that inhibit bacterial DNA gyrase, selected for a fluoroquinolone-resistant hypervirulent strain, ribotype F027, with a *tcdC* mutation in the pathogenicity locus (PaLoc), resulting in toxin de-repression and elevated mortality.[45,230] The PaLoc has a curious history of HGT into the *C. difficile* genome **after** speciation, leading to both toxigenic and non-toxigenic strains.[236] The two major toxins of PaLoc, *tcdA* and *tcdB*, were also the first published sequences of *C. difficile*.[241,708] TcdB, a major virulence factor, has since diverged into several clades.[709]

Beyond resistance and virulence, the influence of *C. difficile* genomic plasticity on metabolic selection driven by nutrient availability also remained an important question to understand the species' evolution. To address this, researchers developed an advanced computational model, named iCN900, to understand genotypic-phenotypic divergences of strain 630 cultures in over 180 nutrient environments to identify metabolic pathways prone to evolution. They observed genes that encode enzymes involved in fiber metabolism and iron acquisition were more likely to diverge, whereas those involved in Stickland fermentation were more conserved.[710] These findings raise the question what is the impact of host diet on the evolution of *C. difficile*.

Hence, the fluoroquinolone-resistant, hypervirulent F027 strain has become a global epidemic lin-

eage with limited treatment options, necessitating the discussion of a global risk assessment.[82] To track the epidemiology of such strains of concern, researchers developed a typing method that amplified the 16S-23S rRNA intergenic spacer region (ISR)[711–713] for noteworthy pathobionts, such as *C. difficile*,[714] and *Staph. aureus*.[715] Over time, the availability of 5' fluorescently-tagged DNA primers led to fluorescence PCR Ribotyping that could be evaluated by capillary electrophoresis[716,717] As a results, technical challenges[718] were addressed by an international schema for the fluorescence ribotyping of *C. difficile*.[719,720]

WHICH ANTIBIOTICS TREAT CDI?

Antibiotic Treatment for CDI

Vancomycin

From bacteria to humans, water is a fundamental molecule to the processes of life. With the aid of aquaporins that allow for the passage of water across cell membranes, differences in salinity determines the osmosis of water into or out of the cell. However, too much water inside a cell can lead to increased swelling, turgor pressure, and catastrophic lysis. To maintain an intracellular volume of water at an otherwise lethal pressure of turgor, different forms of life have evolved rigid external structures known as cell walls to prevent lysis during hypo-osmotic excursions. For example, bacteria have a cell wall made of peptidoglycan, fungi – chitin, and plants – cellulose.

Notably, the absence of a cell wall is a fundamental distinguishing feature of animals from plants, contributing to our increased cellular flexibility, movement and specialization. This distinguishing feature not only separates animals from plants, but also provides the basis for the treatment of bacterial infectious diseases with antibiotics that have a desirable safety profile by targeting cell wall biosynthesis. Although the experimental ascertainment is tricky, previous works suggest Gram-positive bacteria with a thicker peptidoglycan layer, like Bacillus or Clostridium, can tolerate an elevated maximal turgor pressure of up to 20 atmospheres (atm); Gram-negative bacteria, like *E. coli*, have a thinner cell wall and can tolerate

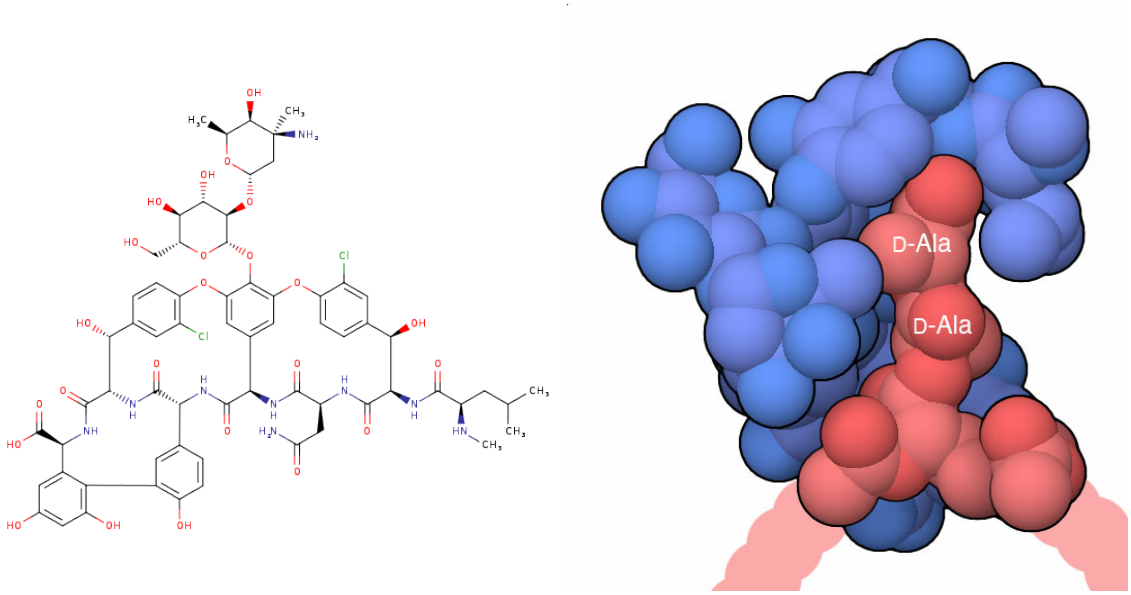

Figure C.1: Vancomycin binding to D-Ala-D-Ala. Target of vancomycin (Table 1.2). (Left) Vancomycin DrugBank (Right) RCSB[464,722,723] Illustration of vancomycin (blue) bound to D-Ala-D-Ala (red) based on PDB 1FVM (Nitanai et al, 2009).

approximately 3 – 5 atm of turgor. That is why videos (from Howard Hughes Medical Institute) show bacteria *burst* in the presence of β-lactam antibiotics.[721] For these reasons, antibiotics that target cell wall biosynthesis are generally considered safe and effective bactericidal agents.

While the cell walls of different organisms serve a similar purpose, they have fundamentally distinguishing features.[431] The cell walls of bacteria form a mesh-like structure that maintains their structure and function.[421] However, while some bacteria have a thick cell wall (monoderm), others have a thin cell wall, protected by an additional outer cell membrane (diderm). In this way, we generally refer to bacteria based on the staining results of cell walls with crystal violet versus a safranin counterstain as either "Gram-positive" or "Gram-negative," respectively.[312] Crucially, while Gram-negative and -positive bacteria possess similar biosynthetic machinery that incorporate lipid II molecules into their outwardly growing peptidoglycan (PG) cell walls, Gram-**positive** bacteria are particularly susceptible to inhibition of the incorporation of PG by the D-Ala-D-Ala terminal dipeptide of the lipid II molecule.

Around the mid-twentieth century, industrial chemists and microbiologists were prospecting soil samples from around the world for antibiotics. Thus, many strains of Streptomyces *spp.* of the phylum Actinomycetota yielded some of our earliest antibiotics, some still used today: In 1947, chloramphenicol

(Chloromycetin) was isolated from a Streptomyces *spp.* of Venezuela;[409–411] In 1948, chlortetracycline (Aureomycin) was isolated from a bacterial species originally identified as *Streptomyces aureofaciens* (renamed to Kitasatospora aureofaciens);[724] In 1952, erythromycin (Ilomycin) was isolated from *Actinomyces erythreus* (twice renamed: [former] *Streptomyces erythreus*; [current] *Saccharopolyspora erythraea*) from the Philippines.[412,413]

Around 1951, a former US Army chaplain on a mission trip to Borneo, Rev. William Conley, sent soil samples to his friend, E.C. Kornfeld. Unable to isolate any antibiotic-producing bacteria, Conley's colleague, William Bouw, sent a second batch to Kornfeld. From this second batch, **compound '05856'** was isolated from a species originally identified as *Streptomyces orientalis* (renamed to *Amycolatopsis orientalis*).[725,726] Compound 05856 would later become known as vancomycin, named for its ability to "**vanquish**" bacteria.[414] However, vancomycin proved difficult to purify, gaining the unfortunate nickname "**Mississippi Mud**". These impurities led to serious adverse infusion reactions, such as the inappropriately named and outdated "Red Man Syndrome", now referred to as the IDSA/HIVMA/SHEA/PIDS/SIDP-endorsed 'Vancomycin Infusion Reaction'.[433,433]

Vancomycin (VAN) (Pubchem CID 14969) is a large (1449.2 g/mol) hydrophilic (-2.6 XLogP3-AA) compound. It has a very low to negligible oral bioavailability and volume of distribution (~0.4 – 1 L/kg) when administered intravenously, achieving high concentrations and intestinal exposure when administered orally *per os*. The mechanism of action of vancomycin is the selective inhibition of the D-Ala-D-Ala terminal dipeptide.[415–417,419] The pharmacokinetics of intravenously administered VAN have been reviewed in detail for the treatment of bloodstream infections due to *Staph.* aureus.[422] Over the years, these physiochemical properties made orally administered (*per os*, or PO) vancomycin (VAN) a treatment for *Clostridioides difficile* infection (CDI). Despite its long utility for CDI, the evaluation of microbiome dynamics in response to orally administered VAN intestinal exposure is an ongoing research endeavor.[465] For instance, following the quantitation of VAN in stool,[439] estimates suggest orally administered VAN can achieve up to 4,000 µg/g stool, but with a slower "T_{max}" of around Day 9 of therapy due to intestinal transit time.[437]

Early clinical studies showed VAN had comparable rates of clinical cure and recurrent CDI (rCDI)

to that of PO metronidazole (MTZ).[418] However, follow-up studies determined VAN was superior to MTZ for more severe cases, but with similar recurrence rates across severity groups.[423] Unfortunately, VAN and MTZ both further disrupt the microbiome, and even promote the growth of *Enterococci* at comparable rates.[424] Such overgrowth of *Enterococci* in particular was long suspected to enhance *C. difficile* pathogenesis,[420] which was definitely confirmed in recent studies.[341] Not long-thereafter, additional findings suggested that VAN and MTZ had similar clinical efficacy, MTZ was associated with delayed and inconsistent microbiological cure.[425] Additional findings further suggested that MTZ was inferior to VAN for CDI.[429]

To combat the recurrence rates associated with VAN, recent investigations have been trying to pair its use with fecal microbiota transplantation (FMT),[727–729] a live biotherapeutic product (LBP) treatment modality that has potential safety concerns.[372] However, not long thereafter, VAN made room for the next antibiotic with comparable clinical cure and superior sustained clinical cure, orally administered fidaxomicin (FDX).[451,452,461,730–732] The evaluation of these data for the strengths and limitations has supported the ongoing guideline recommendation of VAN for CDI.[408,426,432] Orally administered vancomycin profoundly alters the human gut microbiome with impacts on host cardiometabolic signaling and bile acid metabolism[427,428,430]

A prominent mechanism by which Gram-positive bacteria have reduced susceptibility to vancomycin is the D-Ala-D-Ala to D-Ala-D-Ser switch in cell wall biosynthesis.[212,438] *C. difficile* resistance to the antibacterial activity of vancomycin by have been reviewed in the literature.[434,435] Around the turn of the twenty-first century, surveillance studies of collections of *C. difficile* isolates found an estimated 3% of intermediate resistance to vancomycin.[473] However, in the last couple of years there have been increasing concerns for creeping non-susceptibility to vancomycin.[474] Additionally, there is concern for selection of vancomycin-resistant *Enterococci* (VRE) that may enhance *C. difficile* pathogenesis.[341,436] To this end, the extent and clinical relevance of vancomycin "MIC creep' in *C. difficile* remains the subject of active investigation.[475,476] Analyses of publicly available genomic data suggests the genetic determinants of vancomycin resistance are infrequent among globally circulating strains of *C. difficile*.[477] However, critically, reduced *in vitro* susceptibility has been linked to worse clinical outcomes (*in vivo*).[468]

Fidaxomicin

The expression of genetic information, the transcription of DNA to RNA, is a fundamentally essential process to the central dogma of biology.[493] Fundamentally, the ribonucleotide sugar, ribose, differs from deoxyribose by the presence of a 2' hydroxyl.[733,734] The enzyme responsible for this transcription, the DNA-directed RNA polymerase (RNAP),[735] is found in bacteria,[736] archaea,[737] and eukarya.[738] Like that of DNA-directed DNA polymerases, RNAP coordinates the phosphodiester bond formation between the 5' phosphate of an elongating nucleic acid chain and the 3' hydroxyl group of an incoming template-directed ribonucleotide.[556] In bacteria, several proteins come together to form the DNA-directed RNA Polymerase (RNAP) holoenzyme to coordinate the transcription of DNA to RNA.[739] One particular factor in this holoenzyme, the sigma factor, such as σ^{70} in *E. coli*,[740] regulates transcription by directing the RNAP to a particular promoter sequence.[739] Unlike sigma factors that can be swapped in and out, particular subunits are essential to the function of the RNAP holoenzyme. For instance, the *rpoB* subunit of the RNAP holoenzyme is essential in both *E. coli*[741] and *B. subtilis*.[742]

The contemporary endeavors into RNAP inhibitor antibiotics began with the discovery of five substances, named rifamycin A through E, identified from a soil bacterium, *Amycolatopsis mediterranei* (initially identified as *Streptomyces mediterranei*) by Piero Sensi *et al.*[444,458] Among them, Rifamycin B was chemically modified to rifampin (rifampicin), a breakthrough antibiotic that has been reviewed in the literature.[446] Early works showed rifamycins inhibit the initiation of bacterial RNA transcription by binding to the β-subunit of the RNAP holoenzyme,[440] a mechanism later confirmed by structural biology studies.[743]

In parallel to the rifamycins, the distinct class of lipiarmycins[441] from *Actinoplanes deccanensis*,[442] was also characterized for the antibacterial properties at the inhibition of bacterial transcription.[443] In particular, lipiarmycin A3 (fidaxomicin [FDX], OPT-80, tiacumicin B), later referred to as fidaxomicin, was isolated from *Dactylosporangium aurantiacum*.[459,460]

Fidaxomicin (Dificid) is a 1,058.0 g/mol compound with a 6.4 XLogP3-AA (Table 1.2). Although the pharmacokinetic/pharmacodynamic (PK/PD) modeling of FDX in stool is an area of ongoing research, the oral adminsitration of FDX achieves high high intestinal intestinal exposure, upwards of 1,000

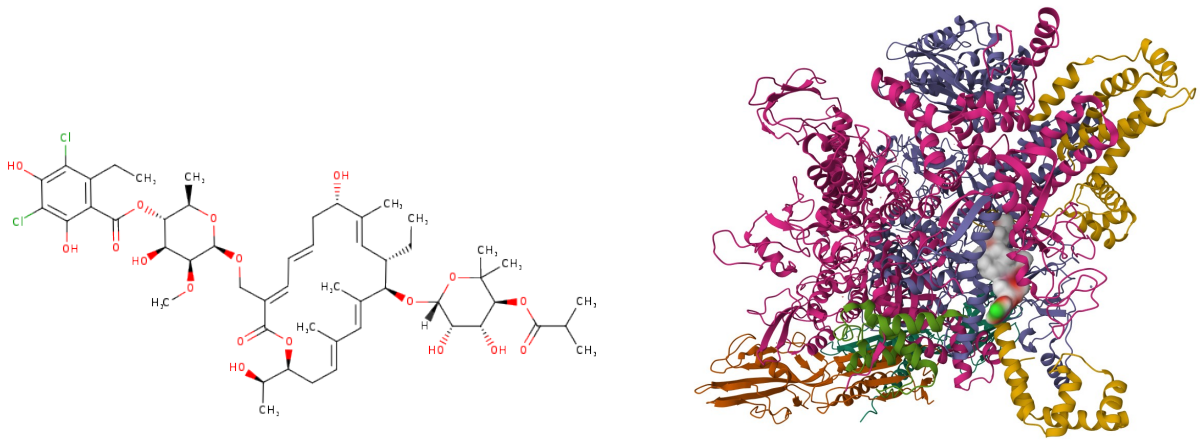

Figure C.2: Fidaxomicin bound to *C. difficile* RNAP. (Left) Chemical structure DB08874.[602,695] (Right) PDB 7L7B[368] Multi-subunit *Clostridioides difficile* RNAP bound to IBZ (Gaussian space-filled structure) in RCSB PDB[464,722,723].

μg/g stool.[455,462,465] These intestinal exposures in stool are dramatically higher than the historically documented range of *in vitro* susceptibility of between 0.001 – 1 ug/mL.[450,453]

FDX possesses a more narrow spectrum as a result of a unique RNAP-binding site that differed slightly across the microbiota,[368] and caused less disruption to the human gut microbiome, a critical concept to the treatment of *Clostridioides difficile* infection (CDI).[447–449,454] This phenomenon has translated into clinical efficacy of FDX for CDI that is non-inferior to VAN and superior for prevention of recurrent CDI (rCDI).[451,452] Furthermore, FDX not only spares the commensal microbiota, but also has reduced impact on the overgrowth of pathogenic *Candida* and vancomycin-resistance *Enterococci* compared to vancomycin.[744] Clinical trials later confirmed the clinical efficacy of FDX for CDI with a more narrow-spectrum, microbiome-sparing activity than vancomycin (VAN).[449,451,452,454,456,461] However, even though several lines of evidence strongly support the place in therapy of FDX, its cost limits the clinical adoption.[457,463] Despite these tremendous efforts, resistance to antibacterial RNAP inhibitors is a classical phenomenon across bacteria, from *E. coli* to *Mycobacterium tuberculosis*,[203,204,445] and *C. difficile* is no exception. For example, an outbreak of CDI with reduced susceptibility to FDX has been recently reported.[478] Genetic studies have determined that *rpoB* mutations in *C. difficile* confer reduced FDX susceptibility *in vitro*, but at a fitness cost.[479] A systematic review identified the Δ *rpoB* T^{3428} that yields Δ

RpoB Val^{1143} may confer *in vitro* MICs as high as 64 μg/mL.[480] To prevent a future without antibiotic treatments for CDI, many ongoing surveillance studies have found very little FDX resistance over the last several years.[467,481–483] However, while one study suggests genetic determinants of FDX resistance are rare among publicly available *C. difficile* genomes,[477] a case study of a 38-year old patient with multiply recurrent CDI suggests that resistance determinants can arise *in vivo* over a single course of FDX, giving us pause.[484]

WHY IS THE MICROBIOME IMPORTANT TO CDI?

Pathophysiology

Colonization Resistance

In 1954, researchers observed mice treated with streptomycin became more susceptible to *Salmonella* infection.[324] In 1964, ten years later, the same researchers observed this streptomycin treatment was associated with an increased (alkaline) pH and decreased short-chain fatty acid (SCFA) production.[325] In 1971 van der Waaiji *et al* first defined "Colonization resistance (CR) was expressed as the log of the oral bacterial dose followed by a persistent take in 50% of the contaminated animals" to quantify the antibiotic-mediated loss of murine intestinal resistance to *E. coli* colonization.[323] In 1994, CR was reviewed and solidified in the scientific literature by Vollard and Clasener.[326]

Today, CR is generally understood to be a highly diverse and interconnected web of competitive mechanisms that have been reviewed.[327–330] For example, *Salmonella*, a well-studied pathogen, has provided foundational insights into our understanding of CR. During gut inflammation, *Salmonella* takes advantage of host inflammation-derived tetrathionate,[331] and ethanolamine.[332] These resources give *Salmonella* the opportunity to grow and compete with butyrate-producing Clostridia that typically dominate the anaer-

obic gut through fermentation.[333] Other bacteria also confer CR to *Salmonella* via SCFAs: acetate-producing *Bifidobacteria*;[334,335] proprionate-producing *Bacteroides*.[336,337] *C. difficile* also takes advantage of nutrients released during host inflammation,[338] including host-derived sorbitol[339] ornithine,[340,341] and heme.[342,343] While the mechanisms of CR nutrient exclusion during inflammation is an ongoing area of research, it plays a major role in mediating CR.[344]

Exogenously administered food,[345,346] drugs[176,177,177,347] and microbiota-derived small molecules[128] can reshape the structure and function of the human gut microboime. Fortunately, extensive research is being done to understand[348] and restore the microbiome.[349] Some noteable classes of these microbiota-derived small molecules being utilized for microbiome-based therapeutic development include lantibiotics[350] bacteriocins[351,352] and antimicrobial peptides.[353] Some specific examples of ongoing research into these molecules include a lipopeptide-producing strain of *Bacillus subtilis* that inhibits *Staphylococcus aureus*[354,355]; a lantibiotic-producing strain of *Blautia producta* that inhibits vancomycin-resistant *Enterococcus faecium* (VRE);[356,357] Clostrdial fermentation of fiber to butyrate can activate host PPAR-γ signaling to inhibit Enterobacteriaceae expansion.[358]

Antibiotics and proton pump inhibitors have extensive, lasting impact on the human gut microbiome.[176,177,177,347,359–364] For example, clindamycin[365] fluoroquinolones[359] and third-generation cephalosporins[366] Yet, the development of narrow-spectrum antibiotics that spare the microbiome,[73] such as loamicin for treatment of Gram-negative bacterial infections,[367] and fidaxomicin for the treatment of CDI,[368] remains limited. To restore the loss of colonization resistance induced by antibiotics and PPIs, researchers have attempted to restore the microbiome through a few different approaches, such as fecal microbiota transplant (FMT), probiotics, and prebiotics.[348,349] However, while many lines of evidence support the effectiveness of FMT for recurrent CDI (rCDI),[369–371] there are major safety concerns regarding the potential for transfer of unknown phenotypes or undetected pathogens.[372] While probiotics are less regulated, their availability and safety have made them safe for over-the-counter use.[373] However, probiotics have historically yielded mixed results for the prevention of antibiotic-associated diarrhea.[374,375] In my opinion, this is most likely due to the failure of strain engraftment.[376] It is possible that CR prevents the invasion of not only pathogens, but any exogenous species, consistent with observations of drivers of FMT strain engraft-

ment.[179,180] To address these several issues, one potentially promising live biotherapeutic product (LBP), VE303, is a defined bacterial consortium comprised of mostly Lachnospiraceae and Oscillospiraceae for the prevention of rCDI.[377,378] To enhance VE303 strain engraftment in CDI patients, investigators employed vancomycin and fidaxomicin, but with limited success due to the susceptibility of the strains to vancomycin and fidaxomicin.[379]

While colonization resistance was first mentioned in the literature in 1971, the first study focus exclusively on CR to *C. difficile* was in 1984 by Rolfe.[380] Rolfe identified *in vivo* inhibitory concentrations of the 4-carbon SCFA, butyrate, for preventing CDI. Today, it is understood that the microbiome plays a central role in CDI increasing the risk of healthy individuals and patients alike to *C. difficile* infection (CDI) through **the loss of colonization resistance**.[327,328,381–384] However, the very antibiotics used to treat CDI, including PO vancomycin (guideline recommended) and metronidazole (no longer guideline recommended), while inhibitory of C. difficile growth, also further disrupt the human gut microbiome and disrupting the restoration of CR.[385] The majority of bacteria in the human gut microbiome, *Bacillota* and Bacteroidota, are typically reduced during antibiotic therapy[386] – and incompletely restore to their initial potential following the removal of the offending agent.[359] Though less well understood, mutations in the genes that encode antibiotic targets are also selected for in commensal bacteria of recovering microbiomes.

Bile is synthesized in the liver and stored in the gall bladder. Upon the presence of food, these bile salts are released from the gall bladder in into the upper duodenum to aid the digestion of fats and lipids. They pass through the small intestine acting as detergents of fats and lipids until they reach the terminal ileum, where they are 95% reabsorbed. However, these bile salts play much more important physiological roles.[145,387–392]

In the large intestine, bacterial bile salt hydrolases (BSH), enzymes that steal the conjugated taurine or glycine, creating de-conjugated (primary) bile acids. Some prominent examples of well charcterized BSHs include that of Lactobacillaceae[393,394] Bacteroidaceae,[395] and *C. difficile*.[396] While *bsh* are widespread across bacteria of the human gut microbiome,[397], the *bai* operon encoding the enzymes necessary for 7α-dehydroxylation in the primary to secondary bile acid conversion, are more phylogenetically restricted to ***OSCILLOSPIRACEAE*** (formerly Ruminococcaceae; *C. leptum* Group; Clostridium Cluster

IV)[398] and ***LACHNOSPIRACEAE*** (formerly *C. coccoides* Group; Clostridium Cluster XIVa)[314,366,399–402]

These taxa possess enzymes that convert host gall bladder-derived ”primary” bile acids to ”secondary” forms (secondary bile acids) through BSH, 7α-dehydroxylation, and epimerization via 3α/β-hydroxysteroid dehydrogenase (3α/β-HSDH), thereby inhibiting *C. difficile* spore germination, vegetative outgrowth, and toxin pathogenicity.[403–406] Notably, the epimers of lithocholate (LCA), including isolithocholate (iLCA) and isoallolithocholate (iaLCA), and that of CDCA, ursodeoxycholic acid (UDCA), further inhibit *C. difficile* growth and pathogenicity while sparing members of the gut microbiota[407]

Germination

Bacterial spores are metabolically dormant and highly resistant to environmental pressures.[299,300] To initiate disease, *C. difficile* spores must pass through the mouth, esophagus, and stomach to reach the small- and large-intestine. Host-derived bile acids synthesized in the liver, stored in the gall bladder and released into the upper duodenum, play a dual role in the process of germination, whereby some act as activators of germination, and others – inhibitors.[314] This bile salt pool significantly influences *C. difficile* spore germination, the critical step in the initiation of the transmission cycle.[304]

The conjugated primary bile salts, taurine- and glycine-conjugated cholate (taurocholate/TCA and glycocholate/GCA, respectively), and unconjugated cholate (CA), act as germinants of *C. difficile* spores.[315] Deoxycholate (DCA), the unconjugated secondary bile acid of CA produced by 7α-dehydroxylating bacteria that posess the *bai* operon, such as Lachnospiraceae and Oscillospiraceae, can also induce spore germination but inhibits vegetative growth.[315] In contrast to the above germinants, chenodeoxycholate (CDCA), another unconjugated primary bile acid like CA, acts as a competitive inhibitor of CA and TCA-mediated germination. The structural basis for this competitive inhibition is thought to be mediated through the absence of the 12α-hydroxyl group found in CA/TCA/GCA/DCA, lending to the crucial nature of this functional group in interactions with the receptor. Similar to DCA, CDCA also inhibits vegetative growth.[316]

While specific bile acids are necessary for germination, they alone are not sufficient.[315,317,318]

CspC, a pseudoprotease bile acid receptor in *C. difficile*, is activated by primary bile salts, such as TCA.[317] However, CspC requires the amino acid glycine as an essential co-germinant.[315] CspA, also a pseudoprotease co-germinant glycine receptor, is thought to directly interact with CspC.[319–321] CspB, a catalytically active protease bile salt receptor is activated upon germinant recognition, cleaves an inhibitory peptide from pro-SleC, a lytic enzyme that degrades the spore cortex.[322]

Sporulation

Bacteria survive harsh environments through formation of metabolically dormant, heavily protected spores.[299] The process of sporulation is an exciting area of research in both the model Gram-positive bacterium, *Bacillus subtilis*,[301–303] and our pathobiont of interest, *C. difficile*.[304–306] Generally speaking, different environmental signals will stimulate histidine kinases to phosphorylate the SpooA protein, a master regulator of sporulation. In doing so, phosphorylated SpooA then increases the abundance of sigma factor-H. (σ^{H} or SigH), leading to the increased expression of several genes required for forespore formation.[307]

While sigma factor regulatory networks in both *B. subtilis* and *C. difficile* control forespore formation, they differ in the architecture of their network, indicating the process is not uniform across the *Bacillota* phylum.[308–310] Generally speaking to location of the sigma factors during mother cell and forespore compartment formation, SigE (σ^{E}) and SigK (σ^{K}) are generally upregulated in the mother cell, whereas while SigF (σ^{F}) and SigG ((σ^{G})) are localized to the forespore in both *B. subtilis* and *C. difficile*[309,311] Critical to the sporulation-germination life-cycle, the pseudoprotease, CspA, is responsible for the insertion of the bile salt germinant receptor, CspC.[318,320] In that way, CspC can sense the presence of taurocholate,[317] indicating to the spore it has successfuly been reintroduced into the anoxic environment of an animals upper duodenum, a warm and nutrient rich environment, free to begin its transmission cycle once again.

Toxigenicity

Even though the two major toxins of *Clostridioides difficile*, Toxin A and Toxin B, are essential virulence factors to *C. difficile* infection (CDI),[231–233] not all lineages within the species are toxigenic (possess toxin-producing genes).[234,235] This has been attributed to the highly dynamic and open nature of the genome that permits the horizontal gene transfer (HGT) of mobile genetic elements (MGEs), like conjugative transposons, to insert the set of genes that encode toxins, the pathogenicity locus (PaLoc), into lineages of the species.[236]

The epidemic strain of ribotype F027 strain of *Clostridioides difficile* was originally reported to possess two separate mutations – one *gyrA* mutation conferring fluoroquinolone resistance; one *tcdC* mutation conferring toxin de-repression.[45,229,237] While genomic epidemiology confirmed the spread of *gyrA* fluoroquinolone resistance, the *tcdC* mutation may have contributed less to the outbreak than initially thought, with *in vitro* assays suggesting no change in toxin expression,[238,239] and the absence of PaLoc mutations in large F027 isolate collections from pre- and post-emergence.[230,240]

The PaLoc is a 19-kb locus that comprises five genes, including *tcdA*, *tcdB*, *tcdC*, *tcdD*, and *tcdE*, and is typically located at a specific chromosomal insertion site in the genomes of toxigenic *C. difficile*.[241] Critically, the sigma factor, TcdR, directs RNA polymerase to the PaLoc, initiating toxin gene expression.[242] The two potent exotoxins of the PaLoc, TcdA[243] (Toxin A) and TcdB[244] (Toxin B), considered part of the "large clostridial toxins" (LCT) family of toxins,[245] were first purified and characterized in 1982.[246] Notably, the *cdtA*- and *cdtB*-encoded binary toxins are present in most F027 and F078-126 ribotypes, and also contribute to disease.[247,248] These exotoxins bind to several human receptors,[249] followed by receptor-mediated endocytosis, endosomal acidification and membrane translocation, cysteine-protease auto-catalytic cleavage and payload delivery,[250,251] followed by Rho glycosylation and GTPase inactivation.[252]

A deeper understanding of how the human body responds to Clostridioides difficile toxins is essential for advancing therapeutic and vaccine strategies. Foundational studies have highlighted the roles of both innate and adaptive immune responses to these toxins,[253–255,338] demonstrated the efficacy of anti-

toxin monoclonal antibodies as therapeutics,[256,257] and explored the potential of vaccination approaches.[5]

Biofilm Production

The attachment and aggregation of microbes to surfaces is what we generally refer to as biofilms.[258,259] The capacity to build biofilms is widespread across bacteria,[266,267] fungi,[268,269] and archaea.[270] The life-cycle of biofilms, including their formation,[271–275] and dispersal[276–278] have been extensively reviewed for the model Gram-negative bacterium, *Escherichia coli*,[279,280] the model Gram-positive bacterium, *Bacillus subtilis*,[281–284] and that of *Clostridioides difficile*.[285–287]

Notably, biofilms play an important role in gastroenteric health and disease[288], such as their ability to survive antimicrobials at concentrations otherwise lethal to the planktonic cells.[289–292] Although experimental models are moving towards more accurate biological recapitulation,[293] notable groups have established experimental models of *C. difficile* biofilms.[294–298] While the data is limited, one study suggests that sub-inhibitory concentrations of metronidazole can induce *C. difficile* biofilm formation;[297] whether this phenomenon is an agent- or class-specific phenomenon limited to metronidazole or extends to other antibiotics, such as vancomycin, fidaxomicin, or other antibiotics, remains yet to be uncovered. In general, evidence suggests biofilms serve as a reservoir for recurrent *C. difficile* infection (rCDI) and warrant further research.[286]

D

Antimicrobial Resistance Crisis

Forecasts estimate an average 2 million deaths attributable to antimicrobial resistance (AMR) could occur annually by the year 2050.[185] Despite the challenges and pressures of anti-infective drug development,[186–192] economic analyses suggest anti-infective approvals return a net benefit to society.[193] The inability of antibiotics to inhibit the growth of bacteria, or bacterial antimicrobial resistance (antibiotic resistance), is a global grand challenge of urgent clinical concern.[185,194–196] Bacteria can sometimes lead to multidrug-resistant (MDR), extensively drug-resistant (XDR) and even pandrug-resistance.[197] To combat the antibiotic resistance crisis, the mechanisms of bacterial antimicrobial resistance have been previously reviewed in the literature.[198–200] In general, there are six broad categories by which bacteria thwart antibiotic activity: (1) decreased influx, (2) increased eflux, (3) antibiotic inactivation, (4) target modification, (5) target protection, and (6) target bypass.[199] However, this is an incomplete list able to incorporate additional categories that have yet to be discovered or established.

Understanding the mechanisms by which oral antibiotics and non-antibiotic drugs remodel the human gut microbiome is an exciting scientific endeavor.[176,177,201,202,205,359,391] While the research unraveling these mechanisms remains ongoing, the resistance determinants in the human gut microbiome are often referred to as "the resistome"[206–208] Although a great amount of attention has, rightfully so, been dedicated towards understanding the resistome in bacterial pathogens and pathobionts, less attention has been given to undestanding the antibiotic susceptibility of commensal and symbiotic bacteria within the

human gut microbiome for their influence on the changing human gut microbiome in response to antibiotic exposure. This discrepancy in attention is likely due to the history of inconsistent results[374,375] with probiotics[209]; only recently have the drivers of strain engraftment been more fully elucidated.[179,180] Despite this, a few prominent examples have demonstrated the potential of commensal and symbiotic bacteria in the prevention of disease caused by pathogenic and pathobiont bacteria. For example, the administration of *Bacillus subtilis* prevents the translocation of *Staphylococcus aureus* from the gut into the bloodstream;[355] *Blautia producta* restores colonization resistance against vancomycin-resistant *Enterococcus* (VRE);[356,357] non-toxigenic *C. difficile* strain prevents against toxigenic *C. difficile* infection (CDI).[234]

ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics[210] boucher 2009 Science Bad Bugs, no Drugs: No ESKAPE![194] arias 2009 nejm antibiotic-resistant bugs in the 21st century - a clinical super-challenge[211] Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis[195] Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050[185] causes, challenges, and responses[213] mechanisms and drivers[214] the need for global solutions[215] WHO priority list of antibiotic-resistant bacteria and tuberculosis[216] utilization of data[217] The scope of the antimicrobial resistance challenge[196] Antimicrobial resistance: a concise update[218] The global consumption rate of antibiotics increased between the years of 2000 and 2015, driven mostly by low- and middle-income countries, and correlated with economic productivity.[219] The increased burden of bacterial antimicrobial resistance have been found in the European economic area[220] South-East Asia,[221] Africa,[222,223] the Americas,[224,225]

E

The Human Gut Microbiome

A microbial biome, or **MICROBIOME**, is the ecosystem of microbial biotic factors - including the communities of bacteria, fungi, and protists; abiotic factors - metabolites and viruses; environmental factors - oxygen, temperature, and water.[84] Microbiomes are found on multicellular organisms, such as plants[85,86] and animals,[87,88] and different environments of the earth,[89–92] such as air,[93] soil,[94] oceans,[95,96] deep-sea hydrothermal vents, glaciers, rivers, and wastewater. The most prominent body sites of human-associated microbiomes include the mouth (oral microbiome),[97] lungs (pulmonary microbiome),[98] intestinal tract (gut microbiome),[99–101] vaginal tract (vaginal microbiome),[102–104] and skin (skin microbiome).[105,106] At the level of biological analysis, the communities of fungi (mycobiome),[107] bacteria (microbiota),[108] viruses (virome),[109] and their metabolites (metabolome) are often characterized by different methods and approaches.

The **HUMAN GUT MICROBIOME** (interchangeably referred to herein and the literature as 'the microbiome') is arguably the pre-eminent human microbiome with significant associations to human health across several large-scale studies of various diseases.[101,108,110–124] This prominence is due to the convenience of sampling human stool, and the majority of resident bacteria in the large intestine.[125,126 127–129] One fascinating feature of the microbiome is its capability of person-to-person transmission[87,130,131] and transfer of host-associated metabolic phenotypes,[132–134] further underscoring its importance at population levels of health and disease. In the search for mechanisms of the microbiome with therapeutic po-

tential, the literature has established biobanks,[135] robust scientific frameworks,[100,136–140,140–147,147] approaches,[148–152], reporting guidelines,[153] and consensus statements[154,155] to guide microbiome researchers.

The **ABIOTIC FACTORS** that determine the enterotypes[101,118] of the microbiome might include the temperature, water, oxygen, pH, nutrient availability that differ along the human alimentary tract. For instance, the mouth and nasopharynx are generally considered rich in oxygen, a slighly acidic to neutral pH, frequent exposure to nutrients from food stuck in teeth, and a temperature of about 35 - 37 °C are favorable to facultative anaerobes such as Streptococcus *spp*. The stomach, known for its acidic pH and lower oxygen generally favors acid-tolerant bacteria, such as *Helicobacter pylori*. The small-intestine, including the duodenum, ileum and cecum, is generally considered the start of a strictly anaerobic,[156,157] nutrient-rich environment favorable to facultative and obligate anaerobic bacteria, such as *Lactobacillus*, *Streptococcus* and *Enterobacteriaceae*. Finally, the large intestine (or colon), comprising the ascending, transverse and descending colon, is generally considered **ANOXIC** and rich in nondigestible carbohydrates and unabsorbed bile acids.

The placement of **BACTERIA** on the tree of life next to other domains, Archaeota and Eukaryota, depends on the 16S rRNA.[158,159] The differences among regions of the 16S rRNA gene of bacteria also allows researchers to quantify the relative abundance of different species in a community[160,161] and even predict their collective functional capacity.[162,163] The bacterial phyla of the human gut microbiome predominantly consist of *Bacillota*, *Bacteroidota*, *Actinomycetota*, *Pseudomonadota*, *Fusobacteriota*, *Verrucomicrobiota*. These communities of microbes interact with each other in interesting and complicated economies, sometimes referred to as **bacterial guilds**. Together, they produce many biomolecules that are beneficial to their human hosts, including the metabolism of dietary biomolecules (such as fiber fermentation)[122,164,165] the production of essential vitamins[166–169] and modulation of inflammation (often through bile acid biotransformation).[170–172]

The human gut microbiome is particularly responsive to dietary fiber as a consequence of the **ANAEROBIOSIS** associated with **FERMENTATION**.[173] Bacterial fermentation of complex carbohydrates from nondigestible fibers into short-chain fatty acids (SCFAs) generally consists of using NADH as an electron donor instead of oxygen. This process is generally considered sensitive to oxygen through

the Fe-S enzymes that are sensitive to oxygen intoxication. An early theory explaining why oxygen was believed to be 'toxic' to some species of bacteria was the absence of a defensive enzyme, superoxide dismutase (SOD), that detoxify the bacterial cell by converting superoxide (O_2^-), a free radical byproduct of molecular oxygen (O_2) – into less harmful molecules.[174] However, recent investigations suggest the absence of SOD insufficiently describes the phylogenetic and biochemical basis of anaerobiosis. Rather, the fermentative enzymes involved in redox reactions during anerobiosis depend on low-potential electron flow and metal coordinated active sites that are critically disrupted by O_2 and O_2^-.[175] Taken together, the environmental conditions, such as the anaerobic nature of the lumen of the small- and large intestine, are a key determinant of the microbial composition of the human gut microbiome.

Finally, major additional abiotic factor that shapes the human gut microbiome include orally administered **ANTIBIOTICS**[176] and **NON-ANTIBIOTIC DRUGS**.[177] Researchers have described profound combinatorial- and dose-dependent impacts of drugs on the human gut microbiome.[178] Several lines of evidence support antibiotic depletion of the microbiome not only reduce diversity, but also reduce colonization resistance to invading bacterial strains. For instance, oral antibiotics are a driver of strain engraftment of orally administered therapeutic bacteria.[179,180] One mechanism by which colonization resistance prevents such invasion is hypothesized to be metabolic nutrient exclusion.[344]

Bacteria

The most prominent **bacteria** found in the human gut include (in descending relative abundance) *Bacteroidota* (formerly *Bacteroidetes*; NCBI:txid976), *Bacillota* (formerly *Firmicutes*; NCBI:txid1239), *Actinomycetota* (formerly *Actinobacteria*; NCBI:txid201174), Verrucomicrobiota (formerly *Verrucomicrobia*; NCBI:txid74201), *Fusobacteriota* (formerly *Fusobacteria*; NCBI:txid32066), *Pseudomonadota* (formerly *Proteobacteria*; NCBI:txid1224). Notably, a majority of these bacteria were historically considered 'unculturable', with recent The relative abundances of these taxa vary across age and geography, with different community structures called enterotypes. For example, the gut microbiomes of infants have elevated levels of *Bififobacteria* (genus of *Actinomycetota*), especially in breastfed infants, and *Pseudomonadota*,

and corresponding diminished levels of *Bacillota* and *Bacteroidota* that increase with age after weaning. That of healthy human adults generally comprise a majority of increased *Bacillota* and *Bacteroidota*, a minor increase in *Verrucomicrobiota*, and decreased *Actinomycetota* and *Pseudomonadota*, relative to infants. Finally, as adults age towards elderly status, their enterotypes change with a decreased abundance of *Bacillota*, varying *Bacteroidota*, and increased *Pseudomonadota* that reflect frailty and inflammation.

The ability to culture anaerobic bacteria in anoxic environments has an essential history to understanding the bacteria in the human gut microbiome, both harmful (e.g. *C. difficile*) and beneficial (e.g. *Lachnospiraceae*, *Oscillospiraceae*). Pasteur is sometimes referred to as the earliest to grow anaerobic bacteria in sealed flasks and displacement by inert gases.[745] Veillon and Zuber is credited with the earliest descriptions of anaerobic cocci, later named *Veillonella* after Veillon.[746] Hungate provided his method, the Hungate Method, that utilized roll tubes, reducing agents (e.g. cysteine and thioglycolate), and CO_2/H_2 gas.[677] More recently, these anaerobic bacteria belonging to the 'unculturable' microbiota, such as *Lachnospiraceae*, *Oscillospiraceae* and *Erysipelotrichaceae*, were able to be grown and isolated on yeast-casitone fatty acid (YCFA) agar.[587]

F

Deoxyribonucleic Acid: a History

In 1871, Johann Friedrich Miescher discovered the fundamental molecule, nucleic acid, from the nuclei of cells.[747,748] In 1893, Albrecht Kossel described the individual components of nucleic acid, including adenine, guanine, cytosine, thymine, and uracil, for which he was awarded the 1910 Nobel Prize in Physiology or Medicine[749] In 1919, the structure of nucleotides was determined by Phoebus Levene for which he was nominated the 1932 Nobel Prize in Physiology or Medicine.[750] In 1928, Frederick Griffith showed a "transforming principle" was capable of transferring traits between strains of *Streptococcus pneumoniae*, however he failed to identify the nature of this principle.[490] In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty made a transformative finding – deoxyribonucleic acid (DNA) is the the molecule of heredity, or the "transforming principle" from their virulent transformation experiments of an avirulent strain of *Streptococcus pneumoniae*.[491] In 1950, Erwin Chargaff published his rules for the base-pairing rules of nucleotides, guanine to cytidine, and adenine to thymidine (or uracil in RNA).[557] In 1958, Meselson and Stahl determined the semi-conservative mode DNA replication.[492] In 1953, Rosalind Franklin and her student, Raymond Gosling, published the iconic Photograph 51, an X-ray diffraction image that proved essential to elucidating the B-form double helix structure of DNA.[751,752]

Bibliography

[1] Wiep Klaas Smits, Dena Lyras, D. Borden Lacy, Mark H. Wilcox, and Ed J. Kuijper. Clostridium difficile infection. *Nature Reviews. Disease Primers*, 2:16020, apr 2016. ISSN 2056-676X. doi: 10.1038/nrdp.2016.20.

[2] Erik R. Dubberke and Margaret A. Olsen. Burden of Clostridium difficile on the healthcare system. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 55 Suppl 2(Suppl 2):S88–92, aug 2012. ISSN 1537-6591. doi: 10.1093/cid/cis335.

[3] F. C. Lessa, L. G. Winston, L. C. McDonald, and Emerging Infections Program C. difficile Surveillance Team. Burden of clostridium difficile infection in the united states. *New England Journal of Medicine*, 372(24):2368–2370, June 2015. ISSN 1533-4406. doi: 10.1056/nejmc1505190. URL http://dx.doi.org/10.1056/NEJMc1505190.

[4] Shelley S. Magill, Erin O'Leary, Sarah J. Janelle, Deborah L. Thompson, Ghinwa Dumyati, Joelle Nadle, Lucy E. Wilson, Marion A. Kainer, Ruth Lynfield, Samantha Greissman, Susan M. Ray, Zintars Beldavs, Cindy Gross, Wendy Bamberg, Marla Sievers, Cathleen Concannon, Nicolai Buhr, Linn Warnke, Meghan Maloney, Valerie Ocampo, Janet Brooks, Tolulope Oyewumi, Shamima Sharmin, Katherine Richards, Jean Rainbow, Monika Samper, Emily B. Hancock, Denise Leaptrot, Eileen Scalise, Farzana Badrun, Ruby Phelps, and Jonathan R. Edwards. Changes in Prevalence of Health Care–Associated Infections in U.S. Hospitals. *New England Journal of Medicine*, 379(18):1732–1744, nov 2018. ISSN 0028-4793. doi: 10.1056/NEJMoa1801550. URL https://www.nejm.org/doi/full/10.1056/NEJMoa1801550.

[5] Vincent B. Young. Vaccinating against Clostridioides difficile Infection. *New England Journal of Medicine*, 392(12):1237–1240, mar 2025. ISSN 0028-4793. doi: 10.1056/NEJMcibr2413794. URL https://www.nejm.org/doi/full/10.1056/NEJMcibr2413794.

[6] Moïra Joëlle Talpaert, Guduru Gopal Rao, Ben Symons Cooper, and Paul Wade. Impact of guidelines and enhanced antibiotic stewardship on reducing broad-spectrum antibiotic usage and its effect on incidence of Clostridium difficile infection. *The Journal of Antimicrobial Chemotherapy*, 66(9):2168–2174, sep 2011. ISSN 1460-2091. doi: 10.1093/jac/dkr253.

[7] Alice Y. Guh, Yi Mu, Lisa G. Winston, Helen Johnston, Danyel Olson, Monica M. Farley, Lucy E. Wilson, Stacy M. Holzbauer, Erin C. Phipps, Ghinwa K. Dumyati, Zintars G. Beldavs, Marion A. Kainer, Maria Karlsson, Dale N. Gerding, and L. Clifford McDonald. Trends in U.S. Burden of Clostridioides difficile Infection and Outcomes. *New England Journal of Medicine*, 382(14):1320–1330, apr 2020. ISSN 0028-4793. doi: 10.1056/NEJMoa1910215. URL https://www.nejm.org/doi/full/10.1056/NEJMoa1910215.

[8] F. Barbut and J. C. Petit. Epidemiology of Clostridium difficile-associated infections. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 7(8):405–410, aug 2001. ISSN 1198-743X. doi: 10.1046/j.1198-743x.2001.00289.x.

[9] Fernanda C. Lessa, Carolyn V. Gould, and L. Clifford McDonald. Current status of Clostridium difficile infection epidemiology. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 55 Suppl 2(Suppl 2):S65–70, aug 2012. ISSN 1537-6591. doi: 10.1093/cid/cis319.

[10] J.S.H. Martin, T.M. Monaghan, and M.H. Wilcox. Clostridium difficile infection: Epidemiology, diagnosis and understanding transmission. *Nature Reviews Gastroenterology and Hepatology*, 13 (4):206–216, 2016. doi: 10.1038/nrgastro.2016.25.

[11] Nicholas A. Turner, Steven C. Grambow, Christopher W. Woods, Vance G. Fowler Jr, Rebekah W. Moehring, Deverick J. Anderson, and Sarah S. Lewis. Epidemiologic trends in Clostridioides difficile infections in a regional community hospital network. *JAMA Network Open*, 2(10): e1914149, 2019. URL https://jamanetwork.com/journals/jamanetworkopen/article-abstract/2753662.

[12] Paul Feuerstadt, Nicolette Theriault, and Glenn Tillotson. The burden of CDI in the United States: a multifactorial challenge. *BMC Infectious Diseases*, 23(1):132, mar 2023. ISSN 1471-2334. doi: 10.1186/s12879-023-08096-0. URL https://doi.org/10.1186/s12879-023-08096-0.

[13] Stefano Di Bella, Gianfranco Sanson, Jacopo Monticelli, Verena Zerbato, Luigi Principe, Mauro Giuffrè, Giuseppe Pipitone, and Roberto Luzzati. Clostridioides difficile infection: history, epidemiology, risk factors, prevention, clinical manifestations, treatment, and future options. *Clinical Microbiology Reviews*, 37(2):e0013523, jun 2024. ISSN 1098-6618. doi: 10.1128/cmr.00135-23.

[14] L. V. McFarland, M. E. Mulligan, R. Y. Kwok, and W. E. Stamm. Nosocomial acquisition of Clostridium difficile infection. *The New England Journal of Medicine*, 320(4):204–210, jan 1989. ISSN 0028-4793. doi: 10.1056/NEJM198901263200402.

[15] Matthew T. Oughton, Vivian G. Loo, Nandini Dendukuri, Susan Fenn, and Michael D. Libman. Hand hygiene with soap and water is superior to alcohol rub and antiseptic wipes for removal of Clostridium difficile. *Infection Control and Hospital Epidemiology*, 30(10):939–944, oct 2009. ISSN 1559-6834. doi: 10.1086/605322.

[16] J. L. Mayfield, T. Leet, J. Miller, and L. M. Mundy. Environmental control to reduce transmission of Clostridium difficile. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 31(4):995–1000, oct 2000. ISSN 1058-4838. doi: 10.1086/318149.

[17] David W. Eyre, Madeleine L. Cule, Daniel J. Wilson, David Griffiths, Alison Vaughan, Lily O'Connor, Camilla L. C. Ip, Tanya Golubchik, Elizabeth M. Batty, John M. Finney, David H. Wyllie, Xavier Didelot, Paolo Piazza, Rory Bowden, Kate E. Dingle, Rosalind M. Harding, Derrick W. Crook, Mark H. Wilcox, Tim E. A. Peto, and A. Sarah Walker. Diverse sources of C. difficile infection identified on whole-genome sequencing. *The New England Journal of Medicine*, 369(13): 1195–1205, sep 2013. ISSN 1533-4406. doi: 10.1056/NEJMoa1216064.

[18] S. C. Lim, D. R. Knight, and T. V. Riley. Clostridium difficile and One Health. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 26(7):857–863, jul 2020. ISSN 1469-0691. doi: 10.1016/j.cmi.2019.10.023.

[19] J. Scott Weese. Clostridium (Clostridioides) difficile in animals. *Journal of Veterinary Diagnostic Investigation*, 32(2):213–221, 3 2020. ISSN 19434936. doi: 10.1177/1040638719899081,. URL https://pubmed.ncbi.nlm.nih.gov/31904312/.

[20] M. Jahangir Alam, Ananna Anu, Seth T. Walk, and Kevin W. Garey. Investigation of potentially pathogenic Clostridium difficile contamination in household environs. *Anaerobe*, 27:31–33, 2014. ISSN 10958274. doi: 10.1016/J.ANAEROBE.2014.03.002.

[21] Sandra Janezic, Ines Blazevic, David W. Eyre, Bozena Kotnik Kevorkijan, Tatjana Remec, and Maja Rupnik. Possible contribution of shoes to Clostridioides difficile transmission within hospitals. *Clinical Microbiology and Infection*, 27(5):797–799, 5 2021. ISSN 14690691. doi: 10.1016/j.cmi.2020.11.001. URL https://pubmed.ncbi.nlm.nih.gov/33166648/.

[22] A. C. Büchler, M. Wicki, R. Frei, V. Hinic, H. M.B. Seth-Smith, A. Egli, and A. F. Widmer. Matching Clostridioides difficile strains obtained from shoe soles of healthcare workers epidemiologically linked to patients and confirmed by whole-genome sequencing. *Journal of Hospital Infection*, 126:10–15, 8 2022. ISSN 15322939. doi: 10.1016/j.jhin.2022.04.016. URL https://pubmed.ncbi.nlm.nih.gov/35562075/.

[23] M. Jahangir Alam, Jacob McPherson, Julie Miranda, Allyson Thrall, Van Ngo, Rebecca Kessinger, Khurshida Begum, Maud Marin, and Kevin W. Garey. Molecular epidemiology of Clostridioides difficile in domestic dogs and zoo animals. *Anaerobe*, 59:107–111, oct 2019. ISSN 1095-8274. doi: 10.1016/j.anaerobe.2019.06.005. URL http://www.ncbi.nlm.nih.gov/pubmed/31207298http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6785390. 2 citations (Semantic Scholar/DOI) [2022-09-12].

[24] Su Chen Lim, Deirdre A. Collins, Korakrit Imwattana, Daniel R Knight, Sicilia Perumalsamy, Natasza M.R. Hain-Saunders, Papanin Putsathit, David Speers, and Thomas V. Riley. Whole-genome sequencing links Clostridium (Clostridioides) difficile in a single hospital to diverse environmental sources in the community. *Journal of Applied Microbiology*, 133(3):1156–1168, 9 2022. ISSN 13652672. doi: 10.1111/JAM.15408,. URL https://pubmed.ncbi.nlm.nih.gov/34894035/.

[25] Jinhee Jo, Anne J. Gonzales-Luna, Chris K. Lancaster, Jacob K. McPherson, Khurshida Begum, M. Jahangir Alam, and Kevin W. Garey. Multi-country surveillance of Clostridioides difficile demonstrates high prevalence of spores in non-healthcare environmental settings. *Anaerobe*, 75, 6 2022. ISSN 10958274. doi: 10.1016/j.anaerobe.2022.102543. URL https://pubmed.ncbi.nlm.nih.gov/35227896/.

[26] Robert Fekety, Kyung Hee Kim, Donald Brown, Donald H. Batts, Margaret Cudmore, and Joseph Silva. Epidemiology of antibiotic-associated colitis: Isolation of clostridium difficile from the hospital environment. *The American Journal of Medicine*, 70(4):906–908, 4 1981. ISSN 0002-9343. doi: 10.1016/0002-9343(81)90553-2. URL https://www.sciencedirect.com/science/article/abs/pii/0002934381905532.

[27] Erik R. Dubberke, Kimberly A. Reske, Judith Noble-Wang, Angela Thompson, George Killgore, Jennie Mayfield, Bernard Camins, Keith Woeltje, Jay R. McDonald, L. Clifford McDonald, and Victoria J. Fraser. Prevalence of Clostridium difficile environmental contamination and strain variability in multiple health care facilities. *American Journal of Infection Control*, 35(5):315–318, 6 2007. ISSN 0196-6553. doi: 10.1016/J.AJIC.2006.12.006. URL https://www.sciencedirect.com/science/article/pii/S0196655307000971?casa_token=Q675fMEW6L8AAAAA:nYYyg10D5bsjavniSO2UqTUEJvlLDlTMU3c5kJAUm0wbLoWIhIWnSpMRE5abClrda2RaMuItWA.

[28] Maury Ellis Mulligan, Rial D. Rolfe, Sydney M. Finegold, and W. Lance George. Contamination of a hospital environment by Clostridium difficile. *Current Microbiology*, 3(3):173–175, 5 1979. ISSN 03438651. doi: 10.1007/BF02601862/METRICS. URL https://link.springer.com/article/10.1007/BF02601862.

[29] H. Malamou-Ladas, S. O'Farrell, J. Q. Nash, and S. Tabaqchali. Isolation of Clostridium difficile from patients and the environment of hospital wards. *Journal of Clinical Pathology*, 36(1):88–92, 1 1983. ISSN 0021-9746. doi: 10.1136/JCP.36.1.88. URL https://jcp.bmj.com/content/36/1/88https://jcp.bmj.com/content/36/1/88.abstract.

[30] Glenn W. Kaatz, Scott D. Gitlin, Dennis R. Schaberg, Kenneth H. Wilson, Carol A. Kauffman, Susan M. Seo, and Robert Fekety. ACQUISITION OF CLOSTRIDIUM DIFFICILE FROM THE HOSPITAL ENVIRONMENT. *American Journal of Epidemiology*, 127(6):1289–1294, 6 1988. ISSN 0002-9262. doi: 10.1093/OXFORDJOURNALS.AJE.A114921. URL https://dx.doi.org/10.1093/oxfordjournals.aje.a114921.

[31] Steven J. Lawrence, Laura A. Puzniak, Brooke N. Shadel, Kathleen N. Gillespie, Marin H. Kollef, and Linda M. Mundy. Clostridium difficile in the Intensive Care Unit: Epidemiology, Costs, and Colonization Pressure. *Infection Control & Hospital Epidemiology*, 28(2):123–130, feb 2007. ISSN 0899-823X, 1559-6834. doi: 10.1086/511793. URL https://www.cambridge.org/core/journals/infection-control-and-hospital-epidemiology/article/abs/clostridium-difficile-in-the-intensive-care-unit-epidemiology-costs-and-colon
D2F9918B4570E0CD38CB8A4C4653251D.

[32] Jay Worley, Mary L. Delaney, Christopher K. Cummins, Andrea DuBois, Michael Klompas, and Lynn Bry. Genomic Determination of Relative Risks for Clostridioides difficile Infection From Asymptomatic Carriage in Intensive Care Unit Patients. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 73(7):e1727–e1736, oct 2021. ISSN 1537-6591. doi: 10.1093/cid/ciaa894.

[33] Arianna Miles-Jay, Evan S. Snitkin, Michael Y. Lin, Teppei Shimasaki, Michael Schoeny, Christine Fukuda, Thelma Dangana, Nicholas Moore, Sarah E. Sansom, Rachel D. Yelin, Pamela Bell, Krishna Rao, Micah Keidan, Alexandra Standke, Christine Bassis, Mary K. Hayden, and Vincent B. Young. Longitudinal genomic surveillance of carriage and transmission of Clostridioides difficile in an intensive care unit. *Nature Medicine*, 29(10):2526–2534, oct 2023. ISSN 1546-170X. doi: 10.1038/s41591-023-02549-4.

[34] Lindsay T. Keegan, Windy Tanner, Brian Orleans, Rachel B. Slayton, John A. Jernigan, L. Clifford McDonald, Judith Noble-Wang, Molly Leecaster, Candace Haroldsen, Karim Khader, Damon J.A. Toth, Tierney O'Sullivan, Matthew H. Samore, William Brazelton, and Michael Rubin.

Environmental and Health Care Personnel Sampling and Unobserved Clostridium difficile Transmission in ICU. *JAMA Network Open*, 8(4):e252787, apr 2025. ISSN 2574-3805. doi: 10.1001/jamanetworkopen.2025.2787. URL https://doi.org/10.1001/jamanetworkopen.2025.2787.

[35] Albert Palleja, Kristian H. Mikkelsen, Sofia K. Forslund, Alireza Kashani, Kristine H. Allin, Trine Nielsen, Tue H. Torben Hansen, Suisha Liang, Qiang Feng, Chenchen Zhang, Paul Theodor Pyl, Luis Pedro Coelho, Huanming Yang, Jun Jian Wang, Athanasios Typas, Morten F. Nielsen, Henrik Bjorn Nielsen, Peer Bork, Jun Jian Wang, Tina Vilsbøll, Tue H. Torben Hansen, Filip K. Knop, Manimozhiyan Arumugam, and Oluf Pedersen. Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nature Microbiology*, 3(11):1255–1265, nov 2018. ISSN 20585276. doi: 10.1038/s41564-018-0257-9. URL https://www.nature.com/articles/s41564-018-0257-9http://www.nature.com/articles/s41564-018-0257-9. 299 citations (Semantic Scholar/DOI) [2022-09-12] ISBN: 4156401802579 Publisher: Nature Publishing Group.

[36] Robert C. Owens, Curtis J. Donskey, Robert P. Gaynes, Vivian G. Loo, and Carlene A. Muto. Antimicrobial-associated risk factors for Clostridium difficile infection. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 46 Suppl 1:S19–31, jan 2008. ISSN 1537-6591. doi: 10.1086/521859.

[37] Claudia Slimings and Thomas V. Riley. Antibiotics and healthcare facility-associated Clostridioides difficile infection: systematic review and meta-analysis 2020 update. *Journal of Antimicrobial Chemotherapy*, 76(7):1676–1688, 2021. URL https://academic.oup.com/jac/article-abstract/76/7/1676/6205941.

[38] Aaron C. Miller, Alan T. Arakkal, Daniel K. Sewell, Alberto M. Segre, Joseph Tholany, Philip M. Polgreen, and CDC MInD-Healthcare Group. Comparison of different antibiotics and the risk for community-associated Clostridioides difficile infection: a case–control study. In *Open forum infectious diseases*, volume 10, page ofad413. Oxford University Press US, 2023. URL https://academic.oup.com/ofid/article-abstract/10/8/ofad413/7237811.

[39] Michael J. Ray, Luke C. Strnad, Kendall J. Tucker, Jon P. Furuno, Eric T. Lofgren, Caitlin M. McCracken, Hiro Park, Jeffrey S. Gerber, and Jessina C. McGregor. Influence of antibiotic exposure intensity on the risk of clostridioides difficile infection. *Clinical Infectious Diseases*, 79(5):1129–1135, 2024. URL https://academic.oup.com/cid/article-abstract/79/5/1129/7672870.

[40] Kevin A. Brown, Nagham Khanafer, Nick Daneman, and David N. Fisman. Meta-Analysis of Antibiotics and the Risk of Community-Associated Clostridium difficile Infection. *Antimicrobial Agents and Chemotherapy*, 57(5):2326–2332, apr 2013. doi: 10.1128/aac.02176-12. URL https://journals.asm.org/doi/full/10.1128/aac.02176-12.

[41] J. G. Bartlett, A. B. Onderdonk, R. L. Cisneros, and D. L. Kasper. Clindamycin-associated colitis due to a toxin-producing species of Clostridium in hamsters. *The Journal of Infectious Diseases*, 136(5):701–705, nov 1977. ISSN 0022-1899. doi: 10.1093/infdis/136.5.701.

[42] J. G. Bartlett, N. Moon, T. W. Chang, N. Taylor, and A. B. Onderdonk. Role of Clostridium difficile in antibiotic-associated pseudomembranous colitis. *Gastroenterology*, 75(5):778–782, nov 1978. ISSN 0016-5085.

[43] J. G. Bartlett. Antibiotic-associated pseudomembranous colitis. *Reviews of Infectious Diseases*, 1 (3):530–539, 1979. ISSN 0162-0886. doi: 10.1093/clinids/1.3.530.

[44] K. W. Garey, S. Sethi, Y. Yadav, and H. L. DuPont. Meta-analysis to assess risk factors for recurrent *Clostridium difficile* infection. *Journal of Hospital Infection*, 70(4):298–304, dec 2008. ISSN 0195-6701. doi: 10.1016/j.jhin.2008.08.012. URL https://www.sciencedirect.com/science/article/pii/S0195670108003526.

[45] Vivian G. Loo, Louise Poirier, Mark A. Miller, Matthew Oughton, Michael D. Libman, Sophie Michaud, Anne-Marie Bourgault, Tuyen Nguyen, Charles Frenette, Mirabelle Kelly, Anne Vibien, Paul Brassard, Susan Fenn, Ken Dewar, Thomas J. Hudson, Ruth Horn, Pierre René, Yury Monczak, and André Dascal. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. *The New England Journal of Medicine*, 353(23):2442–2449, dec 2005. ISSN 1533-4406. doi: 10.1056/NEJMoa051639.

[46] Marjolein P. M. Hensgens, Abraham Goorhuis, Olaf M. Dekkers, and Ed J. Kuijper. Time interval of increased risk for Clostridium difficile infection after exposure to antibiotics. *Journal of Antimicrobial Chemotherapy*, 67(3):742–748, mar 2012. ISSN 0305-7453. doi: 10.1093/jac/dkr508. URL https://doi.org/10.1093/jac/dkr508.

[47] Vanessa Stevens, Ghinwa Dumyati, Lynn S. Fine, Susan G. Fisher, and Edwin van Wijngaarden. Cumulative antibiotic exposures over time and the risk of Clostridium difficile infection. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 53(1): 42–48, jul 2011. ISSN 1537-6591. doi: 10.1093/cid/cir301.

[48] G. E. Bignardi. Risk factors for Clostridium difficile infection. *The Journal of Hospital Infection*, 40(1):1–15, sep 1998. ISSN 0195-6701. doi: 10.1016/s0195-6701(98)90019-6.

[49] Scott R. Curry, Michelle T. Hecker, Justin O'Hagan, Preeta K. Kutty, Heba Alhmidi, Yilen K. Ng-Wong, Jennifer L. Cadnum, Annette L. Jencson, Melany Gonzalez-Orta, Carlos Saldana, Brigid M. Wilson, and Curtis J. Donskey. Natural History of Clostridioides difficile Colonization and Infection Following New Acquisition of Carriage in Healthcare Settings: A Prospective Cohort Study. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 77(1):77–83, jul 2023. ISSN 1537-6591. doi: 10.1093/cid/ciad142.

[50] Sahil Khanna, Darrell S. Pardi, Scott L. Aronson, Patricia P. Kammer, Robert Orenstein, Jennifer L. St. Sauver, W. Scott Harmsen, and Alan R. Zinsmeister. The Epidemiology of Community-acquired Clostridium difficile infection: A population-based study. *The American Journal of Gastroenterology*, 107(1):89–95, jan 2012. ISSN 0002-9270. doi: 10.1038/ajg.2011.398. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3273904/.

[51] Amit S. Chitnis, Stacy M. Holzbauer, Ruth M. Belflower, Lisa G. Winston, Wendy M. Bamberg, Carol Lyons, Monica M. Farley, Ghinwa K. Dumyati, Lucy E. Wilson, Zintars G. Beldavs, John R. Dunn, L. Hannah Gould, Duncan R. MacCannell, Dale N. Gerding, L. Clifford McDonald, and Fernanda C. Lessa. Epidemiology of community-associated Clostridium difficile infection, 2009

through 2011. *JAMA internal medicine*, 173(14):1359–1367, jul 2013. ISSN 2168-6114. doi: 10.1001/jamainternmed.2013.7056.

[52] Jimmy Zhang, Ling Chen, Angela Gomez-Simmonds, Michael T. Yin, and Daniel E. Freedberg. Antibiotic-Specific Risk for Community-Acquired *Clostridioides difficile* Infection in the United States from 2008 to 2020. *Antimicrobial Agents and Chemotherapy*, 66(12):e01129–22, dec 2022. ISSN 0066-4804, 1098-6596. doi: 10.1128/aac.01129-22. URL https://journals.asm.org/doi/10.1128/aac.01129-22.

[53] Sophia V. Kazakova, James Baggs, L. Clifford McDonald, Sarah H. Yi, Kelly M. Hatfield, Alice Guh, Sujan C. Reddy, and John A. Jernigan. Association Between Antibiotic Use and Hospital-onset Clostridioides difficile Infection in US Acute Care Hospitals, 2006-2012: An Ecologic Analysis. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 70(1):11–18, jan 2020. ISSN 1537-6591. doi: 10.1093/cid/ciz169.

[54] Elise Fortin, Daniel J. G. Thirion, Manale Ouakki, Christophe Garenc, Cindy Lalancette, Luc Bergeron, Danielle Moisan, Jasmin Villeneuve, Yves Longtin, and Québec Clostridiodes difficile Infection Surveillance Program (SPIN-CD). Role of high-risk antibiotic use in incidence of health-care-associated Clostridioides difficile infection in Quebec, Canada: a population-level ecological study. *The Lancet. Microbe*, 2(5):e182–e190, may 2021. ISSN 2666-5247. doi: 10.1016/S2666-5247(21)00005-7.

[55] Vered Schechner, Noga Fallach, Tali Braun, Elizabeth Temkin, and Yehuda Carmeli. Antibiotic exposure and the risk of hospital-acquired diarrhoea and Clostridioides difficile infection: a cohort study. *The Journal of Antimicrobial Chemotherapy*, 76(8):2182–2185, jul 2021. ISSN 1460-2091. doi: 10.1093/jac/dkab151.

[56] Brandon J. Webb, Aruna Subramanian, Bert Lopansri, Bruce Goodman, Peter Bjorn Jones, Jeffrey Ferraro, Edward Stenehjem, and Samuel M. Brown. Antibiotic Exposure and Risk for Hospital-Associated Clostridioides difficile Infection. *Antimicrobial Agents and Chemotherapy*, 64(4): e02169–19, mar 2020. ISSN 1098-6596. doi: 10.1128/AAC.02169-19.

[57] Frédéric Barbut, Anne Richard, Kheira Hamadi, Valérie Chomette, Béatrice Burghoffer, and Jean-Claude Petit. Epidemiology of Recurrences or Reinfections ofClostridium difficile-Associated Diarrhea. *Journal of Clinical Microbiology*, 38(6):2386–2388, jun 2000. doi: 10.1128/jcm.38.6.2386-2388.2000. URL https://journals.asm.org/doi/full/10.1128/jcm.38.6.2386-2388.2000.

[58] Gene K. Ma, Colleen M. Brensinger, Qufei Wu, and James D. Lewis. Increasing Incidence of Multiply Recurrent Clostridium difficile Infection in the United States. *Annals of Internal Medicine*, 167(3):152–158, aug 2017. ISSN 0003-4819. doi: 10.7326/M16-2733. URL https://www.acpjournals.org/doi/abs/10.7326/m16-2733.

[59] Jung Hoon Song and You Sun Kim. Recurrent Clostridium difficile Infection: Risk Factors, Treatment, and Prevention. *Gut and Liver*, 13(1):16–24, jan 2019. ISSN 2005-1212. doi: 10.5009/gnl18071.

[60] Abhishek Deshpande, Chaitanya Pant, Vinay Pasupuleti, David D. K. Rolston, Anil Jain, Narayan Deshpande, Priyaleela Thota, Thomas J. Sferra, and Adrian V. Hernandez. Association between

proton pump inhibitor therapy and Clostridium difficile infection in a meta-analysis. *Clinical Gastroenterology and Hepatology: The Official Clinical Practice Journal of the American Gastroenterological Association*, 10(3):225–233, mar 2012. ISSN 1542-7714. doi: 10.1016/j.cgh.2011.09.030.

[61] Amy Linsky, Kalpana Gupta, Elizabeth V. Lawler, Jennifer R. Fonda, and John A. Hermos. Proton pump inhibitors and risk for recurrent Clostridium difficile infection. *Archives of Internal Medicine*, 170(9):772–778, may 2010. ISSN 1538-3679. doi: 10.1001/archinternmed.2010.73.

[62] Nele Moreels, Annelies Boven, Oswaldo Gressani, Fredrik L Andersson, Erika Vlieghe, Steven Callens, Lars Engstrand, Johanna Simin, and Nele Brusselaers. The combined effect of systemic antibiotics and proton pump inhibitors on Clostridioides difficile infection and recurrence. *Journal of Antimicrobial Chemotherapy*, 79(3):608–616, mar 2024. ISSN 0305-7453. doi: 10.1093/jac/dkae012. URL https://doi.org/10.1093/jac/dkae012.

[63] Malin Inghammar, Henrik Svanström, Marianne Voldstedlund, Mads Melbye, Anders Hviid, Kåre Mølbak, and Björn Pasternak. Proton-Pump Inhibitor Use and the Risk of Community-Associated Clostridium difficile Infection. *Clinical Infectious Diseases*, 72(12):e1084–e1089, jun 2021. ISSN 1058-4838. doi: 10.1093/cid/ciaa1857. URL https://doi.org/10.1093/cid/ciaa1857.

[64] Emily G. McDonald, Jonathon Milligan, Charles Frenette, and Todd C. Lee. Continuous Proton Pump Inhibitor Therapy and the Associated Risk of Recurrent Clostridium difficile Infection. *JAMA Internal Medicine*, 175(5):784–791, may 2015. ISSN 2168-6106. doi: 10.1001/jamainternmed.2015.42. URL https://doi.org/10.1001/jamainternmed.2015.42.

[65] Kristin M. D'Silva, Raaj Mehta, Michael Mitchell, Todd C. Lee, Vibha Singhal, Marnie Goodwin Wilson, and Emily G. McDonald. Proton pump inhibitor use and risk for recurrent *Clostridioides difficile* infection: a systematic review and meta-analysis. *Clinical Microbiology and Infection*, 27(5):697–703, may 2021. ISSN 1198-743X. doi: 10.1016/j.cmi.2021.01.008. URL https://www.sciencedirect.com/science/article/pii/S1198743X21000355.

[66] Ciarán P. Kelly Md and J. Thomas LaMont Md. Clostridium difficile Infection. *Annual Review of Medicine*, 49(Volume 49, 1998):375–390, feb 1998. ISSN 0066-4219, 1545-326X. doi: 10.1146/annurev.med.49.1.375. URL https://www.annualreviews.org/content/journals/10.1146/annurev.med.49.1.375.

[67] Daniel A. Leffler and J. Thomas Lamont. Clostridium difficile Infection. *New England Journal of Medicine*, 372(16):1539–1548, apr 2015. ISSN 0028-4793. doi: 10.1056/NEJMra1403772. URL https://www.nejm.org/doi/full/10.1056/NEJMra1403772.

[68] Jacek Czepiel, Mirosław Dróżdż, Hanna Pituch, Ed J. Kuijper, William Perucki, Aleksandra Mielimonka, Sarah Goldman, Dorota Wultańska, Aleksander Garlicki, and Grażyna Biesiada. Clostridium difficile infection: review. *European Journal of Clinical Microbiology & Infectious Diseases*, 38(7):1211–1221, jul 2019. ISSN 1435-4373. doi: 10.1007/s10096-019-03539-6. URL https://doi.org/10.1007/s10096-019-03539-6.

[69] Ciarán P. Kelly and J. Thomas LaMont. Clostridium difficile–more difficult than ever. *The New England Journal of Medicine*, 359(18):1932–1940, oct 2008. ISSN 1533-4406. doi: 10.1056/NEJMra0707500.

[70] Ciaran P. Kelly, Charalabos Pothoulakis, and J. Thomas LaMont. Clostridium difficile Colitis. *New England Journal of Medicine*, 330(4):257–262, jan 1994. ISSN 0028-4793. doi: 10.1056/NEJM199401273300406. URL https://www.nejm.org/doi/full/10.1056/NEJM199401273300406.

[71] Stuart Johnson and Dale N. Gerding. Clostridium difficile - Associated diarrhea. *Clinical Infectious Diseases*, 26(5):1027–1036, 1998. ISSN 10584838. doi: 10.1086/520276,. URL https://pubmed.ncbi.nlm.nih.gov/9597221/.

[72] Rachel E Strength, Shanan R Immel, Ian C Drobish, and John H Powers. Subgroup Analyses in Randomized Trials of Clostridioides difficile–Associated Disease: Recurring Challenges With Analyzing Recurrence. *Clinical Infectious Diseases*, page ciaf188, apr 2025. ISSN 1058-4838. doi: 10.1093/cid/ciaf188. URL https://doi.org/10.1093/cid/ciaf188.

[73] L. Clifford McDonald, Vincent B. Young, Mark H. Wilcox, Alison Laufer Halpin, and Ricardo L. Chaves. Public health case for microbiome-sparing antibiotics and new opportunities for drug development. *mSphere*, 9(8):e0041724, aug 2024. ISSN 2379-5042. doi: 10.1128/msphere.00417-24. URL https://www.ncbi.nlm.nih.gov/pubmed/39092918.

[74] Kelly R. Reveles, Min Yang, Viviana Garcia-Horton, Marie Louise Edwards, Amy Guo, Thomas Lodise, Markian Bochan, Glenn Tillotson, and Erik R. Dubberke. Economic Impact of Recurrent Clostridioides difficile Infection in the USA: A Systematic Literature Review and Cost Synthesis. *Advances in Therapy*, 40(7):3104–3134, jul 2023. ISSN 1865-8652. doi: 10.1007/s12325-023-02498-x. URL https://doi.org/10.1007/s12325-023-02498-x.

[75] Centers for Disease Control and Prevention (CDC). Antibiotic resistance threats in the united states, 2019. Online report, 2019.

[76] Jacques Pépin, Louis Valiquette, Marie-Eve Alary, Philippe Villemure, Annick Pelletier, Karine Forget, Karine Pépin, and Daniel Chouinard. Clostridium difficile-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ: Canadian Medical Association journal = journal de l'Association medicale canadienne*, 171(5):466–472, aug 2004. ISSN 0820-3946. doi: 10.1503/cmaj.1041104.

[77] Matthew D. Redelings, Frank Sorvillo, and Laurene Mascola. Increase in Clostridium difficile-related mortality rates, United States, 1999-2004. *Emerging Infectious Diseases*, 13(9):1417–1419, sep 2007. ISSN 1080-6040. doi: 10.3201/eid1309.061116.

[78] Ed J. Kuijper, Renate J. van den Berg, Sylvia Debast, Caroline E. Visser, Dick Veenendaal, Annet Troelstra, Tjallie van der Kooi, Susan van den Hof, and Daan W. Notermans. Clostridium difficile Ribotype 027, Toxinotype III, the Netherlands. *Emerging Infectious Diseases*, 12(5):827–830, may 2006. ISSN 1080-6040. doi: 10.3201/eid1205.051350. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3374440/.

[79] Thomas V. Riley, Sarah Thean, Graham Hool, and Clayton L. Golledge. First Australian isolation of epidemic Clostridium difficile PCR ribotype 027. *The Medical Journal of Australia*, 190(12):706–708, jun 2009. ISSN 0025-729X. doi: 10.5694/j.1326-5377.2009.tb02644.x.

[80] Michael Richards, James Knox, Briony Elliott, Kate Mackin, Dena Lyras, Lynette J. Waring, and Thomas V. Riley. Severe infection with Clostridium difficile PCR ribotype 027 acquired in Melbourne, Australia. *The Medical Journal of Australia*, 194(7):369–371, apr 2011. ISSN 1326-5377. doi: 10.5694/j.1326-5377.2011.tb03012.x.

[81] H. Kato, Y. Ito, R. van den Berg, E. J. Kuijper, and Y. Arakawa. First isolation of Clostridium difficile 027 in Japan. *Weekly releases (1997–2007)*, 12(2):3110, jan 2007. ISSN 9999-1233. doi: 10.2807/esw.12.02.03110-en. URL https://www.eurosurveillance.org/content/10.2807/esw.12.02.03110-en.

[82] Archie C. A. Clements, Ricardo J. Soares Magalhães, Andrew J. Tatem, David L. Paterson, and Thomas V. Riley. Clostridium difficile PCR ribotype 027: assessing the risks of further worldwide spread. *The Lancet. Infectious Diseases*, 10(6):395–404, jun 2010. ISSN 1474-4457. doi: 10.1016/S1473-3099(10)70080-3.

[83] Wiep Klaas Smits, Kevin W. Garey, Thomas V. Riley, Yiping W. Han, Vincent B. Young, John Heritage, Christian John Lillis, Dena Lyras, Maja Rupnik, and Stuart Johnson. Clostridioides difficile should be considered a bacterial priority pathogen. *The Lancet. Microbe*, page 101184, June 2025. ISSN 2666-5247. doi: 10.1016/j.lanmic.2025.101184.

[84] Julian R. Marchesi and Jacques Ravel. The vocabulary of microbiome research: a proposal. *Microbiome*, 3(1), 12 2015. ISSN 2049-2618. doi: 10.1186/s40168-015-0094-5.

[85] Derek S Lundberg, Sarah L Lebeis, Sandra H Paredes, Scott Yourstone, James Gehring, Stephanie Malfatti, Julien Tremblay, Anna Engelbrektson, Victor Kunin, Tijana Glavina Del Rio, et al. Defining the core arabidopsis thaliana root microbiome. *Nature*, 488(7409):86–90, 2012. doi: 10.1038/nature11237.

[86] Rodrigo Mendes, Paolina Garbeva, and Jos M Raaijmakers. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*, 332(6033):1097–1100, 2011. doi: 10.1126/science.1203980.

[87] Amar Sarkar, Siobhán Harty, Katerina V.-A. Johnson, Andrew H. Moeller, Elizabeth A. Archie, Laura D. Schell, Rachel N. Carmody, Timothy H. Clutton-Brock, Robin I. M. Dunbar, and Philip W. J. Burnet. Microbial transmission in animal social networks and the social microbiome. *Nature Ecology & Evolution*, 4(8):1020–1035, aug 2020. ISSN 2397-334X. doi: 10.1038/s41559-020-1220-8. URL https://www.nature.com/articles/s41559-020-1220-8.

[88] Alex H. Nishida and Howard Ochman. A great-ape view of the gut microbiome. *Nature Reviews Genetics*, 20(4):195–206, apr 2019. ISSN 1471-0056. doi: 10.1038/s41576-018-0085-z. URL https://www.nature.com/articles/s41576-018-0085-zhttp://www.nature.com/articles/s41576-018-0085-z. 46 citations (Crossref) [2025-02-20] 36 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[89] A. P. Alivisatos, M. J. Blaser, E. L. Brodie, M. Chun, J. L. Dangl, T. J. Donohue, P. C. Dorrestein, J. A. Gilbert, J. L. Green, J. K. Jansson, R. Knight, M. E. Maxon, M. J. McFall-Ngai, J. F. Miller, K. S. Pollard, E. G. Ruby, and S. A. Taha. A unified initiative to harness Earth's microbiomes. *Science*, 350(6260):507–508, oct 2015. ISSN 10959203. doi: 10.1126/science.aac8480. 178 citations

(Semantic Scholar/DOI) [2022-09-12] Publisher: American Association for the Advancement of Science.

[90] Jack A Gilbert, Janet K Jansson, and Rob Knight. The Earth Microbiome project: successes and aspirations. *BMC Biology*, 12(1):69, dec 2014. ISSN 1741-7007. doi: 10.1186/s12915-014-0069-1. URL https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-014-0069-1. 653 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: BioMed Central Ltd.

[91] Luke R. Thompson, Jon G. Sanders, Daniel McDonald, Amnon Amir, Joshua Ladau, Kenneth J. Locey, Robert J. Prill, Anupriya Tripathi, Sean M. Gibbons, Gail Ackermann, Jose A. Navas-Molina, Stefan Janssen, Evguenia Kopylova, Yoshiki Vázquez-Baeza, Antonio González, James T. Morton, Siavash Mirarab, Zhenjiang Zech Xu, Lingjing Jiang, Mohamed F. Haroon, Jad Kanbar, Qiyun Zhu, Se Jin Song, Tomasz Kosciolek, Nicholas A. Bokulich, Joshua Lefler, Colin J. Brislawn, Gregory Humphrey, Sarah M. Owens, Jarrad Hampton-Marcell, Donna Berg-Lyons, Valerie McKenzie, Noah Fierer, Jed A. Fuhrman, Aaron Clauset, Rick L. Stevens, Ashley Shade, Katherine S. Pollard, Kelly D. Goodwin, Janet K. Jansson, Jack A. Gilbert, Rob Knight, The Earth Microbiome Project Consortium, Jose L. Agosto Rivera, Lisa Al-Moosawi, John Alverdy, Katherine R. Amato, Jason Andras, Largus T. Angenent, Dionysios A. Antonopoulos, Amy Apprill, David Armitage, Kate Ballantine, Jirˇí Bárta, Julia K. Baum, Allison Berry, Ashish Bhatnagar, Monica Bhatnagar, Jennifer F. Biddle, Lucie Bittner, Bazartseren Boldgiv, Eric Bottos, Donal M. Boyer, Josephine Braun, William Brazelton, Francis Q. Brearley, Alexandra H. Campbell, J. Gregory Caporaso, Cesar Cardona, JoLynn Carroll, S. Craig Cary, Brenda B. Casper, Trevor C. Charles, Haiyan Chu, Danielle C. Claar, Robert G. Clark, Jonathan B. Clayton, Jose C. Clemente, Alyssa Cochran, Maureen L. Coleman, Gavin Collins, Rita R. Colwell, Mónica Contreras, Benjamin B. Crary, Simon Creer, Daniel A. Cristol, Byron C. Crump, Duoying Cui, Sarah E. Daly, Liliana Davalos, Russell D. Dawson, Jennifer Defazio, Frédéric Delsuc, Hebe M. Dionisi, Maria Gloria Dominguez-Bello, Robin Dowell, Eric A. Dubinsky, Peter O. Dunn, Danilo Ercolini, Robert E. Espinoza, Vanessa Ezenwa, Nathalie Fenner, Helen S. Findlay, Irma D. Fleming, Vincenzo Fogliano, Anna Forsman, Chris Freeman, Elliot S. Friedman, Giancarlo Galindo, Liza Garcia, Maria Alexandra Garcia-Amado, David Garshelis, Robin B. Gasser, Gunnar Gerdts, Molly K. Gibson, Isaac Gifford, Ryan T. Gill, Tugrul Giray, Antje Gittel, Peter Golyshin, Donglai Gong, Hans-Peter Grossart, Kristina Guyton, Sarah-Jane Haig, Vanessa Hale, Ross Stephen Hall, Steven J. Hallam, Kim M. Handley, Nur A. Hasan, Shane R. Haydon, Jonathan E. Hickman, Glida Hidalgo, Kirsten S. Hofmockel, Jeff Hooker, Stefan Hulth, Jenni Hultman, Embriette Hyde, Juan Diego Ibáñez-Álamo, Julie D. Jastrow, Aaron R. Jex, L. Scott Johnson, Eric R. Johnston, Stephen Joseph, Stephanie D. Jurburg, Diogo Jurelevicius, Anders Karlsson, Roger Karlsson, Seth Kauppinen, Colleen T. E. Kellogg, Suzanne J. Kennedy, Lee J. Kerkhof, Gary M. King, George W. Kling, Anson V. Koehler, Monika Krezalek, Jordan Kueneman, Regina Lamendella, Emily M. Landon, Kelly Lane-deGraaf, Julie LaRoche, Peter Larsen, Bonnie Laverock, Simon Lax, Miguel Lentino, Iris I. Levin, Pierre Liancourt, Wenju Liang, Alexandra M. Linz, David A. Lipson, Yongqin Liu, Manuel E. Lladser, Mariana Lozada, Catherine M. Spirito, Walter P. MacCormack, Aurora MacRae-Crerar, Magda Magris, Antonio M. Martín-Platero, Manuel Martín-Vivaldi, L. Margarita Martínez, Manuel Martínez-Bueno, Ezequiel M. Marzinelli, Olivia U. Mason, Gregory D. Mayer, Jamie M. McDevitt-Irwin, James E. McDonald, Krista L. McGuire, Katherine D. McMahon, Ryan McMinds, Mónica Medina, Joseph R. Mendelson, Jessica L. Metcalf, Folker Meyer, Fabian Michelangeli, Kim Miller, David A. Mills, Jeremiah Minich, Stefano Mo-

cali, Lucas Moitinho-Silva, Anni Moore, Rachael M. Morgan-Kiss, Paul Munroe, David Myrold, Josh D. Neufeld, Yingying Ni, Graeme W. Nicol, Shaun Nielsen, Jozef I. Nissimov, Kefeng Niu, Matthew J. Nolan, Karen Noyce, Sarah L. O'Brien, Noriko Okamoto, Ludovic Orlando, Yadira Ortiz Castellano, Olayinka Osuolale, Wyatt Oswald, Jacob Parnell, Juan M. Peralta-Sánchez, Peter Petraitis, Catherine Pfister, Elizabeth Pilon-Smits, Paola Piombino, Stephen B. Pointing, F. Joseph Pollock, Caitlin Potter, Bharath Prithiviraj, Christopher Quince, Asha Rani, Ravi Ranjan, Subramanya Rao, Andrew P. Rees, Miles Richardson, Ulf Riebesell, Carol Robinson, Karl J. Rockne, Selena Marie Rodriguezl, Forest Rohwer, Wayne Roundstone, Rebecca J. Safran, Naseer Sangwan, Virginia Sanz, Matthew Schrenk, Mark D. Schrenzel, Nicole M. Scott, Rita L. Seger, Andaine Seguin-Orlando, Lucy Seldin, Lauren M. Seyler, Baddr Shakhsheer, Gabriela M. Sheets, Congcong Shen, Yu Shi, Hakdong Shin, Benjamin D. Shogan, Dave Shutler, Jeffrey Siegel, Steve Simmons, Sara Sjöling, Daniel P. Smith, Juan J. Soler, Martin Sperling, Peter D. Steinberg, Brent Stephens, Melita A. Stevens, Safiyh Taghavi, Vera Tai, Karen Tait, Chia L. Tan, Neslihan Tas¸, D. Lee Taylor, Torsten Thomas, Ina Timling, Benjamin L. Turner, Tim Urich, Luke K. Ursell, Daniel van der Lelie, William Van Treuren, Lukas van Zwieten, Daniela Vargas-Robles, Rebecca Vega Thurber, Paola Vitaglione, Donald A. Walker, William A. Walters, Shi Wang, Tao Wang, Tom Weaver, Nicole S. Webster, Beck Wehrle, Pamela Weisenhorn, Sophie Weiss, Jeffrey J. Werner, Kristin West, Andrew Whitehead, Susan R. Whitehead, Linda A. Whittingham, Eske Willerslev, Allison E. Williams, Stephen A. Wood, Douglas C. Woodhams, Yeqin Yang, Jesse Zaneveld, Iratxe Zarraonaindia, Qikun Zhang, and Hongxia Zhao. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, 551(7681):457–463, nov 2017. ISSN 0028-0836, 1476-4687. doi: 10.1038/nature24621. URL https://www.nature.com/articles/nature24621. 1296 citations (Semantic Scholar/DOI) [2022-09-12].

[92] Stephen Nayfach, Simon Roux, Rekha Seshadri, Daniel Udwary, Neha Varghese, Frederik Schulz, Dongying Wu, David Paez-Espino, I. Min Chen, Marcel Huntemann, Krishna Palaniappan, Joshua Ladau, Supratim Mukherjee, T. B.K. Reddy, Torben Nielsen, Edward Kirton, José P. Faria, Janaka N. Edirisinghe, Christopher S. Henry, Sean P. Jungbluth, Dylan Chivian, Paramvir Dehal, Elisha M. Wood-Charlson, Adam P. Arkin, Susannah G. Tringe, Axel Visel, Helena Abreu, Silvia G. Acinas, Eric Allen, Michelle A. Allen, Gary Andersen, Alexandre M. Anesio, Graeme Attwood, Viridiana Avila-Magaña, Yacine Badis, Jake Bailey, Brett Baker, Petr Baldrian, Hazel A. Barton, David A.C. Beck, Eric D. Becraft, Harry R. Beller, J. Michael Beman, Rizlan Bernier-Latmani, Timothy D. Berry, Anthony Bertagnolli, Stefan Bertilsson, Jennifer M. Bhatnagar, Jordan T. Bird, Sara E. Blumer-Schuette, Brendan Bohannan, Mikayla A. Borton, Allyson Brady, Susan H. Brawley, Juliet Brodie, Steven Brown, Jennifer R. Brum, Andreas Brune, Donald A. Bryant, Alison Buchan, Daniel H. Buckley, Joy Buongiorno, Hinsby Cadillo-Quiroz, Sean M. Caffrey, Ashley N. Campbell, Barbara Campbell, Stephanie Carr, Jo Lynn Carroll, S. Craig Cary, Anna M. Cates, Rose Ann Cattolico, Ricardo Cavicchioli, Ludmila Chistoserdova, Maureen L. Coleman, Philippe Constant, Jonathan M. Conway, Walter P. Mac Cormack, Sean Crowe, Byron Crump, Cameron Currie, Rebecca Daly, Vincent Denef, Stuart E. Denman, Adey Desta, Hebe Dionisi, Jeremy Dodsworth, Nina Dombrowski, Timothy Donohue, Mark Dopson, Timothy Driscoll, Peter Dunfield, Christopher L. Dupont, Katherine A. Dynarski, Virginia Edgcomb, Elizabeth A. Edwards, Mostafa S. Elshahed, Israel Figueroa, Beverly Flood, Nathaniel Fortney, Caroline S. Fortunato, Christopher Francis, Claire M.M. Gachon, Sarahi L. Garcia, Maria C. Gazitua, Terry Gentry, Lena Gerwick, Javad Gharechahi, Peter Girguis, John Gladden, Mary Gradoville, Stephen E. Grasby, Kelly Gravuer, Christen L. Grettenberger, Robert J. Gruninger, Jiarong Guo, Mussie Y. Habtese-

lassie, Steven J. Hallam, Roland Hatzenpichler, Bela Hausmann, Terry C. Hazen, Brian Hedlund, Cynthia Henny, Lydie Herfort, Maria Hernandez, Olivia S. Hershey, Matthias Hess, Emily B. Hollister, Laura A. Hug, Dana Hunt, Janet Jansson, Jessica Jarett, Vitaly V. Kadnikov, Charlene Kelly, Robert Kelly, William Kelly, Cheryl A. Kerfeld, Jeff Kimbrel, Jonathan L. Klassen, Konstantinos T. Konstantinidis, Laura L. Lee, Wen Jun Li, Andrew J. Loder, Alexander Loy, Mariana Lozada, Barbara MacGregor, Cara Magnabosco, Aline Maria da Silva, R. Michael McKay, Katherine McMahon, Chris S. McSweeney, Mónica Medina, Laura Meredith, Jessica Mizzi, Thomas Mock, Lily Momper, Mary Ann Moran, Connor Morgan-Lang, Duane Moser, Gerard Muyzer, David Myrold, Maisie Nash, Camilla L. Nesbø, Anthony P. Neumann, Rebecca B. Neumann, Daniel Noguera, Trent Northen, Jeanette Norton, Brent Nowinski, Klaus Nüsslein, Michelle A. O'Malley, Rafael S. Oliveira, Valeria Maia de Oliveira, Tullis Onstott, Jay Osvatic, Yang Ouyang, Maria Pachiadaki, Jacob Parnell, Laila P. Partida-Martinez, Kabir G. Peay, Dale Pelletier, Xuefeng Peng, Michael Pester, Jennifer Pett-Ridge, Sari Peura, Petra Pjevac, Alvaro M. Plominsky, Anja Poehlein, Phillip B. Pope, Nikolai Ravin, Molly C. Redmond, Rebecca Reiss, Virginia Rich, Christian Rinke, Jorge L.Mazza Rodrigues, Karen Rossmassler, Joshua Sackett, Ghasem Hosseini Salekdeh, Scott Saleska, Matthew Scarborough, Daniel Schachtman, Christopher W. Schadt, Matthew Schrenk, Alexander Sczyrba, Aditi Sengupta, Joao C. Setubal, Ashley Shade, Christine Sharp, David H. Sherman, Olga V. Shubenkova, Isabel Natalia Sierra-Garcia, Rachel Simister, Holly Simon, Sara Sjöling, Joan Slonczewski, Rafael Soares Correa de Souza, John R. Spear, James C. Stegen, Ramunas Stepanauskas, Frank Stewart, Garret Suen, Matthew Sullivan, Dawn Sumner, Brandon K. Swan, Wesley Swingley, Jonathan Tarn, Gordon T. Taylor, Hanno Teeling, Memory Tekere, Andreas Teske, Torsten Thomas, Cameron Thrash, James Tiedje, Claire S. Ting, Benjamin Tully, Gene Tyson, Osvlado Ulloa, David L. Valentine, Marc W. Van Goethem, Jean VanderGheynst, Tobin J. Verbeke, John Vollmers, Aurèle Vuillemin, Nicholas B. Waldo, David A. Walsh, Bart C. Weimer, Thea Whitman, Paul van der Wielen, Michael Wilkins, Timothy J. Williams, Ben Woodcroft, Jamie Woolet, Kelly Wrighton, Jun Ye, Erica B. Young, Noha H. Youssef, Feiqiao Brian Yu, Tamara I. Zemskaya, Ryan Ziels, Tanja Woyke, Nigel J. Mouncey, Natalia N. Ivanova, Nikos C. Kyrpides, and Emiley A. Eloe-Fadrosh. A genomic catalog of Earth's microbiomes. *Nature Biotechnology*, 39(April), 2020. ISSN 15461696. doi: 10.1038/s41587-020-0718-6. 523 citations (Crossref) [2025-02-20] 196 citations (Semantic Scholar/DOI) [2022-09-12].

[93] Jing Zhao, Lei Jin, Dan Wu, Jiwei Xie, Jie Li, Xuwen Fu, Ziyuan Cong, Peiqiang Fu, Yuan Zhang, Xianshu Luo, Xinbin Feng, Gan Zhang, James M Tiedje, and Xiangdong Li. Global airborne bacterial community—interactions with earth's microbiomes and anthropogenic activities. *Proceedings of the National Academy of Sciences*, 119(42):e2204465119, 2022. doi: 10.1073/pnas.2204465119.

[94] Mohammad Bahram, Falk Hildebrand, Sofia K Forslund, Ian Anderson, Nadejda A Soudzilovskaia, Peter M Bodegom, Johan Bengtsson-Palme, Sten Anslan, Luis P Coelho, Harry Harend, et al. Structure and function of the global topsoil microbiome. *Nature*, 560(7717):233–237, 2018. doi: 10.1038/s41586-018-0386-6.

[95] Shinichi Sunagawa, Luis P Coelho, Samuel Chaffron, Jens Roat Kultima, Karine Labadie, Guillem Salazar, Behnam Djahanschiri, Georg Zeller, Daniel R Mende, Adrian Alberti, et al. Structure and function of the global ocean microbiome. *Science*, 348(6237):1261359, 2015. doi: 10.1126/science.1261359.

[96] Stilianos Louca, Laura Wegener Parfrey, and Michael Doebeli. Decoupling function and taxonomy in the global ocean microbiome. *Science*, 353(6305):1272–1277, 2016. doi: 10.1126/science.aaf4507.

[97] William G. Wade. The oral microbiome in health and disease. *Pharmacological Research*, 69(1):137–143, mar 2013. ISSN 10436618. doi: 10.1016/j.phrs.2012.11.006. URL https://linkinghub.elsevier.com/retrieve/pii/S1043661812002277. 755 citations (Semantic Scholar/DOI) [2022-09-12].

[98] Jake G. Natalini, Shivani Singh, and Leopoldo N. Segal. The dynamic lung microbiome in health and disease. *Nature Reviews Microbiology*, 21(4):222–235, 2023. doi: 10.1038/s41579-022-00831-1.

[99] Paul B Eckburg, Elisabeth M Bik, Charles N Bernstein, Elizabeth Purdom, Les Dethlefsen, Michael Sargent, Steven R Gill, Karen E Nelson, and David A Relman. Diversity of the human intestinal microbial flora. *Science*, 308(5728):1635–1638, 2005. doi: 10.1126/science.1110591.

[100] Fredrik Bäckhed, Ruth E Ley, Justin L Sonnenburg, Daniel A Peterson, and Jeffrey I Gordon. Host-bacterial mutualism in the human intestine. *Science*, 307(5717):1915–1920, 2005. doi: 10.1126/science.1104816.

[101] Manimozhiyan Arumugam, Jeroen Raes, Eric Pelletier, Didier Le Paslier, Takuji Yamada, Daniel R Mende, Gabriel R Fernandes, Julien Tap, Tom Bruls, Jean-Michel Batto, et al. Enterotypes of the human gut microbiome. *Nature*, 473(7346):174–180, 2011. doi: 10.1038/nature09944.

[102] Khoudia Diop, Jean-Charles Dufour, Anthony Levasseur, and Florence Fenollar. Exhaustive Repertoire of Human Vaginal Microbiota. *Human Microbiome Journal*, jan 2019. ISSN 2452-2317. doi: 10.1016/J.HUMIC.2018.11.002. URL https://www.sciencedirect.com/science/article/pii/S2452231718300277. 41 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Elsevier.

[103] Jennifer M. Fettweis, Myrna G. Serrano, J. Paul Brooks, David J. Edwards, Philippe H. Girerd, Hardik I. Parikh, Bernice Huang, Tom J. Arodz, Laahirie Edupuganti, Abigail L. Glascock, Jie Xu, Nicole R. Jimenez, Stephany C. Vivadelli, Stephen S. Fong, Nihar U. Sheth, Sophonie Jean, Vladimir Lee, Yahya A. Bokhari, Ana M. Lara, Shreni D. Mistry, Robert A. Duckworth, Steven P. Bradley, Vishal N. Koparde, X. Valentine Orenda, Sarah H. Milton, Sarah K. Rozycki, Andrey V. Matveyev, Michelle L. Wright, Snehalata V. Huzurbazar, Eugenie M. Jackson, Ekaterina Smirnova, Jonas Korlach, Yu Chih Tsai, Molly R. Dickinson, Jamie L. Brooks, Jennifer I. Drake, Donald O. Chaffin, Amber L. Sexton, Michael G. Gravett, Craig E. Rubens, N. Romesh Wijesooriya, Karen D. Hendricks-Muñoz, Kimberly K. Jefferson, Jerome F. Strauss, and Gregory A. Buck. The vaginal microbiome and preterm birth. *Nature Medicine*, 25(6):1012–1021, 6 2019. ISSN 1546170X. doi: 10.1038/s41591-019-0450-2.

[104] Jacques Ravel, Pawel Gajer, Zaid Abdo, G Maria Schneider, Sara S. K. Koenig, Stacey L. McCulle, Shara Karlebach, Reshma Gorle, Jennifer Russell, Carol O Tacket, Rebecca M Brotman, Catherine C Davis, Kevin Ault, Ligia Peralta, and Larry J Forney. Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences*, 108(supplement_1):4680–4687, mar 2011. ISSN 0027-8424. doi: 10.1073/pnas.1002611107. URL www.pnas.org/cgi/doi/10.1073/pnas.1002611107https://pnas.org/doi/full/10.1073/pnas.1002611107. 2440 citations (Semantic Scholar/DOI) [2022-06-24].

[105] Elizabeth A. Grice and Julia A. Segre. The skin microbiome, 4 2011. ISSN 17401526.

[106] Allyson L. Byrd, Yasmine Belkaid, and Julia A. Segre. The human skin microbiome, 2018. ISSN 17401534.

[107] R. Henrik Nilsson, Sten Anslan, Mohammad Bahram, Christian Wurzbacher, Petr Baldrian, and Leho Tedersoo. Mycobiome diversity: high-throughput sequencing and identification of fungi, 11 2019. ISSN 17401534. URL https://www.nature.com/articles/s41579-018-0116-y.

[108] Jeremiah J Faith, Jay R Guruge, Mark Charbonneau, Sasi Subramanian, Henning Seedorf, Andrew L Goodman, Jose C Clemente, Rob Knight, Allison C Heath, Rudolph L Leibel, et al. The long-term stability of the human gut microbiota. *Science*, 341(6141):1237439, 2013. doi: 10.1126/science.1237439.

[109] Guanxiang Liang and Frederic D. Bushman. The human virome: assembly, composition and host interactions. *Nature Reviews Microbiology*, 19(8):514–527, 8 2021. ISSN 1740-1526. doi: 10.1038/s41579-021-00536-5. URL www.nature.com/nrmicrohttp://www.nature.com/articles/s41579-021-00536-5.

[110] Elizabeth K. Costello, Christian L. Lauber, Micah Hamady, Noah Fierer, Jeffrey I. Gordon, and Rob Knight. Bacterial community variation in human body habitats across space and time. *Science*, 326(5960):1694–1697, dec 2009. ISSN 00368075. doi: 10.1126/science.1177486. 2679 citations (Semantic Scholar/DOI) [2022-06-23].

[111] Tanya Yatsunenko, Federico E Rey, Mark J Manary, Indi Trehan, Maria G Dominguez-Bello, Monica Contreras, Magda Magris, Georgia Hidalgo, Robert N Baldassano, Andrey P Anokhin, et al. Human gut microbiome viewed across age and geography. *Nature*, 486(7402):222–227, 2012. doi: 10.1038/nature11053.

[112] Ilseung Cho and Martin J Blaser. The human microbiome: at the interface of health and disease. *Nature Reviews Genetics*, 13(4):260–270, 4 2012. ISSN 1471-0056. doi: 10.1038/nrg3182. URL www.nature.com/reviews/geneticshttp://www.ncbi.nlm.nih.gov/pubmed/22411464%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3418802http://www.nature.com/articles/nrg3182.

[113] Tao Ding and Patrick D. Schloss. Dynamics and associations of microbial community types across the human body. *Nature*, 509(7500):357–360, 5 2014. ISSN 0028-0836. doi: 10.1038/nature13178. URL https://www.nature.com/articles/nature13178http://www.nature.com/articles/nature13178.

[114] Katharine Z. Coyte, Jonas Schluter, and Kevin R. Foster. The ecology of the microbiome: Networks, competition, and stability. *Science*, 350(6261):663–666, nov 2015. ISSN 10959203. doi: 10.1126/science.aad2602. URL https://www.science.org/doi/abs/10.1126/science.aad2602.

[115] Jason Lloyd-Price, Galeb Abu-Ali, and Curtis Huttenhower. The healthy human microbiome. *Genome Medicine*, 8(1), 2016. ISSN 1756994X. doi: 10.1186/s13073-016-0307-y. URL https://com-mendeley-prod-publicsharing-pdfstore.s3.eu-west-1.amazonaws.com/0fa2-PUBMED/10.1186/s13073-016-0307-y/13073_2016_Article_

307_pdf.pdf?X-Amz-Security-Token=FQoGZXIvYXdzENv%2F%2F%2F%2F%2F%2F%2F%2F%2FwEaDLmZpcryQSgwD0b7lSKfBBH4Jcd2Xiz4P%2F80YJr.

[116] Jason Lloyd-Price, Anup Mahurkar, Gholamali Rahnavard, Jonathan Crabtree, Joshua Orvis, Alan B Hall, Ann Brady, Heather H Creasy, Curtis McCracken, Michelle G Giglio, et al. Strains, functions and dynamics in the expanded human microbiome project. *Nature*, 550(7674):61–66, 2017. doi: 10.1038/nature23889.

[117] Gwen Falony, Marie Joossens, Sara Vieira-Silva, Jun Wang, Youssef Darzi, Karoline Faust, Alexander Kurilshikov, Marc Jan Bonder, Mireia Valles-Colomer, Doris Vandeputte, Raul Y. Tito, Samuel Chaffron, Leen Rymenans, Chloë Verspecht, Lise De Sutter, Gipsi Lima-Mendez, Kevin D'hoe, Karl Jonckheere, Daniel Homola, Roberto Garcia, Ettje F. Tigchelaar, Linda Eeckhaudt, Jingyuan Fu, Liesbet Henckaerts, Alexandra Zhernakova, Cisca Wijmenga, and Jeroen Raes. Population-level analysis of gut microbiome variation. *Science*, 352(6285):560–564, 4 2016. ISSN 10959203. doi: 10.1126/science.aad3503. URL http://science.sciencemag.org/.

[118] Paul I. Costea, Falk Hildebrand, Manimozhiyan Arumugam, et al. Enterotypes in the landscape of gut microbial community composition. *Nature Microbiology*, 3:8–16, 2018. doi: 10.1038/s41564-017-0072-8. URL https://doi.org/10.1038/s41564-017-0072-8.

[119] Jack A Gilbert, Martin J Blaser, J Gregory Caporaso, Janet K Jansson, Susan V Lynch, and Rob Knight. Current understanding of the human microbiome. *Nature Medicine*, 24(4):392–400, 2018. doi: 10.1038/nm.4517.

[120] Lita M Proctor, Heather H Creasy, Jennifer M Fettweis, Jason Lloyd-Price, Anup Mahurkar, Wei Zhou, Gerald A Buck, Michael P Snyder, Judith F Strauss, George M Weinstock, et al. The integrative human microbiome project. *Nature*, 569(7758):641–648, 2019. doi: 10.1038/s41586-019-1238-8.

[121] Itai Vujkovic-Cvijin, Jesse Sklar, Lily Jiang, et al. Host variables confound gut microbiota studies of human disease. *Nature*, 587:448–454, 2020. doi: 10.1038/s41586-020-2881-9. URL https://doi.org/10.1038/s41586-020-2881-9.

[122] Yong Fan and Oluf Pedersen. Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology*, 19(1):55–71, jan 2021. ISSN 1740-1534. doi: 10.1038/s41579-020-0433-9. URL https://www.nature.com/articles/s41579-020-0433-9. 2645 citations (Crossref) [2025-02-20] 670 citations (Semantic Scholar/DOI) [2022-09-12] Number: 1 Publisher: Nature Publishing Group.

[123] Giselle McCallum and Carolina Tropini. The gut microbiota and its biogeography. *Nature Reviews Microbiology*, 22(2):105–118, feb 2024. ISSN 1740-1534. doi: 10.1038/s41579-023-00969-0. URL https://www.nature.com/articles/s41579-023-00969-0.

[124] Alexandra Zhernakova, Alexander Kurilshikov, Marc Jan Bonder, Ettje F. Tigchelaar, Melanie Schirmer, Tommi Vatanen, Zlatan Mujagic, Arnau Vich Vila, Gwen Falony, Sara Vieira-Silva, Jun Wang, Floris Imhann, Eelke Brandsma, Soesma A. Jankipersadsing, Marie Joossens, Maria Carmen Cenit, Patrick Deelen, Morris A. Swertz, Rinse K. Weersma, Edith J.M. Feskens, Mihai G. Netea, Dirk Gevers, Daisy Jonkers, Lude Franke, Yurii S. Aulchenko, Curtis Huttenhower, Jeroen Raes, Marten H. Hofker, Ramnik J. Xavier, Cisca Wijmenga, and Jingyuan Fu. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science*, 352

(6285):565–569, apr 2016. ISSN 10959203. doi: 10.1126/science.aad3369. 1092 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: American Association for the Advancement of Science.

[125] Ron Sender, Shai Fuchs, and Ron Milo. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS biology*, 14(8):e1002533, aug 2016. ISSN 1545-7885. doi: 10.1371/journal.pbio.1002533.

[126] Ron Sender, Shai Fuchs, and Ron Milo. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell*, 164(3):337–340, jan 2016. ISSN 1097-4172. doi: 10.1016/j.cell.2016.01.013.

[127] Lan Lin and Jianqiong Zhang. Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. *BMC Immunology*, 18(1):2, jan 2017. ISSN 1471-2172. doi: 10.1186/s12865-016-0187-3. URL https://doi.org/10.1186/s12865-016-0187-3.

[128] Mohamed S. Donia and Michael A. Fischbach. Small molecules from the human microbiota. *Science*, 349(6246):1254766, jul 2015. ISSN 10959203. doi: 10.1126/science.1254766. 453 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: American Association for the Advancement of Science.

[129] Kimberly A. Krautkramer, Jing Fan, and Fredrik Bäckhed. Gut microbial metabolites as multikingdom intermediates. *Nature Reviews. Microbiology*, 19(2):77–94, feb 2021. ISSN 1740-1534. doi: 10.1038/s41579-020-0438-4.

[130] Mireia Valles-Colomer, Aitor Blanco-Míguez, Paolo Manghi, Francesco Asnicar, Leonard Dubois, Davide Golzato, Federica Armanini, Fabio Cumbo, Kun D. Huang, Serena Manara, Giulia Masetti, Federica Pinto, Elisa Piperni, Michal Punčochář, Liviana Ricci, Moreno Zolfo, Olivia Farrant, Adriana Goncalves, Marta Selma-Royo, Ana G. Binetti, Jimmy E. Becerra, Bei Han, John Lusingu, John Amuasi, Loredana Amoroso, Alessia Visconti, Claire M. Steves, Mario Falchi, Michele Filosi, Adrian Tett, Anna Last, Qian Xu, Nan Qin, Huanlong Qin, Jürgen May, Daniel Eibach, Maria Valeria Corrias, Mirco Ponzoni, Edoardo Pasolli, Tim D. Spector, Enrico Domenici, Maria Carmen Collado, and Nicola Segata. The person-to-person transmission landscape of the gut and oral microbiomes. *Nature*, 614(7946):125–135, feb 2023. ISSN 1476-4687. doi: 10.1038/s41586-022-05620-1. 189 citations (Crossref) [2025-02-20].

[131] Vitor Heidrich, Mireia Valles-Colomer, and Nicola Segata. Human microbiome acquisition and transmission. *Nature Reviews. Microbiology*, mar 2025. ISSN 1740-1534. doi: 10.1038/s41579-025-01166-x.

[132] Peter J. Turnbaugh, Ruth E. Ley, Michael A. Mahowald, Vincent Magrini, Elaine R. Mardis, and Jeffrey I. Gordon. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444(7122):1027–1031, 12 2006. ISSN 00280836. doi: 10.1038/nature05414.

[133] Peter J. Turnbaugh, Micah Hamady, Tanya Yatsunenko, Brandi L. Cantarel, Alexis Duncan, Ruth E. Ley, Mitchell L. Sogin, William J. Jones, Bruce A. Roe, Jason P. Affourtit, Michael Egholm, Bernard Henrissat, Andrew C. Heath, Rob Knight, and Jeffrey I. Gordon. A core gut microbiome in obese and lean twins. *Nature*, 457(7228):480–484, jan 2009. ISSN 00280836. doi: 10.1038/nature07540. URL https://www.nature.com/articles/nature07540.pdf. 6554 citations (Semantic Scholar/DOI) [2022-06-23].

[134] Vanessa K. Ridaura, Jeremiah J. Faith, Federico E. Rey, Jiye Cheng, Alexis E. Duncan, Andrew L. Kau, Nicholas W. Griffin, Vincent Lombard, Bernard Henrissat, James R. Bain, Michael J. Muehlbauer, Olga Ilkayeva, Clay F. Semenkovich, Katsuhiko Funai, David K. Hayashi, Barbara J. Lyle, Margaret C. Martini, Luke K. Ursell, Jose C. Clemente, William Van Treuren, William A. Walters, Rob Knight, Christopher B. Newgard, Andrew C. Heath, and Jeffrey I. Gordon. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*, 341(6150), 2013. ISSN 10959203. doi: 10.1126/science.1241214.

[135] Andrew L. Goodman, George Kallstrom, Jeremiah J. Faith, Alejandro Reyes, Aimee Moore, Gautam Dantas, and Jeffrey I. Gordon. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proceedings of the National Academy of Sciences of the United States of America*, 108(15):6252–6257, apr 2011. ISSN 1091-6490. doi: 10.1073/pnas.1102938108.

[136] Steven R Gill, Mihai Pop, Robert T Deboy, Paul B Eckburg, Peter J Turnbaugh, Buck S Samuel, Jeffrey I Gordon, David A Relman, Claire M Fraser-Liggett, and Karen E Nelson. Metagenomic analysis of the human distal gut microbiome. *Science*, 312(5778):1355–1359, 2006. doi: 10.1126/science.1124234.

[137] Peter J. Turnbaugh, Ruth E. Ley, Micah Hamady, Claire M. Fraser-Liggett, Rob Knight, and Jeffrey I. Gordon. The Human Microbiome Project. *Nature*, 449(7164):804–810, oct 2007. ISSN 0028-0836, 1476-4687. doi: 10.1038/nature06244. URL http://www.nature.com/articles/nature06244. 4292 citations (Semantic Scholar/DOI) [2022-06-20].

[138] Jun Qin, Ruiqiang Li, Jeroen Raes, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, 464:59–65, 2010. doi: 10.1038/nature08821. URL https://doi.org/10.1038/nature08821.

[139] Curtis Huttenhower, Dirk Gevers, Rob Knight, Sahar Abubucker, Jonathan H Badger, Asif T Chinwalla, Heather H Creasy, Ashlee M Earl, Michael G FitzGerald, Robert S Fulton, et al. Structure, function and diversity of the healthy human microbiome. *Nature*, 486(7402):207–214, 2012. doi: 10.1038/nature11234.

[140] Human Microbiome Project Consortium. A framework for human microbiome research. *Nature*, 486(7402):215–221, jun 2012. ISSN 1476-4687. doi: 10.1038/nature11209.

[141] Siegfried Schloissnig, Manimozhiyan Arumugam, Shinichi Sunagawa, Makedonka Mitreva, Julien Tap, Ana Zhu, Alison Waller, Daniel R. Mende, Jens Roat Kultima, John Martin, Karthik Kota, Shamil R. Sunyaev, George M. Weinstock, and Peer Bork. Genomic variation landscape of the human gut microbiome. *Nature*, 493(7430):45–50, jan 2013. ISSN 1476-4687. doi: 10.1038/nature11711. URL https://www.nature.com/articles/nature11711. 617 citations (Crossref) [2022-11-02] Number: 7430 Publisher: Nature Publishing Group.

[142] Junhua Li, Huijue Jia, Xiang Cai, Hua Zhong, Qiang Feng, Shinichi Sunagawa, Manimozhiyan Arumugam, Jens Roat Kultima, Edi Prifti, Tina Nielsen, et al. An integrated catalog of reference genes in the human gut microbiome. *Nature Biotechnology*, 32(8):834–841, 2014. doi: 10.1038/nbt.2942.

[143] Doris Vandeputte, Gunter Kathagen, Kevin D'hoe, Sara Vieira-Silva, Mireia Valles-Colomer, Joaõ João Sabino, Jun Wang, Raul Y. Tito, Lindsey De Commer, Youssef Darzi, Séverine Vermeire,

Gwen Falony, Jeroen Raes, Kevin D'Hoe, Sara Vieira-Silva, Mireia Valles-Colomer, Joaõ João Sabino, Jun Wang, Raul Y. Tito, Lindsey De Commer, Youssef Darzi, Séverine Vermeire, Gwen Falony, and Jeroen Raes. Quantitative microbiome profiling links gut community variation to microbial load. *Nature*, 551(7681):507–511, 11 2017. ISSN 14764687. doi: 10.1038/nature24460. URL https://www.nature.com/articles/nature24460.

[144] Alexandre Almeida, Alex L. Mitchell, Miguel Boland, Samuel C. Forster, Gregory B. Gloor, Aleksandra Tarkowska, Trevor D. Lawley, and Robert D. Finn. A new genomic blueprint of the human gut microbiota. *Nature*, 568(7753):499–504, apr 2019. ISSN 14764687. doi: 10.1038/s41586-019-0965-1. 624 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[145] Dari Shalon, Rebecca Neal Culver, Jessica A. Grembi, Jacob Folz, Peter V. Treit, Handuo Shi, Florian A. Rosenberger, Les Dethlefsen, Xiandong Meng, Eitan Yaffe, Andrés Aranda-Díaz, Philipp E. Geyer, Johannes B. Mueller-Reif, Sean Spencer, Andrew D. Patterson, George Triadafilopoulos, Susan P. Holmes, Matthias Mann, Oliver Fiehn, David A. Relman, and Kerwyn Casey Huang. Profiling the human intestinal environment under physiological conditions. *Nature*, 617(7961):581–591, may 2023. ISSN 1476-4687. doi: 10.1038/s41586-023-05989-7. 194 citations (Crossref) [2025-02-20].

[146] Matthias Huelsmann, Olga T. Schubert, and Martin Ackermann. A framework for understanding collective microbiome metabolism. *Nature Microbiology*, 9(12):3097–3109, dec 2024. ISSN 2058-5276. doi: 10.1038/s41564-024-01850-3. URL https://www.nature.com/articles/s41564-024-01850-3.

[147] Jeremy E. Wilkinson, Eric A. Franzosa, Christine Everett, Chengchen Li, Frank B. Hu, Dyann F. Wirth, Mingyang Song, Andrew T. Chan, Eric Rimm, Wendy S. Garrett, and Curtis Huttenhower. A framework for microbiome science in public health. *Nature Medicine*, 27(5):766–774, may 2021. ISSN 1078-8956. doi: 10.1038/s41591-021-01258-0. URL https://www.nature.com/articles/s41591-021-01258-0http://www.nature.com/articles/s41591-021-01258-0. 53 citations (Crossref) [2025-02-20] 20 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[148] Neeraj K. Surana and Dennis L. Kasper. Moving beyond microbiome-wide associations to causal microbe identification. *Nature*, 552(7684):244–247, dec 2017. ISSN 14764687. doi: 10.1038/nature25019. 141 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[149] Jens Walter, Anissa M. Armet, B. Brett Finlay, and Fergus Shanahan. Establishing or Exaggerating Causality for the Gut Microbiome: Lessons from Human Microbiota-Associated Rodents. *Cell*, 180(2):221–232, jan 2020. ISSN 0092-8674. doi: 10.1016/J.CELL.2019.12.025. URL https://doi.org/10.1016/j.cell.2019.12.025. 367 citations (Crossref) [2025-02-20] 195 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Cell Press.

[150] Jordan E. Bisanz, Paola Soto-Perez, Cecilia Noecker, Alexander A. Aksenov, Kathy N. Lam, Grace E. Kenney, Elizabeth N. Bess, Henry J. Haiser, Than S. Kyaw, Feiqiao B. Yu, Vayu M. Rekdal, Connie W. Y. Ha, Suzanne Devkota, Emily P. Balskus, Pieter C. Dorrestein, Emma Allen-Vercoe, and Peter J. Turnbaugh. A Genomic Toolkit for the Mechanistic Dissection of Intractable

Human Gut Bacteria. *Cell Host & Microbe*, 27(6):1001–1013.e9, jun 2020. ISSN 1931-3128. doi: 10.1016/j.chom.2020.04.006. URL https://www.cell.com/cell-host-microbe/abstract/S1931-3128(20)30233-X.

[151] Jukka Corander, William P. Hanage, and Johan Pensar. Causal discovery for the microbiome. *The Lancet Microbe*, 3(11):e881–e887, 2022. doi: 10.1016/S2666-5247(22)00187-6.

[152] Snehal N. Chaudhari, Michael D. McCurry, and A. Sloan Devlin. Chains of evidence from correlations to causal molecules in microbiome-linked diseases. *Nature Chemical Biology*, 17: 1046–1056, 2021. doi: 10.1038/s41589-021-00861-z. URL https://doi.org/10.1038/s41589-021-00861-z.

[153] Christina Mirzayi, Audrey Renson, Genomic Standards Consortium, et al. Reporting guidelines for human microbiome research: the storms checklist. *Nature Medicine*, 27:1885–1892, 2021. doi: 10.1038/s41591-021-01552-x. URL https://doi.org/10.1038/s41591-021-01552-x.

[154] Abdalla Metwaly, Asma Kriaa, Zakaria Hassani, et al. A consensus statement on establishing causality, therapeutic applications and the use of preclinical models in microbiome research. *Nature Reviews Gastroenterology & Hepatology*, 2025. doi: 10.1038/s41575-025-01041-3. URL https://doi.org/10.1038/s41575-025-01041-3.

[155] Serena Porcari, Benjamin H. Mullish, Francesco Asnicar, Siew C. Ng, Liping Zhao, Richard Hansen, Paul W. O'Toole, Jeroen Raes, Georgina Hold, Lorenza Putignani, Christian Lodberg Hvas, Georg Zeller, Omry Koren, Hein Tun, Mireia Valles-Colomer, Maria Carmen Collado, Monika Fischer, Jessica Allegretti, Tariq Iqbal, Benoit Chassaing, Josbert Keller, Simon Mark Baunwall, Maria Abreu, Giovanni Barbara, Faming Zhang, Francesca Romana Ponziani, Sam P. Costello, Sudarshan Paramsothy, Dina Kao, Colleen Kelly, Juozas Kupcinskas, Ilan Youngster, Francesco Franceschi, Sahil Khanna, Maria Vehreschild, Alexander Link, Flavio De Maio, Edoardo Pasolli, Aitor Blanco Miguez, Patrizia Brigidi, Brunella Posteraro, Franco Scaldaferri, Mirjana Rajilic Stojanovic, Francis Megraud, Peter Malfertheiner, Luca Masucci, Manimozhiyan Arumugam, Nadeem Kaakoush, Eran Segal, Jasmohan Bajaj, Rupert Leong, John Cryan, Rinse K. Weersma, Robert Knight, Francisco Guarner, Fergus Shanahan, Patrice D. Cani, Eran Elinav, Maurizio Sanguinetti, Willem M. de Vos, Emad El-Omar, Joel Dorè, Julian Marchesi, Herbert Tilg, Harry Sokol, Nicola Segata, Giovanni Cammarota, Antonio Gasbarrini, and Gianluca Ianiro. International consensus statement on microbiome testing in clinical practice. *The Lancet. Gastroenterology & Hepatology*, 10(2):154–167, feb 2025. ISSN 2468-1253. doi: 10.1016/S2468-1253(24)00311-X.

[156] G. He, R. A. Shankar, M. Chzhan, A. Samouilov, P. Kuppusamy, and J. L. Zweier. Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of living mice with spatial and spectral EPR imaging. *Proceedings of the National Academy of Sciences of the United States of America*, 96(8):4586–4591, apr 1999. ISSN 0027-8424. doi: 10.1073/pnas.96.8.4586.

[157] Rashi Singhal and Yatrik M. Shah. Oxygen battle in the gut: Hypoxia and hypoxia-inducible factors in metabolic and inflammatory responses in the intestine. *The Journal of Biological Chemistry*, 295 (30):10493–10505, jul 2020. ISSN 0021-9258. doi: 10.1074/jbc.REV120.011188. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7383395/.

[158] C. R. Woese and G. E. Fox. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America*, 74(11): 5088–5090, nov 1977. ISSN 0027-8424. doi: 10.1073/pnas.74.11.5088.

[159] Carl R. Woese, O. Kandler, and Mark L. Wheelis. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences*, 87(12):4576–4579, jun 1990. ISSN 0027-8424. doi: 10.1073/pnas.87.12.4576. URL https://pnas.org/doi/full/10.1073/pnas.87.12.4576. 5834 citations (Semantic Scholar/DOI) [2022-06-23].

[160] Gerard Muyzer, Ellen C De Waal, and Anton G Uitterlinden. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16s rrna. *Applied and Environmental Microbiology*, 59(3):695–700, 1993. doi: 10.1128/aem.59.3.695-700.1993.

[161] J Gregory Caporaso, Christian L Lauber, William A Walters, Donna Berg-Lyons, Catherine A Lozupone, Peter J Turnbaugh, Noah Fierer, and Rob Knight. Global patterns of 16s rrna diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, 108(Supplement 1):4516–4522, 2011. doi: 10.1073/pnas.1000080107.

[162] Morgan GI Langille, Jesse Zaneveld, J Gregory Caporaso, Daniel McDonald, Dan Knights, Joshua A Reyes, Jose C Clemente, Deron E Burkepile, Rebecca L Vega Thurber, Rob Knight, et al. Predictive functional profiling of microbial communities using 16s rrna marker gene sequences. *Nature biotechnology*, 31(9):814–821, 2013. doi: 10.1038/nbt.2676.

[163] Gavin M. Douglas, Vincent J. Maffei, Jesse R. Zaneveld, Svetlana N. Yurgel, James R. Brown, Christopher M. Taylor, Curtis Huttenhower, and Morgan G. I. Langille. PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology*, 38(6):685–688, 6 2020. ISSN 1087-0156. doi: 10.1038/s41587-020-0548-6. URL https://github.com/http://www.nature.com/articles/s41587-020-0548-6.

[164] Leah Guthrie, Sarah Wolfson, and Libusha Kelly. The human gut chemical landscape predicts microbe-mediated biotransformation of foods and drugs. *eLife*, 8:e42866, jun 2019. ISSN 2050-084X. doi: 10.7554/eLife.42866. URL https://doi.org/10.7554/eLife.42866. 40 citations (Crossref) [2025-02-20] Publisher: eLife Sciences Publications, Ltd.

[165] Francesco Asnicar, Sarah E. Berry, Ana M. Valdes, Long H. Nguyen, Gianmarco Piccinno, David A. Drew, Emily Leeming, Rachel Gibson, Caroline Le Roy, Haya Al Khatib, Lucy Francis, Mohsen Mazidi, Olatz Mompeo, Mireia Valles-Colomer, Adrian Tett, Francesco Beghini, Léonard Dubois, Davide Bazzani, Andrew Maltez Thomas, Chloe Mirzayi, Asya Khleborodova, Sehyun Oh, Rachel Hine, Christopher Bonnett, Joan Capdevila, Serge Danzanvilliers, Francesca Giordano, Ludwig Geistlinger, Levi Waldron, Richard Davies, George Hadjigeorgiou, Jonathan Wolf, José M. Ordovás, Christopher Gardner, Paul W. Franks, Andrew T. Chan, Curtis Huttenhower, Tim D. Spector, and Nicola Segata. Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nature Medicine*, 27(2):321–332, feb 2021. ISSN 1078-8956. doi: 10.1038/s41591-020-01183-8. URL https://www.nature.com/articles/s41591-020-01183-8http://www.nature.com/articles/s41591-020-01183-8. 591 citations (Crossref) [2025-02-20] 180 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[166] Jessica A. Grembi, Lan H. Nguyen, Thomas D. Haggerty, Christopher D. Gardner, Susan P. Holmes, and Julie Parsonnet. Gut microbiota plasticity is correlated with sustained weight loss on a low-carb or low-fat dietary intervention. *Scientific Reports*, 10(1):1405, dec 2020. ISSN 20452322. doi: 10.1038/s41598-020-58000-y. URL http://www.nature.com/articles/s41598-020-58000-y. 23 citations (Crossref) [2025-02-20] 15 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[167] Brian D. Muegge, Justin Kuczynski, Dan Knights, Jose C. Clemente, Antonio González, Luigi Fontana, Bernard Henrissat, Rob Knight, and Jeffrey I. Gordon. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, 332(6032):970–974, 5 2011. ISSN 10959203. doi: 10.1126/science.1198719.

[168] Niv Zmora, Jotham Suez, and Eran Elinav. You are what you eat: diet, health and the gut microbiota, 1 2019. ISSN 17595053.

[169] Gloria Fackelmann, Paolo Manghi, Niccolò Carlino, Vitor Heidrich, Gianmarco Piccinno, Liviana Ricci, Elisa Piperni, Alberto Arrè, Elco Bakker, Alice C. Creedon, Lucy Francis, Joan Capdevila Pujol, Richard Davies, Jonathan Wolf, Kate M. Bermingham, Sarah E. Berry, Tim D. Spector, Francesco Asnicar, and Nicola Segata. Gut microbiome signatures of vegan, vegetarian and omnivore diets and associated health outcomes across 21,561 individuals. *Nature Microbiology*, 10(1):41–52, jan 2025. ISSN 2058-5276. doi: 10.1038/s41564-024-01870-z. URL https://www.nature.com/articles/s41564-024-01870-z.

[170] Jason Lloyd-Price, Cesar Arze, Ashwin N. Ananthakrishnan, Melanie Schirmer, Julian Avila-Pacheco, Tiffany W. Poon, Elizabeth Andrews, Nadim J. Ajami, Kevin S. Bonham, Colin J. Brislawn, David Casero, Holly Courtney, Antonio Gonzalez, Thomas G. Graeber, A. Brantley Hall, Kathleen Lake, Carol J. Landers, Himel Mallick, Damian R. Plichta, Mahadev Prasad, Gholamali Rahnavard, Jenny Sauk, Dmitry Shungin, Yoshiki Vázquez-Baeza, Richard A. White, Jonathan Braun, Lee A. Denson, Janet K. Jansson, Rob Knight, Subra Kugathasan, Dermot P. B. McGovern, Joseph F. Petrosino, Thaddeus S. Stappenbeck, Harland S. Winter, Clary B. Clish, Eric A. Franzosa, Hera Vlamakis, Ramnik J. Xavier, and Curtis Huttenhower. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature*, 569(7758):655–662, 2019. doi: 10.1038/s41586-019-1237-9.

[171] David Furman, Judith Campisi, Eric Verdin, Pedro Carrera-Bastos, Sasha Targ, Claudio Franceschi, Luigi Ferrucci, Derek W. Gilroy, Alessio Fasano, Gary W. Miller, Andrew H. Miller, Alberto Mantovani, Cornelia M. Weyand, Nir Barzilai, Jorg J. Goronzy, Thomas A. Rando, Rita B. Effros, Alejandro Lucia, Nicole Kleinstreuer, and George M. Slavich. Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, 25(12):1822–1832, dec 2019. ISSN 1546170X. doi: 10.1038/s41591-019-0675-0. 2764 citations (Crossref) [2025-02-20] 1007 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Research.

[172] Netusha Thevaranjan, Alicja Puchta, Christian Schulz, Avee Naidoo, J. C. Szamosi, Chris P. Verschoor, Dessi Loukov, Louis P. Schenck, Jennifer Jury, Kevin P. Foley, Jonathan D. Schertzer, Maggie J. Larché, Donald J. Davidson, Elena F. Verdú, Michael G. Surette, and Dawn M. E. Bowdish. Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction. *Cell Host & Microbe*, 21(4):455–466.e4, apr 2017. ISSN 1934-6069. doi: 10.1016/j.chom.2017.03.002.

[173] Hongbin Liu, Chen Liao, Lu Wu, Jinhui Tang, Junyu Chen, Chaobi Lei, Linggang Zheng, Chenhong Zhang, Yang-Yu Liu, Joao Xavier, and Lei Dai. Ecological dynamics of the gut microbiome in response to dietary fiber. *The ISME journal*, 16(8):2040–2055, aug 2022. ISSN 1751-7370. doi: 10.1038/s41396-022-01253-4.

[174] Joe M. McCord, Bernard B. Keele, and Irwin Fridovich. An Enzyme-Based Theory of Obligate Anaerobiosis: The Physiological Function of Superoxide Dismutase. *Proceedings of the National Academy of Sciences of the United States of America*, 68(5):1024–1027, may 1971. ISSN 0027-8424. doi: 10.1073/pnas.68.5.1024. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC389105/.

[175] Zheng Lu and James A. Imlay. When anaerobes encounter oxygen: mechanisms of oxygen toxicity, tolerance and defence. *Nature Reviews. Microbiology*, 19(12):774–785, dec 2021. ISSN 1740-1534. doi: 10.1038/s41579-021-00583-y.

[176] Lisa Maier, Camille V. Goemans, Jakob Wirbel, Michael Kuhn, Claudia Eberl, Mihaela Pruteanu, Patrick Müller, Sarela Garcia-Santamarina, Elisabetta Cacace, Boyao Zhang, Cordula Gekeler, Tisya Banerjee, Exene Erin Anderson, Alessio Milanese, Ulrike Löber, Sofia K. Forslund, Kiran Raosaheb Patil, Michael Zimmermann, Bärbel Stecher, Georg Zeller, Peer Bork, and Athanasios Typas. Unravelling the collateral damage of antibiotics on gut bacteria. *Nature*, pages 1–5, oct 2021. ISSN 0028-0836. doi: 10.1038/s41586-021-03986-2. URL https://www.nature.com/articles/s41586-021-03986-2. 218 citations (Crossref) [2025-02-20] 33 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[177] Lisa Maier, Mihaela Pruteanu, Michael Kuhn, Georg Zeller, Anja Telzerow, Exene Erin Anderson, Ana Rita Brochado, Keith Conrad Fernandez, Hitomi Dose, Hirotada Mori, Kiran Raosaheb Patil, Peer Bork, and Athanasios Typas. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, 555(7698):623–628, mar 2018. ISSN 14764687. doi: 10.1038/nature25979. URL http://www.ncbi.nlm.nih.gov/pubmed/29555994. 964 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[178] Sofia K. Forslund, Rima Chakaroun, Maria Zimmermann-Kogadeeva, Lajos Markó, Judith Aron-Wisnewsky, Trine Nielsen, Lucas Moitinho-Silva, Thomas S. B. Schmidt, Gwen Falony, Sara Vieira-Silva, Solia Adriouch, Renato J. Alves, Karen Assmann, Jean-Philippe Bastard, Till Birkner, Robert Caesar, Julien Chilloux, Luis Pedro Coelho, Leopold Fezeu, Nathalie Galleron, Gerard Helft, Richard Isnard, Boyang Ji, Michael Kuhn, Emmanuelle Le Chatelier, Antonis Myridakis, Lisa Olsson, Nicolas Pons, Edi Prifti, Benoit Quinquis, Hugo Roume, Joe-Elie Salem, Nataliya Sokolovska, Valentina Tremaroli, Mireia Valles-Colomer, Christian Lewinter, Nadja B. Søndertoft, Helle Krogh Pedersen, Tue H. Hansen, MetaCardis Consortium*, Jens Peter Gøtze, Lars Køber, Henrik Vestergaard, Torben Hansen, Jean-Daniel Zucker, Serge Hercberg, Jean-Michel Oppert, Ivica Letunic, Jens Nielsen, Fredrik Bäckhed, S. Dusko Ehrlich, Marc-Emmanuel Dumas, Jeroen Raes, Oluf Pedersen, Karine Clément, Michael Stumvoll, and Peer Bork. Combinatorial, additive and dose-dependent drug-microbiome associations. *Nature*, 600(7889):500–505, dec 2021. ISSN 1476-4687. doi: 10.1038/s41586-021-04177-9.

[179] Gianluca Ianiro, Michal Punčochář, Nicolai Karcher, Serena Porcari, Federica Armanini, Francesco Asnicar, Francesco Beghini, Aitor Blanco-Míguez, Fabio Cumbo, Paolo Manghi, Federica Pinto,

Luca Masucci, Gianluca Quaranta, Silvia De Giorgi, Giusi Desirè Sciumè, Stefano Bibbò, Federica Del Chierico, Lorenza Putignani, Maurizio Sanguinetti, Antonio Gasbarrini, Mireia Valles-Colomer, Giovanni Cammarota, and Nicola Segata. Variability of strain engraftment and predictability of microbiome composition after fecal microbiota transplantation across different diseases. *Nature Medicine*, pages 1–11, sep 2022. ISSN 1546-170X. doi: 10.1038/s41591-022-01964-3. URL https://www.nature.com/articles/s41591-022-01964-3. 146 citations (Crossref) [2025-02-20] Publisher: Nature Publishing Group.

[180] Thomas S. B. Schmidt, Simone S. Li, Oleksandr M. Maistrenko, Wasiu Akanni, Luis Pedro Coelho, Sibasish Dolai, Anthony Fullam, Anna M. Glazek, Rajna Hercog, Hilde Herrema, Ferris Jung, Stefanie Kandels, Askarbek Orakov, Roman Thielemann, Moritz von Stetten, Thea Van Rossum, Vladimir Benes, Thomas J. Borody, Willem M. de Vos, Cyriel Y. Ponsioen, Max Nieuwdorp, and Peer Bork. Drivers and determinants of strain dynamics following fecal microbiota transplantation. *Nature Medicine*, 28(9):1902–1912, sep 2022. ISSN 1546-170X. doi: 10.1038/s41591-022-01913-0. URL https://www.nature.com/articles/s41591-022-01913-0.

[181] Verena Zimorski, Chuan Ku, William F Martin, and Sven B Gould. Endosymbiotic theory for organelle origins. *Current Opinion in Microbiology*, 22:38–48, 2014. doi: 10.1016/j.mib.2014.09.008.

[182] Joran Martijn, Julian Vosseberg, Lionel Guy, Pierre Offre, and Thijs J.G. Ettema. Deep mitochondrial origin outside the sampled alphaproteobacteria. *Nature*, 557(7703):101–105, 2018. doi: 10.1038/s41586-018-0059-5.

[183] Cymon J. Cox, Peter G. Foster, Robert P. Hirt, Simon R. Harris, and T. Martin Embley. The archaebacterial origin of eukaryotes. *Proceedings of the National Academy of Sciences*, 105(51): 20356–20361, 2008. doi: 10.1073/pnas.0810647105.

[184] Hatim Sati, Elena Carrara, Alessia Savoldi, Paul Hansen, Jacopo Garlasco, Enrica Campagnaro, Simone Boccia, Juan Antonio Castillo-Polo, Eugenia Magrini, Pilar Garcia-Vello, Eve Wool, Valeria Gigante, Erin Duffy, Alessandro Cassini, Benedikt Huttner, Pilar Ramon Pardo, Mohsen Naghavi, Fuad Mirzayev, Matteo Zignol, Alexandra Cameron, Evelina Tacconelli, and WHO Bacterial Priority Pathogens List Advisory Group. The WHO Bacterial Priority Pathogens List 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. *The Lancet. Infectious Diseases*, pages S1473–3099(25)00118–5, apr 2025. ISSN 1474-4457. doi: 10.1016/S1473-3099(25)00118-5.

[185] GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. *Lancet (London, England)*, 404 (10459):1199–1226, sep 2024. ISSN 1474-547X. doi: 10.1016/S0140-6736(24)01867-1.

[186] S. Ragnar Norrby, Carl Erik Nord, Roger Finch, and European Society of Clinical Microbiology and Infectious Diseases. Lack of development of new antimicrobial drugs: a potential serious threat to public health. *The Lancet. Infectious Diseases*, 5(2):115–119, feb 2005. ISSN 1473-3099. doi: 10.1016/S1473-3099(05)01283-1.

[187] Anthony Coates, Yanmin Hu, Richard Bax, and Clive Page. The future challenges facing the development of new antimicrobial drugs. *Nature Reviews. Drug Discovery*, 1(11):895–910, nov 2002. ISSN 1474-1776. doi: 10.1038/nrd940.

[188] John R. Perfect. The antifungal pipeline: a reality check. *Nature Reviews. Drug Discovery*, 16(9): 603–616, sep 2017. ISSN 1474-1784. doi: 10.1038/nrd.2017.46.

[189] Annette von Delft, Matthew D. Hall, Ann D. Kwong, Lisa A. Purcell, Kumar Singh Saikatendu, Uli Schmitz, John A. Tallarico, and Alpha A. Lee. Accelerating antiviral drug discovery: lessons from COVID-19. *Nature Reviews. Drug Discovery*, 22(7):585–603, jul 2023. ISSN 1474-1784. doi: 10.1038/s41573-023-00692-8.

[190] Marcus Miethke, Marco Pieroni, Tilmann Weber, Mark Brönstrup, Peter Hammann, Ludovic Halby, Paola B. Arimondo, Philippe Glaser, Bertrand Aigle, Helge B. Bode, Rui Moreira, Yanyan Li, Andriy Luzhetskyy, Marnix H. Medema, Jean-Luc Pernodet, Marc Stadler, José Rubén Tormo, Olga Genilloud, Andrew W. Truman, Kira J. Weissman, Eriko Takano, Stefano Sabatini, Evi Stegmann, Heike Brötz-Oesterhelt, Wolfgang Wohlleben, Myriam Seemann, Martin Empting, Anna K. H. Hirsch, Brigitta Loretz, Claus-Michael Lehr, Alexander Titz, Jennifer Herrmann, Timo Jaeger, Silke Alt, Thomas Hesterkamp, Mathias Winterhalter, Andrea Schiefer, Kenneth Pfarr, Achim Hoerauf, Heather Graz, Michael Graz, Mika Lindvall, Savithri Ramurthy, Anders Karlén, Maarten van Dongen, Hrvoje Petkovic, Andreas Keller, Frédéric Peyrane, Stefano Donadio, Laurent Fraisse, Laura J. V. Piddock, Ian H. Gilbert, Heinz E. Moser, and Rolf Müller. Towards the sustainable discovery and development of new antibiotics. *Nature Reviews. Chemistry*, 5(10):726–749, 2021. ISSN 2397-3358. doi: 10.1038/s41570-021-00313-1.

[191] Laura J. V. Piddock, Yewande Alimi, James Anderson, Damiano de Felice, Catrin E. Moore, John-Arne Røttingen, Henry Skinner, and Peter Beyer. Advancing global antibiotic research, development and access. *Nature Medicine*, 30(9):2432–2443, sep 2024. ISSN 1546-170X. doi: 10.1038/s41591-024-03218-w.

[192] Ursula Theuretzbacher, Ravindra P. Jumde, Alan Hennessy, Jennifer Cohn, and Laura J. V. Piddock. Global health perspectives on antibacterial drug discovery and the preclinical pipeline. *Nature Reviews. Microbiology*, 23(8):474–490, aug 2025. ISSN 1740-1534. doi: 10.1038/s41579-025-01167-w.

[193] Huseyin Naci, Peter Murphy, Beth Woods, James Lomas, Jinru Wei, and Irene Papanicolas. Population-health impact of new drugs recommended by the National Institute for Health and Care Excellence in England during 2000-20: a retrospective analysis. *Lancet (London, England)*, 405 (10472):50–60, jan 2025. ISSN 1474-547X. doi: 10.1016/S0140-6736(24)02352-3.

[194] Helen W. Boucher, George H. Talbot, John S. Bradley, John E. Edwards, David Gilbert, Louis B. Rice, Michael Scheld, Brad Spellberg, and John Bartlett. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 48(1):1–12, jan 2009. ISSN 1537-6591. doi: 10.1086/595011.

[195] Christopher JL Murray, Kevin Shunji Ikuta, Fablina Sharara, Lucien Swetschinski, Gisela Robles Aguilar, Authia Gray, Chieh Han, Catherine Bisignano, Puja Rao, Eve Wool, Sarah C. Johnson, Annie J. Browne, Michael Give Chipeta, Frederick Fell, Sean Hackett, Georgina Haines-Woodhouse, Bahar H. Kashef Hamadani, Emmanuelle A.P. P Kumaran, Barney McManigal, Ramesh Agarwal, Samuel Akech, Samuel Albertson, John Amuasi, Jason Andrews, Aleskandr Aravkin, Elizabeth Ashley, Freddie Bailey, Stephen Baker, Buddha Basnyat, Adrie Bekker, Rose

Bender, Adhisivam Bethou, Julia Bielicki, Suppawat Boonkasidecha, James Bukosia, Cristina Carvalheiro, Carlos Castañeda-Orjuela, Vilada Chansamouth, Suman Chaurasia, Sara Chiurchiù, Fazle Chowdhury, Aislinn J. Cook, Ben Cooper, Tim R. Cressey, Elia Criollo-Mora, Matthew Cunningham, Saffiatou Darboe, Nicholas P.J. J Day, Maia De Luca, Klara Dokova, Angela Dramowski, Susanna J. Dunachie, Tim Eckmanns, Daniel Eibach, Amir Emami, Nicholas Feasey, Natasha Fisher-Pearson, Karen Forrest, Denise Garrett, Petra Gastmeier, Ababi Zergaw Giref, Rachel Claire Greer, Vikas Gupta, Sebastian Haller, Andrea Haselbeck, Simon I. Hay, Marianne Holm, Susan Hopkins, Kenneth C. Iregbu, Jan Jacobs, Daniel Jarovsky, Fatemeh Javanmardi, Meera Khorana, Niranjan Kissoon, Elsa Kobeissi, Tomislav Kostyanev, Fiorella Krapp, Ralf Krumkamp, Ajay Kumar, Hmwe Hmwe Kyu, Cherry Lim, Direk Limmathurotsakul, Michael James Loftus, Miles Lunn, Jianing Ma, Neema Mturi, Tatiana Munera-Huertas, Patrick Musicha, Marisa Marcia Mussi-Pinhata, Tomoka Nakamura, Ruchi Nanavati, Sushma Nangia, Paul Newton, Chanpheaktra Ngoun, Amanda Novotney, Davis Nwakanma, Christina W. Obiero, Antonio Olivas-Martinez, Piero Olliaro, Ednah Ooko, Edgar Ortiz-Brizuela, Anton Yariv Peleg, Carlo Perrone, Nishad Plakkal, Alfredo Ponce-de Leon, Mathieu Raad, Tanusha Ramdin, Amy Riddell, Tamalee Roberts, Julie Victoria Robotham, Anna Roca, Kristina E. Rudd, Neal Russell, Jesse Schnall, John Anthony Gerard Scott, Madhusudhan Shivamallappa, Jose Sifuentes-Osornio, Nicolas Steenkeste, Andrew James Stewardson, Temenuga Stoeva, Nidanuch Tasak, Areerat Thaiprakong, Guy Thwaites, Claudia Turner, Paul Turner, H. Rogier van Doorn, Sithembiso Velaphi, Avina Vongpradith, Huong Vu, Timothy Walsh, Seymour Waner, Tri Wangrangsimakul, Teresa Wozniak, Peng Zheng, Benn Sartorius, Alan D. Lopez, Andy Stergachis, Catrin Moore, Christiane Dolecek, and Mohsen Naghavi. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325):629–655, feb 2022. ISSN 01406736. doi: 10.1016/S0140-6736(21)02724-0. URL http://www.thelancet.com/article/S0140673621027240/fulltexthttp://www.thelancet.com/article/S0140673621027240/abstracthttps://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)02724-0/abstracthttps://linkinghub.elsevier.com/retrieve/pii/S01406. 7308 citations (Crossref) [2025-02-20] 533 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Elsevier.

[196] Iruka N. Okeke, Marlieke E. A. de Kraker, Thomas P. Van Boeckel, Chirag K. Kumar, Heike Schmitt, Ana C. Gales, Silvia Bertagnolio, Mike Sharland, and Ramanan Laxminarayan. The scope of the antimicrobial resistance challenge. *Lancet (London, England)*, 403(10442):2426–2438, jun 2024. ISSN 1474-547X. doi: 10.1016/S0140-6736(24)00876-6.

[197] A.-P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, and D. L. Monnet. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 18(3):268–281, mar 2012. ISSN 1469-0691. doi: 10.1111/j.1469-0691.2011.03570.x.

[198] Jose M. Munita and Cesar A. Arias. Mechanisms of Antibiotic Resistance. *Microbiology Spectrum*, 4(2), apr 2016. ISSN 2165-0497. doi: 10.1128/microbiolspec.VMBF-0016-2015.

[199] Elizabeth M. Darby, Eleftheria Trampari, Pauline Siasat, Maria Solsona Gaya, Ilyas Alav, Mark A. Webber, and Jessica M. A. Blair. Molecular mechanisms of antibiotic resistance re-

visited. *Nature Reviews. Microbiology*, 21(5):280–295, may 2023. ISSN 1740-1534. doi: 10.1038/s41579-022-00820-y. URL https://www.ncbi.nlm.nih.gov/pubmed/36411397.

[200] William P. J. Smith, Benjamin R. Wucher, Carey D. Nadell, and Kevin R. Foster. Bacterial defences: mechanisms, evolution and antimicrobial resistance. *Nature Reviews. Microbiology*, 21(8): 519–534, aug 2023. ISSN 1740-1534. doi: 10.1038/s41579-023-00877-3.

[201] Martin J. Blaser. Antibiotic use and its consequences for the normal microbiome. *Science (New York, N.Y.)*, 352(6285):544–545, apr 2016. ISSN 1095-9203. doi: 10.1126/science.aad9358.

[202] Skye R. S. Fishbein, Bejan Mahmud, and Gautam Dantas. Antibiotic perturbations to the gut microbiome. *Nature Reviews. Microbiology*, 21(12):772–788, dec 2023. ISSN 1740-1534. doi: 10.1038/s41579-023-00933-y.

[203] A. Telenti, P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M. J. Colston, L. Matter, K. Schopfer, and T. Bodmer. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. *Lancet (London, England)*, 341(8846):647–650, mar 1993. ISSN 0140-6736. doi: 10.1016/0140-6736(93)90417-f.

[204] V. Kapur, L. L. Li, S. Iordanescu, M. R. Hamrick, A. Wanger, B. N. Kreiswirth, and J. M. Musser. Characterization by automated DNA sequencing of mutations in the gene (rpoB) encoding the RNA polymerase beta subunit in rifampin-resistant Mycobacterium tuberculosis strains from New York City and Texas. *Journal of Clinical Microbiology*, 32(4):1095–1098, apr 1994. ISSN 0095-1137. doi: 10.1128/jcm.32.4.1095-1098.1994.

[205] Laura de Nies, Carolin M. Kobras, and Mathew Stracy. Antibiotic-induced collateral damage to the microbiota and associated infections. *Nature Reviews. Microbiology*, 21(12):789–804, dec 2023. ISSN 1740-1534. doi: 10.1038/s41579-023-00936-9.

[206] Willem van Schaik. The human gut resistome. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 370(1670):20140087, jun 2015. ISSN 1471-2970. doi: 10.1098/rstb.2014.0087.

[207] A.J. Andrew J. Gasparrini, Bin Wang, Xiaoqing Sun, Elizabeth A. E.A. Kennedy, Ariel Hernandez-Leyva, I.M. Malick Ndao, Phillip I. Tarr, B.B. Barbara B. Warner, and Gautam Dantas. Persistent metagenomic signatures of early-life hospitalization and antibiotic treatment in the infant gut microbiota and resistome. *Nature Microbiology*, 4(12):2285–2297, sep 2019. ISSN 20585276. doi: 10.1038/s41564-019-0550-2. URL https://www.nature.com/articles/s41564-019-0550-2. 219 citations (Crossref) [2025-02-20] 92 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[208] Achal Dhariwal, Polona Rajar, Gabriela Salvadori, Heidi Aarø Åmdal, Dag Berild, Ola Didrik Saugstad, Drude Fugelseth, Gorm Greisen, Ulf Dahle, Kirsti Haaland, and Fernanda Cristina Petersen. Prolonged hospitalization signature and early antibiotic effects on the nasopharyngeal resistome in preterm infants. *Nature Communications*, 15(1):6024, jul 2024. ISSN 2041-1723. doi: 10.1038/s41467-024-50433-7.

[209] Colin Hill, Francisco Guarner, Gregor Reid, Glenn R. Gibson, Daniel J. Merenstein, Bruno Pot, Lorenzo Morelli, Roberto Berni Canani, Harry J. Flint, Seppo Salminen, Philip C. Calder, and

Mary Ellen Sanders. Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*, 11(8):506–514, 2014. ISSN 17595053. doi: 10.1038/nrgastro.2014.66. 4201 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[210] William R. Miller and Cesar A. Arias. ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics. *Nature Reviews. Microbiology*, 22(10):598–616, oct 2024. ISSN 1740-1534. doi: 10.1038/s41579-024-01054-w. URL https://www.ncbi.nlm.nih.gov/pubmed/38831030.

[211] Cesar A. Arias and Barbara E. Murray. Antibiotic-resistant bugs in the 21st century–a clinical superchallenge. *The New England Journal of Medicine*, 360(5):439–443, jan 2009. ISSN 1533-4406. doi: 10.1056/NEJMp0804651.

[212] Patrice Courvalin. Vancomycin resistance in gram-positive cocci. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 42 Suppl 1:S25–34, jan 2006. ISSN 1537-6591. doi: 10.1086/491711.

[213] Stuart B. Levy and Bonnie Marshall. Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine*, 10(12 Suppl):S122–129, dec 2004. ISSN 1078-8956. doi: 10.1038/nm1145.

[214] Alison H. Holmes, Luke S. P. Moore, Arnfinn Sundsfjord, Martin Steinbakk, Sadie Regmi, Abhilasha Karkey, Philippe J. Guerin, and Laura J. V. Piddock. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet (London, England)*, 387(10014):176–187, jan 2016. ISSN 1474-547X. doi: 10.1016/S0140-6736(15)00473-0.

[215] Ramanan Laxminarayan, Adriano Duse, Chand Wattal, Anita K. M. Zaidi, Heiman F. L. Wertheim, Nithima Sumpradit, Erika Vlieghe, Gabriel Levy Hara, Ian M. Gould, Herman Goossens, Christina Greko, Anthony D. So, Maryam Bigdeli, Göran Tomson, Will Woodhouse, Eva Ombaka, Arturo Quizhpe Peralta, Farah Naz Qamar, Fatima Mir, Sam Kariuki, Zulfiqar A. Bhutta, Anthony Coates, Richard Bergstrom, Gerard D. Wright, Eric D. Brown, and Otto Cars. Antibiotic resistance-the need for global solutions. *The Lancet. Infectious Diseases*, 13(12):1057–1098, dec 2013. ISSN 1474-4457. doi: 10.1016/S1473-3099(13)70318-9.

[216] Evelina Tacconelli, Elena Carrara, Alessia Savoldi, Stephan Harbarth, Marc Mendelson, Dominique L. Monnet, Céline Pulcini, Gunnar Kahlmeter, Jan Kluytmans, Yehuda Carmeli, Marc Ouellette, Kevin Outterson, Jean Patel, Marco Cavaleri, Edward M. Cox, Chris R. Houchens, M. Lindsay Grayson, Paul Hansen, Nalini Singh, Ursula Theuretzbacher, Nicola Magrini, and WHO Pathogens Priority List Working Group. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet. Infectious Diseases*, 18(3):318–327, mar 2018. ISSN 1474-4457. doi: 10.1016/S1473-3099(17)30753-3.

[217] Scott John Charles Pallett, Esmita Charani, Georgia Lamb, Andrea Mazzella, Saleh A. Alqahtani, and Luke Stephen Prockter Moore. Optimising utilisation of GLASS data to inform cross-border antimicrobial resistance strategy is a priority. *Lancet Regional Health. Americas*, 29:100655, jan 2024. ISSN 2667-193X. doi: 10.1016/j.lana.2023.100655.

[218] Charlotte S. Ho, Carlos T. H. Wong, Thet Tun Aung, Rajamani Lakshminarayanan, Jodhbir S. Mehta, Saaeha Rauz, Alan McNally, Balint Kintses, Sharon J. Peacock, Cesar de la Fuente-Nunez, Robert E. W. Hancock, and Darren S. J. Ting. Antimicrobial resistance: a concise update. *The Lancet. Microbe*, 6(1):100947, jan 2025. ISSN 2666-5247. doi: 10.1016/j.lanmic.2024.07.010.

[219] Eili Y. Klein, Thomas P. Van Boeckel, Elena M. Martinez, Suraj Pant, Sumanth Gandra, Simon A. Levin, Herman Goossens, and Ramanan Laxminarayan. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences of the United States of America*, 115(15):E3463–E3470, apr 2018. ISSN 1091-6490. doi: 10.1073/pnas.1717295115.

[220] Alessandro Cassini, Liselotte Diaz Högberg, Diamantis Plachouras, Annalisa Quattrocchi, Ana Hoxha, Gunnar Skov Simonsen, Mélanie Colomb-Cotinat, Mirjam E. Kretzschmar, Brecht Devleesschauwer, Michele Cecchini, Driss Ait Ouakrim, Tiago Cravo Oliveira, Marc J. Struelens, Carl Suetens, Dominique L. Monnet, and Burden of AMR Collaborative Group. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *The Lancet. Infectious Diseases*, 19(1):56–66, jan 2019. ISSN 1474-4457. doi: 10.1016/S1473-3099(18)30605-4.

[221] Benyamin Sihombing, Rajesh Bhatia, Rahul Srivastava, Tjandra Yoga Aditama, Ramanan Laxminarayan, and Suman Rijal. Response to antimicrobial resistance in South-East Asia Region. *The Lancet Regional Health. Southeast Asia*, 18:100306, nov 2023. ISSN 2772-3682. doi: 10.1016/j.lansea.2023.100306.

[222] Antimicrobial Resistance Collaborators. The burden of bacterial antimicrobial resistance in the WHO African region in 2019: a cross-country systematic analysis. *The Lancet. Global Health*, 12 (2):e201–e216, feb 2024. ISSN 2214-109X. doi: 10.1016/S2214-109X(23)00539-9.

[223] Sabiha Y. Essack and Annick Lenglet. Bacterial antimicrobial resistance burden in Africa: accuracy, action, and alternatives. *The Lancet. Global Health*, 12(2):e171–e172, feb 2024. ISSN 2214-109X. doi: 10.1016/S2214-109X(23)00587-9.

[224] Antimicrobial Resistance Collaborators. The burden of antimicrobial resistance in the Americas in 2019: a cross-country systematic analysis. *Lancet Regional Health. Americas*, 25:100561, sep 2023. ISSN 2667-193X. doi: 10.1016/j.lana.2023.100561.

[225] Eduardo A. Undurraga, Anne Peters, Cesar A. Arias, and Jose M. Munita. Lives lost and disease burden related to antimicrobial resistance in the Americas can no longer be ignored. *Lancet Regional Health. Americas*, 26:100596, oct 2023. ISSN 2667-193X. doi: 10.1016/j.lana.2023.100596.

[226] Léo C. Caulat, Aurélie Lotoux, Maria C. Martins, Nicolas Kint, Cyril Anjou, Miguel Teixeira, Filipe Folgosa, Claire Morvan, and Isabelle Martin-Verstraete. Physiological role and complex regulation of O2-reducing enzymes in the obligate anaerobe Clostridioides difficile. *mBio*, 15(10): e0159124, October 2024. ISSN 2150-7511. doi: 10.1128/mbio.01591-24.

[227] Nicole Giordano, Jessica L. Hastie, Ashley D. Smith, Elissa D. Foss, Daniela F. Gutierrez-Munoz, and Paul E. Carlson. Cysteine Desulfurase IscS2 Plays a Role in Oxygen Resistance in Clostridium difficile. *Infection and Immunity*, 86(8):e00326–18, aug 2018. ISSN 1098-5522. doi: 10.1128/IAI.00326-18.

[228] Nicolas Kint, Carolina Alves Feliciano, Maria C. Martins, Claire Morvan, Susana F. Fernandes, Filipe Folgosa, Bruno Dupuy, Miguel Texeira, and Isabelle Martin-Verstraete. How the Anaerobic Enteropathogen Clostridioides difficile Tolerates Low O2 Tensions. *mBio*, 11(5):e01559–20, September 2020. ISSN 2150-7511. doi: 10.1128/mBio.01559-20.

[229] L. Clifford McDonald, George E. Killgore, Angela Thompson, Robert C. Owens, Sophia V. Kazakova, Susan P. Sambol, Stuart Johnson, and Dale N. Gerding. An epidemic, toxin gene-variant strain of Clostridium difficile. *The New England Journal of Medicine*, 353(23):2433–2441, dec 2005. ISSN 1533-4406. doi: 10.1056/NEJMoa051590.

[230] Miao He, Fabio Miyajima, Paul Roberts, Louise Ellison, Derek J. Pickard, Melissa J. Martin, Thomas R. Connor, Simon R. Harris, Derek Fairley, Kathleen B. Bamford, Stephanie D'Arc, Jon Brazier, Derek Brown, John E. Coia, Gill Douce, Dale Gerding, Hee Jung Kim, Tse Hsien Koh, Haru Kato, Mitsutoshi Senoh, Tom Louie, Stephen Michell, Emma Butt, Sharon J. Peacock, Nick M. Brown, Tom Riley, Glen Songer, Mark Wilcox, Munir Pirmohamed, Ed Kuijper, Peter Hawkey, Brendan W. Wren, Gordon Dougan, Julian Parkhill, and Trevor D. Lawley. Emergence and global spread of epidemic healthcare-associated Clostridium difficile. *Nature Genetics*, 45(1): 109–113, jan 2013. ISSN 1546-1718. doi: 10.1038/ng.2478. URL https://www.nature.com/articles/ng.2478.

[231] Dena Lyras, Jennifer R. O'Connor, Pauline M. Howarth, Susan P. Sambol, Glen P. Carter, Tongted Phumoonna, Rachael Poon, Vicki Adams, Gayatri Vedantam, Stuart Johnson, Dale N. Gerding, and Julian I. Rood. Toxin B is essential for virulence of Clostridium difficile. *Nature*, 458(7242):1176–1179, apr 2009. ISSN 1476-4687. doi: 10.1038/nature07822.

[232] Sarah A. Kuehne, Stephen T. Cartman, John T. Heap, Michelle L. Kelly, Alan Cockayne, and Nigel P. Minton. The role of toxin A and toxin B in Clostridium difficile infection. *Nature*, 467 (7316):711–713, oct 2010. ISSN 1476-4687. doi: 10.1038/nature09397.

[233] Ramyavardhanee Chandrasekaran and D. Borden Lacy. The role of toxins in Clostridium difficile infection. *FEMS microbiology reviews*, 41(6):723–750, nov 2017. ISSN 1574-6976. doi: 10.1093/femsre/fux048.

[234] Dale N. Gerding, Thomas Meyer, Christine Lee, Stuart H. Cohen, Uma K. Murthy, Andre Poirier, Trevor C. Van Schooneveld, Darrell S. Pardi, Antonio Ramos, Michelle A. Barron, Hongzi Chen, and Stephen Villano. Administration of spores of nontoxigenic Clostridium difficile strain M3 for prevention of recurrent C. difficile infection: a randomized clinical trial. *JAMA*, 313(17):1719–1727, may 2015. ISSN 1538-3598. doi: 10.1001/jama.2015.3725.

[235] Mukil Natarajan, Seth T. Walk, Vincent B. Young, and David M. Aronoff. A clinical and epidemiological review of non-toxigenic Clostridium difficile. *Anaerobe*, 22:1–5, aug 2013. ISSN 1095-8274. doi: 10.1016/j.anaerobe.2013.05.005.

[236] Kate E. Dingle, Briony Elliott, Esther Robinson, David Griffiths, David W. Eyre, Nicole Stoesser, Alison Vaughan, Tanya Golubchik, Warren N. Fawley, Mark H. Wilcox, Timothy E. Peto, A. Sarah Walker, Thomas V. Riley, Derrick W. Crook, and Xavier Didelot. Evolutionary History of the Clostridium difficile Pathogenicity Locus. *Genome Biology and Evolution*, 6(1):36–52, jan 2014. ISSN 1759-6653. doi: 10.1093/gbe/evt204. URL https://doi.org/10.1093/gbe/evt204.

[237] Michel Warny, Jacques Pepin, Aiqi Fang, George Killgore, Angela Thompson, Jon Brazier, Eric Frost, and L. Clifford McDonald. Toxin production by an emerging strain of Clostridium difficile associated with outbreaks of severe disease in North America and Europe. *The Lancet*, 2005. ISSN 01406736. doi: 10.1016/S0140-6736(05)67420-X. 1432 citations (Semantic Scholar/DOI) [2022-06-24] ISBN: 1474-547X (Electronic)\no140-6736 (Linking).

[238] Susana Matamouros, Patrick England, and Bruno Dupuy. Clostridium difficile toxin expression is inhibited by the novel regulator TcdC. *Molecular Microbiology*, 64(5):1274–1288, jun 2007. ISSN 0950-382X. doi: 10.1111/j.1365-2958.2007.05739.x.

[239] B. Dupuy, R. Govind, A. Antunes, and S. Matamouros. Clostridium difficile toxin synthesis is negatively regulated by TcdC. *Journal of Medical Microbiology*, 57(Pt 6):685–689, jun 2008. ISSN 0022-2615. doi: 10.1099/jmm.0.47775-0.

[240] Richard A. Stabler, Miao He, Lisa Dawson, Melissa Martin, Esmeralda Valiente, Craig Corton, Trevor D. Lawley, Mohammed Sebaihia, Michael A. Quail, Graham Rose, Dale N. Gerding, Maryse Gibert, Michel R. Popoff, Julian Parkhill, Gordon Dougan, and Brendan W. Wren. Comparative genome and phenotypic analysis of Clostridium difficile 027 strains provides insight into the evolution of a hypervirulent bacterium. *Genome Biology*, 10(9):R102, 2009. ISSN 1474-760X. doi: 10.1186/gb-2009-10-9-r102. URL https://www.ncbi.nlm.nih.gov/pubmed/19781061.

[241] Veit Braun, Thomas Hundsberger, Petra Leukel, Markus Sauerborn, and Christoph von Eichel-Streiber. Definition of the single integration site of the pathogenicity locus in *Clostridium difficile*. *Gene*, 181(1):29–38, nov 1996. ISSN 0378-1119. doi: 10.1016/S0378-1119(96)00398-8. URL https://www.sciencedirect.com/science/article/pii/S0378111996003988.

[242] Bruno Dupuy and Susana Matamouros. Regulation of toxin and bacteriocin synthesis in Clostridium species by a new subgroup of RNA polymerase sigma-factors. *Research in Microbiology*, 157 (3):201–205, apr 2006. ISSN 0923-2508. doi: 10.1016/j.resmic.2005.11.004.

[243] G. Hecht, C. Pothoulakis, J.T. LaMont, and J.L. Madara. Clostridium difficile toxin A perturbs cytoskeletal structure and tight junction permeability of cultured human intestinal epithelial monolayers. *Journal of Clinical Investigation*, 82(5):1516–1524, 1988. doi: 10.1172/JCI113760.

[244] M. Riegler, R. Sedivy, C. Pothoulakis, G. Hamilton, J. Zacherl, G. Bischof, E. Cosentini, W. Feil, R. Schiessel, J.T. LaMont, and E. Wenzl. Clostridium difficile toxin B is more potent than toxin A in damaging human colonic epithelium in vitro. *Journal of Clinical Investigation*, 95(5):2004–2011, 1995. doi: 10.1172/JCI117885.

[245] Kathleen E. Orrell and Roman A. Melnyk. Large Clostridial Toxins: Mechanisms and Roles in Disease. *Microbiology and molecular biology reviews: MMBR*, 85(3):e0006421, aug 2021. ISSN 1098-5557. doi: 10.1128/MMBR.00064-21.

[246] N. M. Sullivan, S. Pellett, and T. D. Wilkins. Purification and characterization of toxins A and B of Clostridium difficile. *Infection and Immunity*, 35(3):1032–1040, mar 1982. ISSN 0019-9567. doi: 10.1128/iai.35.3.1032-1040.1982.

[247] Dale N Gerding, Stuart Johnson, Maja Rupnik, and Klaus Aktories. Clostridium difficile binary toxin CDT. *Gut Microbes*, 5(1):15–27, jan 2014. ISSN 1949-0976. doi: 10.4161/gmic.26854. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4049931/.

[248] David M. Anderson, Michael J. Sheedlo, Jaime L. Jensen, and D. Borden Lacy. Structural insights into the transition of Clostridioides difficile binary toxin from prepore to pore. *Nature Microbiology*, 5(1):102–107, jan 2020. ISSN 2058-5276. doi: 10.1038/s41564-019-0601-8.

[249] Shannon L. Kordus, Audrey K. Thomas, and D. Borden Lacy. Clostridioides difficile toxins: mechanisms of action and antitoxin therapeutics. *Nature Reviews. Microbiology*, 20(5):285–298, may 2022. ISSN 1740-1534. doi: 10.1038/s41579-021-00660-2.

[250] Jessica Reineke, Stefan Tenzer, Maja Rupnik, Andreas Koschinski, Oliver Hasselmayer, André Schrattenholz, Hansjörg Schild, and Christoph von Eichel-Streiber. Autocatalytic cleavage of Clostridium difficile toxin B. *Nature*, 446(7134):415–419, mar 2007. ISSN 1476-4687. doi: 10.1038/nature05622.

[251] Martina Egerer, Torsten Giesemann, Thomas Jank, Karla J. Fullner Satchell, and Klaus Aktories. Auto-catalytic cleavage of Clostridium difficile toxins A and B depends on cysteine protease activity. *The Journal of Biological Chemistry*, 282(35):25314–25321, aug 2007. ISSN 0021-9258. doi: 10.1074/jbc.M703062200.

[252] I. Just, J. Selzer, M. Wilm, C. von Eichel-Streiber, M. Mann, and K. Aktories. Glucosylation of Rho proteins by Clostridium difficile toxin B. *Nature*, 375(6531):500–503, jun 1995. ISSN 0028-0836. doi: 10.1038/375500a0.

[253] L. Kyne, M. Warny, A. Qamar, and C.P. Kelly. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. *New England Journal of Medicine*, 342(6):390–397, 2000. doi: 10.1056/NEJM200002103420604.

[254] L. Kyne, M. Warny, A. Qamar, and C.P. Kelly. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhoea. *Lancet*, 357(9251):189–193, 2001. doi: 10.1016/S0140-6736(00)03592-3.

[255] H. Xu, J. Yang, W. Gao, L. Li, P. Li, L. Zhang, Y.-N. Gong, X. Peng, J.J. Xi, S. Chen, F. Wang, and F. Shao. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature*, 513(7517):237–241, 2014. doi: 10.1038/nature13449.

[256] G.J. Babcock, T.J. Broering, H.J. Hernandez, R.B. Mandell, K. Donahue, N. Boatright, A.M. Stack, I. Lowy, R. Graziano, D. Molrine, D.M. Ambrosino, and W.D. Thomas Jr. Human monoclonal antibodies directed against toxins A and B prevent Clostridium difficile-induced mortality in hamsters. *Infection and Immunity*, 74(11):6339–6347, 2006. doi: 10.1128/IAI.00982-06.

[257] M. H. Wilcox, D. N. Gerding, I. R. Poxton, C. Kelly, R. Nathan, T. Birch, O. A. Cornely, G. Rahav, E. Bouza, C. Lee, G. Jenkin, W. Jensen, Y. S. Kim, J. Yoshida, L. Gabryelski, A. Pedley, K. Eves, R. Tipping, D. Guris, N. Kartsonis, and M. B. Dorr. Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection. *New England Journal of Medicine*, 376(4):305–317, jan 2017. ISSN 15334406. doi: 10.1056/NEJMoa1602615. 334 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Massachussetts Medical Society.

[258] Anahit Penesyan, Ian T. Paulsen, Staffan Kjelleberg, and Michael R. Gillings. Three faces of biofilms: a microbial lifestyle, a nascent multicellular organism, and an incubator for diversity. *NPJ biofilms and microbiomes*, 7(1):80, nov 2021. ISSN 2055-5008. doi: 10.1038/s41522-021-00251-2.

[259] Dennis Claessen, Daniel E. Rozen, Oscar P. Kuipers, Lotte Søgaard-Andersen, and Gilles P. van Wezel. Bacterial solutions to multicellularity: a tale of biofilms, filaments and fruiting bodies. *Nature Reviews. Microbiology*, 12(2):115–124, feb 2014. ISSN 1740-1534. doi: 10.1038/nrmicro3178.

[260] J. A. Shapiro. Thinking about bacterial populations as multicellular organisms. *Annual Review of Microbiology*, 52:81–104, 1998. ISSN 0066-4227. doi: 10.1146/annurev.micro.52.1.81.

[261] Nicholas A. Lyons and Roberto Kolter. On the evolution of bacterial multicellularity. *Current Opinion in Microbiology*, 24:21–28, apr 2015. ISSN 1879-0364. doi: 10.1016/j.mib.2014.12.007.

[262] Luke L. M. Heaton, Nick S. Jones, and Mark D. Fricker. A mechanistic explanation of the transition to simple multicellularity in fungi. *Nature Communications*, 11(1):2594, may 2020. ISSN 2041-1723. doi: 10.1038/s41467-020-16072-4.

[263] Shunsuke Sogabe, William L. Hatleberg, Kevin M. Kocot, Tahsha E. Say, Daniel Stoupin, Kathrein E. Roper, Selene L. Fernandez-Valverde, Sandie M. Degnan, and Bernard M. Degnan. Pluripotency and the origin of animal multicellularity. *Nature*, 570(7762):519–522, jun 2019. ISSN 1476-4687. doi: 10.1038/s41586-019-1290-4.

[264] Thibaut Brunet and Nicole King. The Origin of Animal Multicellularity and Cell Differentiation. *Developmental Cell*, 43(2):124–140, oct 2017. ISSN 1878-1551. doi: 10.1016/j.devcel.2017.09.016.

[265] Emma U. Hammarlund, Emily Flashman, Sofie Mohlin, and Francesco Licausi. Oxygen-sensing mechanisms across eukaryotic kingdoms and their roles in complex multicellularity. *Science (New York, N.Y.)*, 370(6515):eaba3512, oct 2020. ISSN 1095-9203. doi: 10.1126/science.aba3512.

[266] J. W. Costerton, K. J. Cheng, G. G. Geesey, T. I. Ladd, J. C. Nickel, M. Dasgupta, and T. J. Marrie. Bacterial biofilms in nature and disease. *Annual Review of Microbiology*, 41:435–464, 1987. ISSN 0066-4227. doi: 10.1146/annurev.mi.41.100187.002251.

[267] Luanne Hall-Stoodley, J. William Costerton, and Paul Stoodley. Bacterial biofilms: From the natural environment to infectious diseases. *Nature Reviews Microbiology*, 2(2):95–108, feb 2004. ISSN 17401526. doi: 10.1038/nrmicro821. URL http://www.nature.com/articles/nrmicro821. 5673 citations (Semantic Scholar/DOI) [2022-06-29].

[268] Mary Ann Jabra-Rizk, William A. Falkler, and Timothy F. Meiller. Fungal Biofilms and Drug Resistance. *Emerging Infectious Diseases*, 10(1):14–19, jan 2004. ISSN 1080-6040. doi: 10.3201/eid1001.030119. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3031105/.

[269] Gordon Ramage, Ryan Kean, Riina Rautemaa-Richardson, Craig Williams, and Jose L. Lopez-Ribot. Fungal biofilms in human health and disease. *Nature Reviews Microbiology*, 23(6):355–370, jun 2025. ISSN 1740-1534. doi: 10.1038/s41579-025-01147-0. URL https://www.nature.com/articles/s41579-025-01147-0.

[270] Marleen van Wolferen, Alvaro Orell, and Sonja-Verena Albers. Archaeal biofilm formation. *Nature Reviews Microbiology*, 16(11):699–713, nov 2018. ISSN 1740-1534. doi: 10.1038/s41579-018-0058-4. URL https://www.nature.com/articles/s41579-018-0058-4.

[271] Karin Sauer, Paul Stoodley, Darla M. Goeres, Luanne Hall-Stoodley, Mette Burmølle, Philip S. Stewart, and Thomas Bjarnsholt. The biofilm life cycle: expanding the conceptual model of biofilm formation. *Nature Reviews Microbiology*, 20(10):608–620, 10 2022. ISSN 17401534. doi: 10.1038/S41579-022-00767-0,. URL https://pubmed.ncbi.nlm.nih.gov/35922483/.

[272] Masanori Toyofuku, Tomohiro Inaba, Tatsunori Kiyokawa, Nozomu Obana, Yutaka Yawata, and Nobuhiko Nomura. Environmental factors that shape biofilm formation. *Bioscience, Biotechnology, and Biochemistry*, 80(1):7–12, 2016. ISSN 1347-6947. doi: 10.1080/09168451.2015.1058701.

[273] Lars D. Renner and Douglas B. Weibel. Physicochemical regulation of biofilm formation. *MRS bulletin / Materials Research Society*, 36(5):347–355, may 2011. ISSN 0883-7694. doi: 10.1557/mrs.2011.65. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3224470/.

[274] G. O'Toole, H. B. Kaplan, and R. Kolter. Biofilm formation as microbial development. *Annual Review of Microbiology*, 54:49–79, 2000. ISSN 0066-4227. doi: 10.1146/annurev.micro.54.1.49.

[275] Hans-Curt Flemming and Jost Wingender. The biofilm matrix. *Nature Reviews Microbiology*, 8(9):623–633, sep 2010. ISSN 1740-1534. doi: 10.1038/nrmicro2415. URL https://www.nature.com/articles/nrmicro2415. 5616 citations (Crossref) [2022-11-16] Number: 9 Publisher: Nature Publishing Group.

[276] Diane McDougald, Scott A. Rice, Nicolas Barraud, Peter D. Steinberg, and Staffan Kjelleberg. Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. *Nature Reviews. Microbiology*, 10(1):39–50, nov 2011. ISSN 1740-1534. doi: 10.1038/nrmicro2695.

[277] Cyril Guilhen, Christiane Forestier, and Damien Balestrino. Biofilm dispersal: multiple elaborate strategies for dissemination of bacteria with unique properties. *Molecular Microbiology*, 105(2):188–210, jul 2017. ISSN 1365-2958. doi: 10.1111/mmi.13698.

[278] Shaochi Wang, Yanteng Zhao, Alexandra P. Breslawec, Tingting Liang, Zhifen Deng, Laura L. Kuperman, and Qiuning Yu. Strategy to combat biofilms: a focus on biofilm dispersal enzymes. *NPJ Biofilms and Microbiomes*, 9:63, sep 2023. ISSN 2055-5008. doi: 10.1038/s41522-023-00427-y. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10485009/.

[279] Christophe Beloin, Agnès Roux, and Jean-Marc Ghigo. Escherichia coli biofilms. *Current Topics in Microbiology and Immunology*, 322:249–289, 2008. ISSN 0070-217X. doi: 10.1007/978-3-540-75418-3_12. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2864707/.

[280] G. Sharma, S. Sharma, P. Sharma, D. Chandola, S. Dang, S. Gupta, and R. Gabrani. Escherichia coli biofilm: development and therapeutic strategies. *Journal of Applied Microbiology*, 121(2):309–319, aug 2016. ISSN 1365-2672. doi: 10.1111/jam.13078.

[281] Daniel López, Hera Vlamakis, and Roberto Kolter. Biofilms. *Cold Spring Harbor Perspectives in Biology*, 2(7):a000398, jul 2010. ISSN 1943-0264. doi: 10.1101/cshperspect.a000398. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2890205/.

[282] Hera Vlamakis, Yunrong Chai, Pascale Beauregard, Richard Losick, and Roberto Kolter. Sticking together: Building a biofilm the Bacillus subtilis way. *Nature Reviews Microbiology*, 11(3):157–168,

3 2013. ISSN 17401526. doi: 10.1038/NRMICRO2960,. URL https://pubmed.ncbi.nlm.nih.gov/23353768/.

[283] Sofia Arnaouteli, Natalie C. Bamford, Nicola R. Stanley-Wall, and Ákos T. Kovács. Bacillus subtilis biofilm formation and social interactions. *Nature Reviews Microbiology*, 19(9):600–614, 9 2021. ISSN 17401534. doi: 10.1038/S41579-021-00540-9,. URL https://pubmed.ncbi.nlm.nih.gov/33824496/.

[284] Christine A. Arbour, Rupa Nagar, Hannah M. Bernstein, Soumi Ghosh, Yusra Al-Sammarraie, Helge C. Dorfmueller, Michael A.J. Ferguson, Nicola R. Stanley-Wall, and Barbara Imperiali. Defining early steps in Bacillus subtilis biofilm biosynthesis. *mBio*, 14(5), 9 2023. ISSN 21507511. doi: 10.1128/MBIO.00948-23,. URL https://pubmed.ncbi.nlm.nih.gov/37650625/.

[285] Claudia Vuotto, Gianfranco Donelli, Anthony Buckley, and Caroline Chilton. Clostridium difficile Biofilm. *Advances in Experimental Medicine and Biology*, 1050:97–115, 2018. ISSN 0065-2598. doi: 10.1007/978-3-319-72799-8_7.

[286] Charmaine Normington, Ines B. Moura, Jessica A. Bryant, Duncan J. Ewin, Emma V. Clark, Morgan J. Kettle, Hannah C. Harris, William Spittal, Georgina Davis, Matthew R. Henn, Christopher B. Ford, Mark H. Wilcox, and Anthony M. Buckley. Biofilms harbour Clostridioides difficile, serving as a reservoir for recurrent infection. *NPJ biofilms and microbiomes*, 7(1):16, feb 2021. ISSN 2055-5008. doi: 10.1038/s41522-021-00184-w.

[287] Yannick Dn Tremblay and Bruno Dupuy. The blueprint for building a biofilm the Clostridioides difficile way. *Current Opinion in Microbiology*, 66:39–45, apr 2022. ISSN 1879-0364. doi: 10.1016/j.mib.2021.12.002.

[288] Jean-Paul Motta, John L. Wallace, André G. Buret, Céline Deraison, and Nathalie Vergnolle. Gastrointestinal biofilms in health and disease. *Nature Reviews. Gastroenterology & Hepatology*, 18(5):314–334, may 2021. ISSN 1759-5053. doi: 10.1038/s41575-020-00397-y.

[289] Niels Høiby, Thomas Bjarnsholt, Michael Givskov, Søren Molin, and Oana Ciofu. Antibiotic resistance of bacterial biofilms. *International Journal of Antimicrobial Agents*, 35(4):322–332, apr 2010. ISSN 1872-7913. doi: 10.1016/j.ijantimicag.2009.12.011.

[290] P. S. Stewart and J. W. Costerton. Antibiotic resistance of bacteria in biofilms. *Lancet (London, England)*, 358(9276):135–138, jul 2001. ISSN 0140-6736. doi: 10.1016/s0140-6736(01)05321-1.

[291] Victor Choi, Jennifer L. Rohn, Paul Stoodley, Dario Carugo, and Eleanor Stride. Drug delivery strategies for antibiofilm therapy. *Nature Reviews Microbiology*, 21(9):555–572, sep 2023. ISSN 1740-1534. doi: 10.1038/s41579-023-00905-2. URL https://www.nature.com/articles/s41579-023-00905-2.

[292] Ho Yu Liu, Emma L. Prentice, and Mark A. Webber. Mechanisms of antimicrobial resistance in biofilms. *npj Antimicrobials and Resistance*, 2(1):27, oct 2024. ISSN 2731-8745. doi: 10.1038/s44259-024-00046-3. URL https://www.nature.com/articles/s44259-024-00046-3.

[293] Kendra P. Rumbaugh and Marvin Whiteley. Towards improved biofilm models. *Nature Reviews Microbiology*, 23(1), 1 2024. ISSN 17401534. doi: 10.1038/S41579-024-01086-2,. URL https://pubmed.ncbi.nlm.nih.gov/39112554/.

[294] Tanja Đapa, Rosanna Leuzzi, Yen K. Ng, Soza T. Baban, Roberto Adamo, Sarah A. Kuehne, Maria Scarselli, Nigel P. Minton, Davide Serruto, and Meera Unnikrishnan. Multiple factors modulate biofilm formation by the anaerobic pathogen Clostridium difficile. *Journal of Bacteriology*, 195(3): 545–555, feb 2013. ISSN 1098-5530. doi: 10.1128/JB.01980-12.

[295] Garth A. James, Laurent Chesnel, Laura Boegli, Elinor deLancey Pulcini, Steve Fisher, and Philip S. Stewart. Analysis of Clostridium difficile biofilms: imaging and antimicrobial treatment. *The Journal of Antimicrobial Chemotherapy*, 73(1):102–108, jan 2018. ISSN 1460-2091. doi: 10.1093/jac/dkx353.

[296] Harsh Mathur, Mary C. Rea, Paul D. Cotter, Colin Hill, and R. Paul Ross. The efficacy of thuricin CD, tigecycline, vancomycin, teicoplanin, rifampicin and nitazoxanide, independently and in paired combinations against Clostridium difficile biofilms and planktonic cells. *Gut Pathogens*, 8:20, 2016. ISSN 1757-4749. doi: 10.1186/s13099-016-0102-8.

[297] Claudia Vuotto, Ines Moura, Fabrizio Barbanti, Gianfranco Donelli, and Patrizia Spigaglia. Subinhibitory concentrations of metronidazole increase biofilm formation in Clostridium difficile strains. *Pathogens and Disease*, 74(2):ftv114, mar 2016. ISSN 2049-632X. doi: 10.1093/femspd/ftv114.

[298] Grace S. Crowther, Caroline H. Chilton, Sharie L. Todhunter, Scott Nicholson, Jane Freeman, Simon D. Baines, and Mark H. Wilcox. Development and validation of a chemostat gut model to study both planktonic and biofilm modes of growth of Clostridium difficile and human microbiota. *PloS One*, 9(2):e88396, 2014. ISSN 1932-6203. doi: 10.1371/journal.pone.0088396.

[299] P. Setlow. Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals. *Journal of Applied Microbiology*, 101(3):514–525, sep 2006. ISSN 1364-5072. doi: 10.1111/j.1365-2672.2005.02736.x.

[300] Peter Setlow. Spore Resistance Properties. *Microbiology Spectrum*, 2(5), oct 2014. ISSN 2165-0497. doi: 10.1128/microbiolspec.TBS-0003-2012.

[301] J. Errington. Bacillus subtilis sporulation: regulation of gene expression and control of morphogenesis. *Microbiological Reviews*, 57(1):1–33, mar 1993. ISSN 0146-0749. doi: 10.1128/mr.57.1.1-33.1993.

[302] Patrick J. Piggot and David W. Hilbert. Sporulation of Bacillus subtilis. *Current Opinion in Microbiology*, 7(6):579–586, dec 2004. ISSN 1369-5274. doi: 10.1016/j.mib.2004.10.001.

[303] Douglas Higgins and Jonathan Dworkin. Recent progress in Bacillus subtilis sporulation. *FEMS microbiology reviews*, 36(1):131–148, jan 2012. ISSN 1574-6976. doi: 10.1111/j.1574-6976.2011.00310.x.

[304] Daniel Paredes-Sabja, Aimee Shen, and Joseph A. Sorg. Clostridium difficile spore biology: sporulation, germination, and spore structural proteins. *Trends in microbiology*, 22(7):406–416, jul 2014. ISSN 0966-842X. doi: 10.1016/j.tim.2014.04.003. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4098856/.

[305] Aimee Shen. Clostridioides difficile Spore Formation and Germination: New Insights and Opportunities for Intervention. *Annual Review of Microbiology*, 74(Volume 74, 2020):545–566, sep 2020. ISSN 0066-4227, 1545-3251. doi: 10.1146/annurev-micro-011320-011321. URL https://www.annualreviews.org/content/journals/10.1146/annurev-micro-011320-011321.

[306] Cheyenne D Lee, Arshad Rizvi, Adrianne N Edwards, Michael A DiCandia, Germán G Vargas Cuebas, Marcos P Monteiro, and Shonna M McBride. Genetic mechanisms governing sporulation initiation in *Clostridioides difficile*. *Current Opinion in Microbiology*, 66:32–38, apr 2022. ISSN 1369-5274. doi: 10.1016/j.mib.2021.12.001. URL https://www.sciencedirect.com/science/article/pii/S1369527421001764.

[307] Laure Saujet, Marc Monot, Bruno Dupuy, Olga Soutourina, and Isabelle Martin-Verstraete. The Key Sigma Factor of Transition Phase, SigH, Controls Sporulation, Metabolism, and Virulence Factor Expression in Clostridium difficile . *Journal of Bacteriology*, 193(13):3186–3196, jul 2011. ISSN 0021-9193. doi: 10.1128/JB.00272-11. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3133256/.

[308] Kelly A. Fimlaid, Jeffrey P. Bond, Kristin C. Schutz, Emily E. Putnam, Jacqueline M. Leung, Trevor D. Lawley, and Aimee Shen. Global analysis of the sporulation pathway of Clostridium difficile. *PLoS genetics*, 9(8):e1003660, 2013. ISSN 1553-7404. doi: 10.1371/journal.pgen.1003660.

[309] Kelly A. Fimlaid and Aimee Shen. Diverse Mechanisms Regulate Sporulation Sigma Factor Activity in the Firmicutes. *Current opinion in microbiology*, 24:88–95, apr 2015. ISSN 1369-5274. doi: 10.1016/j.mib.2015.01.006. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4380625/.

[310] Aimee Shen, Adrianne N. Edwards, Mahfuzur R. Sarker, and Daniel Paredes-Sabja. Sporulation and Germination in Clostridial Pathogens. *Microbiology Spectrum*, 7(6): 10.1128/microbiolspec.gpp3–0017–2018, nov 2019. ISSN 2165-0497. doi: 10.1128/microbiolspec.GPP3-0017-2018. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6927485/.

[311] M.C. Abt, P.T. McKenney, and E.G. Pamer. Clostridium difficile colitis: Pathogenesis and host defence. *Nature Reviews Microbiology*, 14(10):609–620, 2016. doi: 10.1038/nrmicro.2016.108.

[312] C. GRAM. Ueber die isolirte Farbung der Schizomyceten in Schnitt-und Trockenpraparaten. *Fortschritte der Medicin*, 2:185–189, 1884. URL https://cir.nii.ac.jp/crid/1572261549731981952.

[313] Nishant Tripathi, Muhammad Zubair, and Amit Sapra. Gram Staining. In *StatPearls*. StatPearls Publishing, Treasure Island (FL), 2025. URL http://www.ncbi.nlm.nih.gov/books/NBK562156/.

[314] Matthew K. Schnizlein and Vincent B. Young. Capturing the environment of the Clostridioides difficile infection cycle. *Nature Reviews Gastroenterology & Hepatology 2022*, pages 1–13, 4 2022. ISSN 1759-5053. doi: 10.1038/s41575-022-00610-0. URL https://www.nature.com/articles/s41575-022-00610-0.

[315] Joseph A. Sorg and Abraham L. Sonenshein. Bile salts and glycine as cogerminants for Clostridium difficile spores. *Journal of Bacteriology*, 190(7):2505–2512, apr 2008. ISSN 1098-5530. doi: 10.1128/JB.01765-07.

[316] Joseph A. Sorg and Abraham L. Sonenshein. Chenodeoxycholate is an inhibitor of Clostridium difficile spore germination. *Journal of Bacteriology*, 191(3):1115–1117, feb 2009. ISSN 1098-5530. doi: 10.1128/JB.01260-08.

[317] Michael B. Francis, Charlotte A. Allen, Ritu Shrestha, and Joseph A. Sorg. Bile acid recognition by the Clostridium difficile germinant receptor, CspC, is important for establishing infection. *PLoS pathogens*, 9(5):e1003356, may 2013. ISSN 1553-7374. doi: 10.1371/journal.ppat.1003356.

[318] Yuzo Kevorkian and Aimee Shen. Revisiting the Role of Csp Family Proteins in Regulating Clostridium difficile Spore Germination. *Journal of Bacteriology*, 199(22):e00266–17, oct 2017. ISSN 0021-9193. doi: 10.1128/JB.00266-17. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5648855/.

[319] Marko Baloh, Hailee N. Nerber, and Joseph A. Sorg. Imaging clostridioides difficile spore germination and germination proteins. *Journal of Bacteriology*, 204(7), July 2022. ISSN 1098-5530. doi: 10.1128/jb.00210-22. URL http://dx.doi.org/10.1128/jb.00210-22.

[320] Yuzo Kevorkian, David J. Shirley, and Aimee Shen. Regulation of Clostridium difficile spore germination by the CspA pseudoprotease domain. *Biochimie*, 122:243–254, mar 2016. ISSN 0300-9084. doi: 10.1016/j.biochi.2015.07.023. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4732931/.

[321] Ritu Shrestha, Alicia M. Cochran, and Joseph A. Sorg. The requirement for co-germinants during Clostridium difficile spore germination is influenced by mutations in yabG and cspA. *PLoS Pathogens*, 15(4):e1007681, apr 2019. ISSN 1553-7366. doi: 10.1371/journal.ppat.1007681. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6464247/.

[322] Chloe M. Adams, Brian E. Eckenroth, Emily E. Putnam, Sylvie Doublié, and Aimee Shen. Structural and functional analysis of the CspB protease required for Clostridium spore germination. *PLoS pathogens*, 9(2):e1003165, feb 2013. ISSN 1553-7374. doi: 10.1371/journal.ppat.1003165.

[323] D. van der Waaij, J. M. Berghuis-de Vries, and null Lekkerkerk Lekkerkerk-v. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *The Journal of Hygiene*, 69 (3):405–411, sep 1971. ISSN 0022-1724. doi: 10.1017/s0022172400021653.

[324] M. Bohnhoff, B. L. Drake, and C. P. Miller. Effect of streptomycin on susceptibility of intestinal tract to experimental Salmonella infection. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)*, 86(1):132–137, may 1954. ISSN 0037-9727. doi: 10.3181/00379727-86-21030.

[325] M. Bohnhoff, C. P. Miller, and W. R. Martin. RESISTANCE OF THE MOUSE'S INTESTINAL TRACT TO EXPERIMENTAL SALMONELLA INFECTION. II. FACTORS RESPONSIBLE FOR ITS LOSS FOLLOWING STREPTOMYCIN TREATMENT. *The Journal of Experimental Medicine*, 120(5):817–828, nov 1964. ISSN 0022-1007. doi: 10.1084/jem.120.5.817.

[326] E. J. Vollaard and H. A. Clasener. Colonization resistance. *Antimicrobial Agents and Chemotherapy*, 38(3):409–414, mar 1994. ISSN 0066-4804. doi: 10.1128/AAC.38.3.409.

[327] Charlie G. Buffie and Eric G. Pamer. Microbiota-mediated colonization resistance against intestinal pathogens. *Nature Reviews. Immunology*, 13(11):790–801, nov 2013. ISSN 1474-1741. doi: 10.1038/nri3535.

[328] Trevor D. Lawley and Alan W. Walker. Intestinal colonization resistance. *Immunology*, 138(1):1–11, jan 2013. ISSN 1365-2567. doi: 10.1111/j.1365-2567.2012.03616.x.

[329] Gustavo Caballero-Flores, Joseph M. Pickard, and Gabriel Núñez. Microbiota-mediated colonization resistance: mechanisms and regulation. *Nature Reviews. Microbiology*, 21(6):347–360, jun 2023. ISSN 1740-1534. doi: 10.1038/s41579-022-00833-7.

[330] Simon Woelfel, Marta Salvado Silva, and Bärbel Stecher. Intestinal colonization resistance in the context of environmental, host, and microbial determinants. *Cell Host & Microbe*, 32(6):820–836, jun 2024. ISSN 1934-6069. doi: 10.1016/j.chom.2024.05.002.

[331] Sebastian E. Winter, Parameth Thiennimitr, Maria G. Winter, Brian P. Butler, Douglas L. Huseby, Robert W. Crawford, Joseph M. Russell, Charles L. Bevins, L. Garry Adams, Renée M. Tsolis, John R. Roth, and Andreas J. Bäumler. Gut inflammation provides a respiratory electron acceptor for Salmonella. *Nature*, 467(7314):426–429, sep 2010. ISSN 1476-4687. doi: 10.1038/nature09415.

[332] Parameth Thiennimitr, Sebastian E. Winter, Maria G. Winter, Mariana N. Xavier, Vladimir Tolstikov, Douglas L. Huseby, Torsten Sterzenbach, Renée M. Tsolis, John R. Roth, and Andreas J. Bäumler. Intestinal inflammation allows Salmonella to use ethanolamine to compete with the microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 108 (42):17480–17485, oct 2011. ISSN 1091-6490. doi: 10.1073/pnas.1107857108.

[333] Fabian Rivera-Chávez, Lillian F. Zhang, Franziska Faber, Christopher A. Lopez, Mariana X. Byndloss, Erin E. Olsan, Gege Xu, Eric M. Velazquez, Carlito B. Lebrilla, Sebastian E. Winter, and Andreas J. Bäumler. Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. *Cell Host & Microbe*, 19(4):443–454, apr 2016. ISSN 1934-6069. doi: 10.1016/j.chom.2016.03.004.

[334] Shoji Yamazaki, Hiroshi Kamimura, Hiroshi Momose, Takuji Kawashima, and Katsumoto Ueda. Protective Effect of Bifidobacterium-Monoassociation against Lethal Activity of Escherichia coli. *Bifidobacteria and Microflora*, 1(1):55–59, 1982. doi: 10.12938/bifidus1982.1.1_55.

[335] Shinji Fukuda, Hidehiro Toh, Koji Hase, Kenshiro Oshima, Yumiko Nakanishi, Kazutoshi Yoshimura, Toru Tobe, Julie M. Clarke, David L. Topping, Tohru Suzuki, Todd D. Taylor, Kikuji Itoh, Jun Kikuchi, Hidetoshi Morita, Masahira Hattori, and Hiroshi Ohno. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*, 469(7331):543–547, jan 2011. ISSN 1476-4687. doi: 10.1038/nature09646.

[336] Amanda Jacobson, Lilian Lam, Manohary Rajendram, Fiona Tamburini, Jared Honeycutt, Trung Pham, Will Van Treuren, Kali Pruss, Stephen Russell Stabler, Kyler Lugo, Donna M. Bouley, Jose G. Vilches-Moure, Mark Smith, Justin L. Sonnenburg, Ami S. Bhatt, Kerwyn Casey Huang, and Denise Monack. A Gut Commensal-Produced Metabolite Mediates Colonization Resistance to

Salmonella Infection. *Cell Host & Microbe*, 24(2):296–307.e7, aug 2018. ISSN 1934-6069. doi: 10.1016/j.chom.2018.07.002.

[337] Catherine D. Shelton, Woongjae Yoo, Nicolas G. Shealy, Teresa P. Torres, Jacob K. Zieba, M. Wade Calcutt, Nora J. Foegeding, Dajeong Kim, Jinshil Kim, Sangryeol Ryu, and Mariana X. Byndloss. Salmonella enterica serovar Typhimurium uses anaerobic respiration to overcome propionate-mediated colonization resistance. *Cell Reports*, 38(1):110180, jan 2022. ISSN 2211-1247. doi: 10.1016/j.celrep.2021.110180.

[338] Joshua R. Fletcher, Colleen M. Pike, Ruth J. Parsons, Alissa J. Rivera, Matthew H. Foley, Michael R. McLaren, Stephanie A. Montgomery, and Casey M. Theriot. Clostridioides difficile exploits toxin-mediated inflammation to alter the host nutritional landscape and exclude competitors from the gut microbiota. *Nature Communications*, 12(1):462, jan 2021. ISSN 2041-1723. doi: 10.1038/s41467-020-20746-4.

[339] Kali M. Pruss and Justin L. Sonnenburg. C. difficile exploits a host metabolite produced during toxin-mediated disease. *Nature*, 593(7858):261–265, may 2021. ISSN 1476-4687. doi: 10.1038/s41586-021-03502-6.

[340] Kali M. Pruss, Fatima Enam, Eric Battaglioli, Mary DeFeo, Oscar R. Diaz, Steven K. Higginbottom, Curt R. Fischer, Andrew J. Hryckowian, William Van Treuren, Dylan Dodd, Purna Kashyap, and Justin L. Sonnenburg. Oxidative ornithine metabolism supports non-inflammatory C. difficile colonization. *Nature Metabolism*, 4(1):19–28, jan 2022. ISSN 2522-5812. doi: 10.1038/s42255-021-00506-4.

[341] Alexander B. Smith, Matthew L. Jenior, Orlaith Keenan, Jessica L. Hart, Jonathan Specker, Arwa Abbas, Paula C. Rangel, Chao Di, Jamal Green, Katelyn A. Bustin, Jennifer A. Gaddy, Maribeth R. Nicholson, Clare Laut, Brendan J. Kelly, Megan L. Matthews, Daniel R. Evans, Daria Van Tyne, Emma E. Furth, Jason A. Papin, Frederic D. Bushman, Jessi Erlichman, Robert N. Baldassano, Michael A. Silverman, Gary M. Dunny, Boone M. Prentice, Eric P. Skaar, and Joseph P. Zackular. Enterococci enhance Clostridioides difficile pathogenesis. *Nature*, 611(7937):780–786, nov 2022. ISSN 1476-4687. doi: 10.1038/s41586-022-05438-x.

[342] Reece J. Knippel, Aaron G. Wexler, Jeanette M. Miller, William N. Beavers, Andy Weiss, Valérie de Crécy-Lagard, Katherine A. Edmonds, David P. Giedroc, and Eric P. Skaar. Clostridioides difficile Senses and Hijacks Host Heme for Incorporation into an Oxidative Stress Defense System. *Cell Host & Microbe*, 28(3):411–421.e6, sep 2020. ISSN 1931-3128. doi: 10.1016/j.chom.2020.05.015. URL https://www.cell.com/cell-host-microbe/abstract/S1931-3128(20)30297-3.

[343] Alexander B. Smith, Jonathan T. Specker, Katharine K. Hewlett, Troy R. Scoggins, Montana Knight, Abigail M. Lustig, Yanhong Li, Kirsten M. Evans, Yingchan Guo, Qianxuan She, Michael W. Christopher, Timothy J. Garrett, Ahmed M. Moustafa, Daria Van Tyne, Boone M. Prentice, and Joseph P. Zackular. Liberation of host heme by Clostridioides difficile-mediated damage enhances Enterococcus faecalis fitness during infection. *mBio*, 15(1):e0165623, jan 2024. ISSN 2150-7511. doi: 10.1128/mbio.01656-23.

[344] Frances Spragge, Erik Bakkeren, Martin T. Jahn, Elizete B. N. Araujo, Claire F. Pearson, Xuedan Wang, Louise Pankhurst, Olivier Cunrath, and Kevin R. Foster. Microbiome diversity protects

against pathogens by nutrient blocking. *Science*, dec 2023. doi: 10.1126/science.adj3502. URL https://www.science.org/doi/10.1126/science.adj3502.

[345] Reiner Jumpertz von Schwartzenberg, Jordan E. Bisanz, Svetlana Lyalina, Peter Spanogiannopoulos, Qi Yan Ang, Jingwei Cai, Sophia Dickmann, Marie Friedrich, Su-Yang Liu, Stephanie L. Collins, Danielle Ingebrigtsen, Steve Miller, Jessie A. Turnbaugh, Andrew D. Patterson, Katherine S. Pollard, Knut Mai, Joachim Spranger, and Peter J. Turnbaugh. Caloric restriction disrupts the microbiota and colonization resistance. *Nature*, 595(7866):272–277, jul 2021. ISSN 1476-4687. doi: 10.1038/s41586-021-03663-4.

[346] K. Leigh Greathouse and Abigail J. Johnson. Does caloric restriction prime the microbiome for pathogenic bacteria? *Cell Host & Microbe*, 29(8):1209–1211, aug 2021. ISSN 1934-6069. doi: 10.1016/j.chom.2021.07.009.

[347] Arnau Vich Vila, Valerie Collij, Serena Sanna, Trishla Sinha, Floris Imhann, Arno R. Bourgonje, Zlatan Mujagic, Daisy M.A.E. A. E. Jonkers, Ad A.M. M. Masclee, Jingyuan Fu, Alexander Kurilshikov, Cisca Wijmenga, Alexandra Zhernakova, Rinse K. Weersma, and A Vich Vila. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nature Communications*, 11(1):1–11, dec 2020. ISSN 20411723. doi: 10.1038/s41467-019-14177-z. URL http://www.nature.com/articles/s41467-019-14177-zhttps://doi.org/10.1038/s41467-019-14177-zhttps://www.nature.com/articles/s41467-019-14177-z. 464 citations (Crossref) [2025-02-20] 235 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Research.

[348] Eric G. Pamer. Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. *Science (New York, N.Y.)*, 352(6285):535–538, apr 2016. ISSN 1095-9203. doi: 10.1126/science.aad9382.

[349] Matthew T. Sorbara and Eric G. Pamer. Microbiome-based therapeutics, 1 2022. ISSN 1740-1526. URL https://www.nature.com/articles/s41579-021-00667-9.

[350] Cody Cole, Zhenrun Zhang, Shravan Dommaraju, Qiwen Dong, Rosemary Pope, Sophie Son, Emma McSpadden, Che Woodson, Huaiying Lin, Nicholas Dylla, Ashley Sidebottom, Anitha Sundararajan, Douglas Mitchell, and Eric Pamer. Lantibiotic-producing bacteria impact microbiome resilience and colonization resistanc, 2025. URL https://doi.org/10.1101/2025.05.06.652565.

[351] Simon Heilbronner, Bernhard Krismer, Heike Brötz-Oesterhelt, and Andreas Peschel. The microbiome-shaping roles of bacteriocins. *Nature Reviews. Microbiology*, 19(11):726–739, nov 2021. ISSN 1740-1534. doi: 10.1038/s41579-021-00569-w.

[352] Pascal Hols, Laura Ledesma-García, Philippe Gabant, and Johann Mignolet. Mobilization of Microbiota Commensals and Their Bacteriocins for Therapeutics. *Trends in Microbiology*, 27(8):690–702, aug 2019. ISSN 1878-4380. doi: 10.1016/j.tim.2019.03.007.

[353] Yue Ma, Zhengyan Guo, Binbin Xia, Yuwei Zhang, Xiaolin Liu, Ying Yu, Na Tang, Xiaomei Tong, Min Wang, Xin Ye, Jie Feng, Yihua Chen, and Jun Wang. Identification of antimicrobial peptides from the human gut microbiome using deep learning. *Nature Biotechnology*, 40(6):921–931, jun 2022. ISSN 1546-1696. doi: 10.1038/s41587-022-01226-0.

[354] Pipat Piewngam, Yue Zheng, Thuan H. Nguyen, Seth W. Dickey, Hwang-Soo Joo, Amer E. Villaruz, Kyle A. Glose, Emilie L. Fisher, Rachelle L. Hunt, Barry Li, Janice Chiou, Sujiraphong Pharkjaksu, Sunisa Khongthong, Gordon Y. C. Cheung, Pattarachai Kiratisin, and Michael Otto. Pathogen elimination by probiotic Bacillus via signalling interference. *Nature*, 562(7728):532–537, oct 2018. ISSN 1476-4687. doi: 10.1038/s41586-018-0616-y.

[355] Pipat Piewngam, Sunisa Khongthong, Natthrit Roekngam, Yongyuth Theapparat, Somkiat Sunpaweravong, Damrongsak Faroongsarng, and Michael Otto. Probiotic for pathogen-specific Staphylococcus aureus decolonisation in Thailand: a phase 2, double-blind, randomised, placebo-controlled trial. *The Lancet. Microbe*, 4(2):e75–e83, feb 2023. ISSN 2666-5247. doi: 10.1016/S2666-5247(22)00322-6.

[356] Silvia Caballero, Sohn Kim, Rebecca A. Carter, Ingrid M. Leiner, Bože Sušac, Liza Miller, Grace J. Kim, Lilan Ling, and Eric G. Pamer. Cooperating Commensals Restore Colonization Resistance to Vancomycin-Resistant Enterococcus faecium. *Cell Host & Microbe*, 21(5):592–602.e4, may 2017. ISSN 1934-6069. doi: 10.1016/j.chom.2017.04.002.

[357] Sohn G. Kim, Simone Becattini, Thomas U. Moody, Pavel V. Shliaha, Eric R. Littmann, Ruth Seok, Mergim Gjonbalaj, Vincent Eaton, Emily Fontana, Luigi Amoretti, Roberta Wright, Silvia Caballero, Zhong-Min X. Wang, Hea-Jin Jung, Sejal M. Morjaria, Ingrid M. Leiner, Weige Qin, Ruben J. J. F. Ramos, Justin R. Cross, Seiko Narushima, Kenya Honda, Jonathan U. Peled, Ronald C. Hendrickson, Ying Taur, Marcel R. M. van den Brink, and Eric G. Pamer. Microbiota-derived lantibiotic restores resistance against vancomycin-resistant Enterococcus. *Nature*, 572(7771):665–669, aug 2019. ISSN 1476-4687. doi: 10.1038/s41586-019-1501-z.

[358] Mariana X. Byndloss, Erin E. Olsan, Fabian Rivera-Chávez, Connor R. Tiffany, Stephanie A. Cevallos, Kristen L. Lokken, Teresa P. Torres, Austin J. Byndloss, Franziska Faber, Yandong Gao, Yael Litvak, Christopher A. Lopez, Gege Xu, Eleonora Napoli, Cecilia Giulivi, Renée M. Tsolis, Alexander Revzin, Carlito B. Lebrilla, and Andreas J. Bäumler. Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science (New York, N.Y.)*, 357(6351): 570–575, aug 2017. ISSN 1095-9203. doi: 10.1126/science.aam9949.

[359] Les Dethlefsen and David A. Relman. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proceedings of the National Academy of Sciences of the United States of America*, 108 Suppl 1(Suppl 1):4554–4561, mar 2011. ISSN 1091-6490. doi: 10.1073/pnas.1000087107.

[360] Daniel E. Freedberg, Nora C. Toussaint, Sway P. Chen, Adam J. Ratner, Susan Whittier, Timothy C. Wang, Harris H. Wang, and Julian A. Abrams. Proton Pump Inhibitors Alter Specific Taxa in the Human Gastrointestinal Microbiome: A Crossover Trial. *Gastroenterology*, 149(4):883–885.e9, oct 2015. ISSN 1528-0012. doi: 10.1053/j.gastro.2015.06.043.

[361] Ayumi Tsuda, Wataru Suda, Hidetoshi Morita, Kageyasu Takanashi, Atsushi Takagi, Yasuhiro Koga, and Masahira Hattori. Influence of Proton-Pump Inhibitors on the Luminal Microbiota in the Gastrointestinal Tract. *Clinical and Translational Gastroenterology*, 6(6):e89, jun 2015. ISSN 2155-384X. doi: 10.1038/ctg.2015.20.

[362] Floris Imhann, Marc Jan Bonder, Arnau Vich Vila, Jingyuan Fu, Zlatan Mujagic, Lisa Vork, Ettje F. Tigchelaar, Soesma A. Jankipersadsing, Maria Carmen Cenit, Hermie J.M. M Harmsen, Gerard Dijkstra, Lude Franke, Ramnik J. Xavier, Daisy Jonkers, Cisca Wijmenga, Rinse K. Weersma, Alexandra Zhernakova, Arnau Vich Vila, Jingyuan Fu, Zlatan Mujagic, Lisa Vork, Ettje F. Tigchelaar, Soesma A. Jankipersadsing, Maria Carmen Cenit, Hermie J.M. M Harmsen, Gerard Dijkstra, Lude Franke, Ramnik J. Xavier, Daisy Jonkers, Cisca Wijmenga, Rinse K. Weersma, and Alexandra Zhernakova. Proton pump inhibitors affect the gut microbiome. *Gut*, 65(5):740–8, may 2016. ISSN 0017-5749. doi: 10.1136/gutjnl-2015-310376. URL https://gut.bmj.com/content/65/5/740https://gut.bmj.com/content/65/5/740.abstract/labs/pmc/articles/PMC4853569//labs/pmc/articles/PMC4853569/?report=abstracthttps://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC4853569/http://www.ncbi.nlm.nih.gov/pubmed/. 680 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: BMJ Publishing Group.

[363] Martina Klünemann, Sergej Andrejev, Sonja Blasche, Andre Mateus, Prasad Phapale, Saravanan Devendran, Johanna Vappiani, Bernd Simon, Timothy A. Scott, Eleni Kafkia, Dimitrios Konstantinidis, Katharina Zirngibl, Eleonora Mastrorilli, Manuel Banzhaf, Marie-Therese Therese Mackmull, Felix Hövelmann, Leo Nesme, Ana Rita Brochado, Lisa Maier, Thomas Bock, Vinita Periwal, Manjeet Kumar, Yongkyu Kim, Melanie Tramontano, Carsten Schultz, Martin Beck, Janosch Hennig, Michael Zimmermann, Daniel C. Sévin, Filipe Cabreiro, Mikhail M. Savitski, Peer Bork, Athanasios Typas, and Kiran R. Patil. Bioaccumulation of therapeutic drugs by human gut bacteria. *Nature*, 597(7877):533–538, sep 2021. ISSN 14764687. doi: 10.1038/s41586-021-03891-8. URL https://www.nature.com/articles/s41586-021-03891-8. 183 citations (Crossref) [2025-02-20] 47 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[364] Andrew A. Verdegaal and Andrew L. Goodman. Integrating the gut microbiome and pharmacology. *Science Translational Medicine*, 16(732):eadg8357, jan 2024. ISSN 1946-6242. doi: 10.1126/scitranslmed.adg8357.

[365] Charlie G. Buffie, Irene Jarchum, Michele Equinda, Lauren Lipuma, Asia Gobourne, Agnes Viale, Carles Ubeda, Joao Xavier, and Eric G. Pamer. Profound Alterations of Intestinal Microbiota following a Single Dose of Clindamycin Results in Sustained Susceptibility to Clostridium difficile-Induced Colitis. *Infection and Immunity*, 80(1):62, jan 2012. ISSN 00199567. doi: 10.1128/IAI.05496-11. URL /pmc/articles/PMC3255689//pmc/articles/PMC3255689/?report=abstracthttps://www.ncbi.nlm.nih.gov/pmc/articles/PMC3255689/. 441 citations (Semantic Scholar/DOI) [2022-06-23] Publisher: American Society for Microbiology (ASM).

[366] Casey M. Theriot, Mark J. Koenigsknecht, Paul E. Carlson, Gabrielle E. Hatton, Adam M. Nelson, Bo Li, Gary B. Huffnagle, Jun Z Li, and Vincent B. Young. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. *Nature Communications*, 5:3114, 2014. ISSN 2041-1723. doi: 10.1038/ncomms4114.

[367] Kristen A. Muñoz, Rebecca J. Ulrich, Archit K. Vasan, Matt Sinclair, Po-Chao Wen, Jessica R. Holmes, Hyang Yeon Lee, Chien-Che Hung, Christopher J. Fields, Emad Tajkhorshid, Gee W. Lau, and Paul J. Hergenrother. A Gram-negative-selective antibiotic that spares the gut microbiome. *Nature*, 630(8016):429–436, jun 2024. ISSN 1476-4687. doi: 10.1038/s41586-024-07502-0.

[368] Xinyun Cao, Hande Boyaci, James Chen, Yu Bao, Robert Landick, and Elizabeth A. Campbell. Basis of narrow-spectrum activity of fidaxomicin on Clostridioides difficile. *Nature*, 604(7906): 541–545, apr 2022. ISSN 1476-4687. doi: 10.1038/s41586-022-04545-z.

[369] Zain Kassam, Christine H. Lee, Yuhong Yuan, and Richard H. Hunt. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. *The American Journal of Gastroenterology*, 108(4):500–508, apr 2013. ISSN 1572-0241. doi: 10.1038/ajg.2013.59.

[370] Colleen R. Kelly, Alexander Khoruts, Christopher Staley, Michael J. Sadowsky, Mortadha Abd, Mustafa Alani, Brianna Bakow, Patrizia Curran, Joyce McKenney, Allison Tisch, Steven E. Reinert, Jason T. Machan, and Lawrence J. Brandt. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. *Annals of Internal Medicine*, 165(9):609–616, nov 2016. ISSN 1539-3704. doi: 10.7326/M16-0271.

[371] Christian Lodberg Hvas, Simon Mark Dahl Jørgensen, Søren Peter Jørgensen, Merete Storgaard, Lars Lemming, Mette Mejlby Hansen, Christian Erikstrup, and Jens Frederik Dahlerup. Fecal Microbiota Transplantation Is Superior to Fidaxomicin for Treatment of Recurrent Clostridium difficile Infection. *Gastroenterology*, 156(5):1324–1332.e3, apr 2019. ISSN 1528-0012. doi: 10.1053/j.gastro.2018.12.019.

[372] Zachariah DeFilipp, Patricia P. Bloom, Mariam Torres Soto, Michael K. Mansour, Mohamad R. A. Sater, Miriam H. Huntley, Sarah Turbett, Raymond T. Chung, Yi-Bin Chen, and Elizabeth L. Hohmann. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. *The New England Journal of Medicine*, 381(21):2043–2050, nov 2019. ISSN 1533-4406. doi: 10.1056/NEJMoa1910437.

[373] Jotham Suez, Niv Zmora, Eran Segal, and Eran Elinav. The pros, cons, and many unknowns of probiotics. *Nature Medicine*, 25(5):716–729, may 2019. ISSN 1546-170X. doi: 10.1038/s41591-019-0439-x. URL https://www.nature.com/articles/s41591-019-0439-x. 869 citations (Crossref) [2025-02-20] 397 citations (Semantic Scholar/DOI) [2022-09-12] Number: 5 Publisher: Nature Publishing Group.

[374] Aloysius L. D'Souza, Chakravarthi Rajkumar, Jonathan Cooke, and Christopher J. Bulpitt. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. *BMJ*, 324(7350):1361, jun 2002. ISSN 0959-8138, 1468-5833. doi: 10.1136/bmj.324.7350.1361. URL https://www.bmj.com/content/324/7350/1361.

[375] Susanne Hempel, Sydne J. Newberry, Alicia R. Maher, Zhen Wang, Jeremy N. V. Miles, Roberta Shanman, Breanne Johnsen, and Paul G. Shekelle. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA*, 307(18):1959–1969, may 2012. ISSN 1538-3598. doi: 10.1001/jama.2012.3507.

[376] Niv Zmora, Gili Zilberman-Schapira, Jotham Suez, Uria Mor, Mally Dori-Bachash, Stavros Bashiardes, Eran Kotler, Maya Zur, Dana Regev-Lehavi, Rotem Ben Zeev Brik, Sara Federici, Yotam Cohen, Raquel Linevsky, Daphna Rothschild, Andreas E. Moor, Shani Ben-Moshe, Alon Harmelin, Shalev Itzkovitz, Nitsan Maharshak, Oren Shibolet, Hagit Shapiro, Meirav Pevsner-Fischer, Itai Sharon, Zamir Halpern, Eran Segal, and Eran Elinav. Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Fea-

tures. *Cell*, 174(6):1388–1405.e21, sep 2018. ISSN 10974172. doi: 10.1016/j.cell.2018.08.041. 703 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Cell Press.

[377] Melissa Dsouza, Rajita Menon, Emily Crossette, Shakti K. Bhattarai, Jessica Schneider, Yun-Gi Kim, Shilpa Reddy, Silvia Caballero, Cintia Felix, Louis Cornacchione, Jared Hendrickson, Andrea R. Watson, Samuel S. Minot, Nick Greenfield, Lisa Schopf, Rose Szabady, Juan Patarroyo, William Smith, Pratibha Harrison, Ed J. Kuijper, Ciaran P. Kelly, Bernat Olle, Dmitri Bobilev, Jeffrey L. Silber, Vanni Bucci, Bruce Roberts, Jeremiah Faith, and Jason M. Norman. Colonization of the live biotherapeutic product VE303 and modulation of the microbiota and metabolites in healthy volunteers. *Cell Host & Microbe*, 30(4):583–598.e8, apr 2022. ISSN 1934-6069. doi: 10.1016/j.chom.2022.03.016.

[378] Thomas Louie, Yoav Golan, Sahil Khanna, Dmitri Bobilev, Nathalie Erpelding, Candida Fratazzi, Meg Carini, Rajita Menon, Mary Ruisi, Jason M. Norman, Jeremiah J. Faith, Bernat Olle, Minran Li, Jeffrey L. Silber, and Darrell S. Pardi. VE303, a Defined Bacterial Consortium, for Prevention of Recurrent Clostridioides difficile Infection: A Randomized Clinical Trial. *JAMA*, 329(16):1356–1366, apr 2023. ISSN 1538-3598. doi: 10.1001/jama.2023.4314.

[379] Rajita Menon, Shakti K. Bhattarai, Emily Crossette, Amanda L. Prince, Bernat Olle, Jeffrey L. Silber, Vanni Bucci, Jeremiah Faith, and Jason M. Norman. Multi-omic profiling a defined bacterial consortium for treatment of recurrent Clostridioides difficile infection. *Nature Medicine*, 31(1):223–234, jan 2025. ISSN 1546-170X. doi: 10.1038/s41591-024-03337-4.

[380] R. D. Rolfe. Role of volatile fatty acids in colonization resistance to Clostridium difficile. *Infection and Immunity*, 45(1):185–191, jul 1984. ISSN 0019-9567. doi: 10.1128/iai.45.1.185-191.1984.

[381] Robert A. Britton and Vincent B. Young. Role of the intestinal microbiota in resistance to colonization by Clostridium difficile. *Gastroenterology*, 146(6):1547–1553, may 2014. ISSN 1528-0012. doi: 10.1053/j.gastro.2014.01.059.

[382] Casey M. Theriot and Vincent B. Young. Interactions Between the Gastrointestinal Microbiome and Clostridium difficile. *Annual Review of Microbiology*, 69:445–461, 2015. ISSN 1545-3251. doi: 10.1146/annurev-micro-091014-104115.

[383] Monique J. T. Crobach, Jonathan J. Vernon, Vivian G. Loo, Ling Yuan Kong, Séverine Péchiné, Mark H. Wilcox, and Ed J. Kuijper. Understanding Clostridium difficile Colonization. *Clinical Microbiology Reviews*, 31(2):e00021–17, apr 2018. ISSN 1098-6618. doi: 10.1128/CMR.00021-17.

[384] Anna Maria Seekatz, Nasia Safdar, and Sahil Khanna. The role of the gut microbiome in colonization resistance and recurrent Clostridioides difficile infection. *Therapeutic Advances in Gastroenterology*, 15:17562848221134396, 2022. ISSN 1756-283X. doi: 10.1177/17562848221134396.

[385] Brittany B. Lewis, Charlie G. Buffie, Rebecca A. Carter, Ingrid Leiner, Nora C. Toussaint, Liza C. Miller, Asia Gobourne, Lilan Ling, and Eric G. Pamer. Loss of Microbiota-Mediated Colonization Resistance to Clostridium difficile Infection With Oral Vancomycin Compared With Metronidazole. *The Journal of Infectious Diseases*, 212(10):1656–1665, nov 2015. ISSN 1537-6613. doi: 10.1093/infdis/jiv256.

[386] Amir Zarrinpar, Amandine Chaix, Zhenjiang Z. Xu, Max W. Chang, Clarisse A. Marotz, Alan Saghatelian, Rob Knight, and Satchidananda Panda. Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. *Nature Communications*, 9(1):2872, jul 2018. ISSN 2041-1723. doi: 10.1038/s41467-018-05336-9.

[387] Robert A. Quinn, Alexey V. Melnik, Alison Vrbanac, Ting Fu, Kathryn A. Patras, Mitchell Christy, Zsolt Bodai, Pedro Belda-Ferre, Anupriya Tripathi, Lawton K. Chung, Michael Downes, Ryan D. Welch, Melissa Quinn, Greg Humphrey, Morgan Panitchpakdi, Kelly Weldon, Alexander Aksenov, Ricardo da Silva, Julian Avila-Pacheco, Clary Clish, Sena Bae, Himel Mallick, Eric A. Franzosa, Jason Lloyd-Price, Robert Bussell, Taren Thron, Andrew T. Nelson, Mingxun Wang, Eric Leszczynski, Fernando Vargas, Julia M. Gauglitz, Michael J. Meehan, Emily Gentry, Timothy D. Arthur, Alexis C. Komor, Orit Poulsen, Brigid S. Boland, John T. Chang, William J. Sandborn, Meerana Lim, Neha Garg, Julie C. Lumeng, Ramnik J. Xavier, Barbara I. Kazmierczak, Ruchi Jain, Marie Egan, Kyung E. Rhee, David Ferguson, Manuela Raffatellu, Hera Vlamakis, Gabriel G. Haddad, Dionicio Siegel, Curtis Huttenhower, Sarkis K. Mazmanian, Ronald M. Evans, Victor Nizet, Rob Knight, and Pieter C. Dorrestein. Global Chemical Impact of the Microbiome Includes Novel Bile Acid Conjugations. *Nature*, 579(7797):123–129, mar 2020. ISSN 0028-0836. doi: 10.1038/s41586-020-2047-9. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7252668/.

[388] Stephanie L. Collins, Jonathan G. Stine, Jordan E. Bisanz, C. Denise Okafor, and Andrew D. Patterson. Bile acids and the gut microbiota: metabolic interactions and impacts on disease. *Nature Reviews. Microbiology*, 21(4):236–247, apr 2023. ISSN 1740-1534. doi: 10.1038/s41579-022-00805-x.

[389] Ipsita Mohanty, Celeste Allaband, Helena Mannochio-Russo, Yasin El Abiead, Lee R. Hagey, Rob Knight, and Pieter C. Dorrestein. The changing metabolic landscape of bile acids - keys to metabolism and immune regulation. *Nature Reviews. Gastroenterology & Hepatology*, 21(7):493–516, jul 2024. ISSN 1759-5053. doi: 10.1038/s41575-024-00914-3.

[390] Shakti K. Bhattarai, Muxue Du, Abigail L. Zeamer, Benedikt M Morzfeld, Tasia D. Kellogg, Kaya Firat, Anna Benjamin, James M. Bean, Matthew Zimmerman, Gertrude Mardi, Stalz Charles Vilbrun, Kathleen F. Walsh, Daniel W. Fitzgerald, Michael S. Glickman, and Vanni Bucci. Commensal antimicrobial resistance mediates microbiome resilience to antibiotic disruption. *Science Translational Medicine*, 16(730):eadi9711, jan 2024. ISSN 1946-6242. doi: 10.1126/scitranslmed.adi9711.

[391] Eitan Yaffe, Les Dethlefsen, Arati V. Patankar, Chen Gui, Susan Holmes, and David A. Relman. Brief antibiotic use drives human gut bacteria towards low-cost resistance. *Nature*, 641(8061):182–191, may 2025. ISSN 0028-0836. doi: 10.1038/s41586-025-08781-x. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC12168174/.

[392] Michael H. Lee, Sean-Paul Nuccio, Ipsita Mohanty, Lee R. Hagey, Pieter C. Dorrestein, Hiutung Chu, and Manuela Raffatellu. How bile acids and the microbiota interact to shape host immunity. *Nature Reviews. Immunology*, 24(11):798–809, nov 2024. ISSN 1474-1741. doi: 10.1038/s41577-024-01057-x.

[393] Matthew H. Foley, Sarah O'Flaherty, Garrison Allen, Alissa J. Rivera, Allison K. Stewart, Rodolphe Barrangou, and Casey M. Theriot. Lactobacillus bile salt hydrolase substrate specificity governs bacterial fitness and host colonization. *Proceedings of the National Academy of*

Sciences of the United States of America, 118(6):e2017709118, feb 2021. ISSN 1091-6490. doi: 10.1073/pnas.2017709118.

[394] Matthew H. Foley, Morgan E. Walker, Allison K. Stewart, Sarah O'Flaherty, Emily C. Gentry, Shakshi Patel, Violet V. Beaty, Garrison Allen, Meichen Pan, Joshua B. Simpson, Caroline Perkins, Molly E. Vanhoy, Michael K. Dougherty, Sarah K. McGill, Ajay S. Gulati, Pieter C. Dorrestein, Erin S. Baker, Matthew R. Redinbo, Rodolphe Barrangou, and Casey M. Theriot. Bile salt hydrolases shape the bile acid landscape and restrict Clostridioides difficile growth in the murine gut. *Nature Microbiology*, 8(4):611–628, apr 2023. ISSN 2058-5276. doi: 10.1038/s41564-023-01337-7.

[395] Bipin Rimal, Stephanie L. Collins, Ceylan E. Tanes, Edson R. Rocha, Megan A. Granda, Sumeet Solanki, Nushrat J. Hoque, Emily C. Gentry, Imhoi Koo, Erin R. Reilly, Fuhua Hao, Devendra Paudel, Vishal Singh, Tingting Yan, Min Soo Kim, Kyle Bittinger, Joseph P. Zackular, Kristopher W. Krausz, Dhimant Desai, Shantu Amin, James P. Coleman, Yatrik M. Shah, Jordan E. Bisanz, Frank J. Gonzalez, John P. Vanden Heuvel, Gary D. Wu, Babette S. Zemel, Pieter C. Dorrestein, Emily E. Weinert, and Andrew D. Patterson. Bile salt hydrolase catalyses formation of amine-conjugated bile acids. *Nature*, 626(8000):859–863, feb 2024. ISSN 1476-4687. doi: 10.1038/s41586-023-06990-w.

[396] Andrea Martinez Aguirre, Adegoke Oyeleye Adegbite, and Joseph A. Sorg. Clostridioides difficile bile salt hydrolase activity has substrate specificity and affects biofilm formation. *npj Biofilms and Microbiomes*, 8(1):94, nov 2022. ISSN 2055-5008. doi: 10.1038/s41522-022-00358-0. URL https://www.nature.com/articles/s41522-022-00358-0.

[397] Ziwei Song, Yuanyuan Cai, Xingzhen Lao, Xue Wang, Xiaoxuan Lin, Yingyun Cui, Praveen Kumar Kalavagunta, Jun Liao, Liang Jin, Jing Shang, and Jing Li. Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes based on worldwide human gut microbiome. *Microbiome*, 7(1):9, jan 2019. ISSN 2049-2618. doi: 10.1186/s40168-019-0628-3.

[398] Kyung Hyun Kim, Dongbin Park, Baolei Jia, Ju Hye Baek, Yoonsoo Hahn, and Che Ok Jeon. Identification and Characterization of Major Bile Acid 7α-Dehydroxylating Bacteria in the Human Gut. *mSystems*, 7(4):e0045522, aug 2022. ISSN 2379-5077. doi: 10.1128/msystems.00455-22.

[399] Angela E. Reeves, Mark J. Koenigsknecht, Ingrid L. Bergin, and Vincent B. Young. Suppression of Clostridium difficile in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family Lachnospiraceae. *Infection and Immunity*, 80(11):3786–3794, nov 2012. ISSN 1098-5522. doi: 10.1128/IAI.00647-12.

[400] C.G. Charlie G. Buffie, Vanni Bucci, Richard R.R. Stein, Peter T. Mckenney, Lilan Ling, Asia Gobourne, Daniel No, Hui Liu, Melissa Kinnebrew, Agnes Viale, Eric Littmann, Marcel R.M. M Van Den Brink, Robert R. Jenq, Ying Taur, Chris Sander, Justin R. Cross, Nora C. Toussaint, Joao B. Xavier, Eric G. Pamer, and et Al. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature*, 517(7533):205–208, jan 2015. ISSN 14764687. doi: 10.1038/nature13828. URL https://www.nature.com/articles/nature13828.pdf. 913 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[401] Casey M. Theriot, Alison A. Bowman, and Vincent B. Young. Antibiotic-Induced Alterations of the Gut Microbiota Alter Secondary Bile Acid Production and Allow for Clostridium difficile Spore

Germination and Outgrowth in the Large Intestine. *mSphere*, 1(1):e00045–15, 2016. ISSN 2379-5042. doi: 10.1128/mSphere.00045-15.

[402] Juan Noriega Tejada, William A. Walters, Yanling Wang, Melissa Kordahi, Benoit Chassaing, Joseph Pickard, Gabriel Nunez, Ruth Ley, and Andrew T. Gewirtz. Prevention and cure of murine C. difficile infection by a Lachnospiraceae strain. *Gut Microbes*, 16(1):2392872, 2024. ISSN 1949-0984. doi: 10.1080/19490976.2024.2392872.

[403] Jenessa A. Winston and Casey M. Theriot. Impact of microbial derived secondary bile acids on colonization resistance against Clostridium difficile in the gastrointestinal tract. *Anaerobe*, 41:44–50, oct 2016. ISSN 1095-8274. doi: 10.1016/j.anaerobe.2016.05.003.

[404] Rajani Thanissery, Jenessa A. Winston, and Casey M. Theriot. Inhibition of spore germination, growth, and toxin activity of clinically relevant C. difficile strains by gut microbiota derived secondary bile acids. *Anaerobe*, 45:86–100, jun 2017. ISSN 1095-8274. doi: 10.1016/j.anaerobe.2017.03.004.

[405] John Tam, Simoun Icho, Evelyn Utama, Kathleen E. Orrell, Rodolfo F. Gómez-Biagi, Casey M. Theriot, Heather K. Kroh, Stacey A. Rutherford, D. Borden Lacy, and Roman A. Melnyk. Intestinal bile acids directly modulate the structure and function of C. difficile TcdB toxin. *Proceedings of the National Academy of Sciences of the United States of America*, 117(12):6792–6800, mar 2020. ISSN 1091-6490. doi: 10.1073/pnas.1916965117.

[406] Simoun Icho, Jennifer S. Ward, John Tam, Larry K. Kociolek, Casey M. Theriot, and Roman A. Melnyk. Intestinal bile acids provide a surmountable barrier against C. difficile TcdB-induced disease pathogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 120(19):e2301252120, may 2023. ISSN 1091-6490. doi: 10.1073/pnas.2301252120.

[407] Samantha C. Kisthardt, Rajani Thanissery, Colleen M. Pike, Matthew H. Foley, and Casey M. Theriot. The microbial-derived bile acid lithocholate and its epimers inhibit Clostridioides difficile growth and pathogenicity while sparing members of the gut microbiota. *Journal of Bacteriology*, 205(9):e0018023, sep 2023. ISSN 1098-5530. doi: 10.1128/jb.00180-23.

[408] Stuart Johnson, Valéry Lavergne, Andrew M Skinner, Anne J Gonzales-Luna, Kevin W Garey, Ciaran P Kelly, and Mark H Wilcox. Clinical Practice Guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA): 2021 Focused Update Guidelines on Management of *Clostridioides difficile* Infection in Adults. *Clinical Infectious Diseases*, 73(5):e1029–e1044, sep 2021. ISSN 1058-4838, 1537-6591. doi: 10.1093/cid/ciab549. URL https://academic.oup.com/cid/article/73/5/e1029/6298219. 324 citations (Crossref) [2025-02-20] 55 citations (Semantic Scholar/DOI) [2022-09-12].

[409] J. E. Smadel and E. B. Jackson. Chloromycetin, an Antibiotic With Chemotherapeutic Activity in Experimental Rickettsial and Viral Infections. *Science (New York, N.Y.)*, 106(2757):418–419, oct 1947. ISSN 0036-8075. doi: 10.1126/science.106.2757.418.

[410] J. Ehrlich, Q. R. Bartz, R. M. Smith, D. A. Joslyn, and P. R. Burkholder. Chloromycetin, a New Antibiotic From a Soil Actinomycete. *Science (New York, N.Y.)*, 106(2757):417, oct 1947. ISSN 0036-8075. doi: 10.1126/science.106.2757.417.

[411] William L. Hewitt and Bryan Williams. Chloromycetin (Chloramphenicol) in the Treatment of Infections. *New England Journal of Medicine*, 242(4):119–127, jan 1950. ISSN 0028-4793. doi: 10.1056/NEJM195001262420402. URL https://www.nejm.org/doi/full/10.1056/NEJM195001262420402.

[412] J. M. McGUIRE, R. L. Bunch, R. C. Anderson, H. E. Boaz, E. H. Flynn, H. M. Powell, and J. W. Smith. Ilotycin, a new antibiotic. *Antibiotics & Chemotherapy (Northfield, Ill.)*, 2(6):281–283, jun 1952. ISSN 0570-3123.

[413] Thomas H. Haight and Maxwell Finland. Laboratory and Clinical Studies on Erythromycin. *New England Journal of Medicine*, 247(7):227–232, aug 1952. ISSN 0028-4793. doi: 10.1056/NEJM195208142470701. URL https://www.nejm.org/doi/full/10.1056/NEJM195208142470701.

[414] M. H. Mccormick, J. M. Mcguire, G. E. Pittenger, R. C. Pittenger, and W. M. Stark. Vancomycin, a new antibiotic. I. Chemical and biologic properties. *Antibiotics Annual*, 3:606–611, 1955. ISSN 0570-3131.

[415] H. R. Perkins. Specificity of combination between mucopeptide precursors and vancomycin or ristocetin. *The Biochemical Journal*, 111(2):195–205, jan 1969. ISSN 0264-6021. doi: 10.1042/bj1110195.

[416] Walter P. Hammes and Francis C. Neuhaus. On the Mechanism of Action of Vancomycin: Inhibition of Peptidoglycan Synthesis in Gaffkya homari. *Antimicrobial Agents and Chemotherapy*, 6(6):722–728, dec 1974. doi: 10.1128/aac.6.6.722. URL https://journals.asm.org/doi/abs/10.1128/aac.6.6.722.

[417] George M. Sheldrick, Peter G. Jones, Olga Kennard, Dudley H. Williams, and Gerald A. Smith. Structure of vancomycin and its complex with acetyl-D-alanyl-D-alanine. *Nature*, 271(5642):223–225, jan 1978. ISSN 0028-0836. doi: 10.1038/271223a0. URL https://www.nature.com/articles/271223a0http://www.nature.com/articles/271223a0. 242 citations (Semantic Scholar/DOI) [2022-06-23] Publisher: Nature Publishing Group.

[418] D. G. Teasley, D. N. Gerding, M. M. Olson, L. R. Peterson, R. L. Gebhard, M. J. Schwartz, and J. T. Lee. Prospective randomised trial of metronidazole versus vancomycin for Clostridium-difficile-associated diarrhoea and colitis. *Lancet (London, England)*, 2(8358):1043–1046, nov 1983. ISSN 0140-6736. doi: 10.1016/s0140-6736(83)91036-x.

[419] P. E. Reynolds. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology*, 8(11):943–950, nov 1989. ISSN 0934-9723. doi: 10.1007/BF01967563.

[420] D. N. Gerding. Is there a relationship between vancomycin-resistant enterococcal infection and Clostridium difficile infection? *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 25 Suppl 2:S206–210, sep 1997. ISSN 1058-4838. doi: 10.1086/516247.

[421] Samy O. Meroueh, Krisztina Z. Bencze, Dusan Hesek, Mijoon Lee, Jed F. Fisher, Timothy L. Stemmler, and Shahriar Mobashery. Three-dimensional structure of the bacterial cell wall peptidoglycan. *Proceedings of the National Academy of Sciences of the United States of America*, 103 (12):4404–4409, mar 2006. ISSN 0027-8424. doi: 10.1073/pnas.0510182103.

[422] Michael J. Rybak. The pharmacokinetic and pharmacodynamic properties of vancomycin. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 42 Suppl 1:S35–39, jan 2006. ISSN 1537-6591. doi: 10.1086/491712.

[423] Fred A. Zar, Srinivasa R. Bakkanagari, K. M. L. S. T. Moorthi, and Melinda B. Davis. A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 45(3):302–307, aug 2007. ISSN 1537-6591. doi: 10.1086/519265.

[424] Wafa N. Al-Nassir, Ajay K. Sethi, Yuejin Li, Michael J. Pultz, Michelle M. Riggs, and Curtis J. Donskey. Both oral metronidazole and oral vancomycin promote persistent overgrowth of vancomycin-resistant enterococci during treatment of Clostridium difficile-associated disease. *Antimicrobial Agents and Chemotherapy*, 52(7):2403–2406, jul 2008. ISSN 1098-6596. doi: 10.1128/AAC.00090-08.

[425] Wafa N. Al-Nassir, Ajay K. Sethi, Michelle M. Nerandzic, Greg S. Bobulsky, Robin L. P. Jump, and Curtis J. Donskey. Comparison of clinical and microbiological response to treatment of Clostridium difficile-associated disease with metronidazole and vancomycin. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 47(1):56–62, jul 2008. ISSN 1537-6591. doi: 10.1086/588293.

[426] Stuart H. Cohen, Dale N. Gerding, Stuart Johnson, Ciaran P. Kelly, Vivian G. Loo, L. Clifford McDonald, Jacques Pepin, Mark H. Wilcox, Society for Healthcare Epidemiology of America, and Infectious Diseases Society of America. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infection Control and Hospital Epidemiology*, 31(5): 431–455, may 2010. ISSN 1559-6834. doi: 10.1086/651706.

[427] Anne Vrieze, Carolien Out, Susana Fuentes, Lisanne Jonker, Isaie Reuling, Ruud S. Kootte, Els van Nood, Frits Holleman, Max Knaapen, Johannes A. Romijn, Maarten R. Soeters, Ellen E. Blaak, Geesje M. Dallinga-Thie, Dorien Reijnders, Mariëtte T. Ackermans, Mireille J. Serlie, Filip K. Knop, Jenst J. Holst, Claude van der Ley, Ido P. Kema, Erwin G. Zoetendal, Willem M. de Vos, Joost B. L. Hoekstra, Erik S. Stroes, Albert K. Groen, and Max Nieuwdorp. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. *Journal of Hepatology*, 60 (4):824–831, apr 2014. ISSN 1600-0641. doi: 10.1016/j.jhep.2013.11.034.

[428] Sandrine Isaac, Jose U. Scher, Ana Djukovic, Nuria Jiménez, Dan R. Littman, Steven B. Abramson, Eric G. Pamer, and Carles Ubeda. Short- and long-term effects of oral vancomycin on the human intestinal microbiota. *The Journal of Antimicrobial Chemotherapy*, 72(1):128–136, jan 2017. ISSN 1460-2091. doi: 10.1093/jac/dkw383.

[429] Stuart Johnson, Thomas J. Louie, Dale N. Gerding, Oliver A. Cornely, Scott Chasan-Taber, David Fitts, Steven P. Gelone, Colin Broom, David M. Davidson, and Polymer Alternative for CDI Treatment (PACT) investigators. Vancomycin, metronidazole, or tolevamer for Clostridium difficile

infection: results from two multinational, randomized, controlled trials. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 59(3):345–354, aug 2014. ISSN 1537-6591. doi: 10.1093/cid/ciu313.

[430] Alessio Basolo, Maximilian Hohenadel, Qi Yan Ang, Paolo Piaggi, Sascha Heinitz, Mary Walter, Peter Walter, Shannon Parrington, Donovan D. Trinidad, Reiner Jumpertz von Schwartzenberg, Peter J. Turnbaugh, and Jonathan Krakoff. Effects of underfeeding and oral vancomycin on gut microbiome and nutrient absorption in humans. *Nature Medicine*, 26(4):589–598, apr 2020. ISSN 1546-170X. doi: 10.1038/s41591-020-0801-z.

[431] Lisa Brown, Julie M. Wolf, Rafael Prados-Rosales, and Arturo Casadevall. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. *Nature Reviews Microbiology*, 13(10):620–630, oct 2015. ISSN 1740-1534. doi: 10.1038/nrmicro3480. URL https://www.nature.com/articles/nrmicro3480.

[432] L. Clifford McDonald, Dale N. Gerding, Stuart Johnson, Johan S. Bakken, Karen C. Carroll, Susan E. Coffin, Erik R. Dubberke, Kevin W. Garey, Carolyn V. Gould, Ciaran Kelly, Vivian Loo, Julia Shaklee Sammons, Thomas J. Sandora, and Mark H. Wilcox. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 66(7): e1–e48, mar 2018. ISSN 1537-6591. doi: 10.1093/cid/cix1085.

[433] Santiago Alvarez-Arango, S. Michelle Ogunwole, Thomas D. Sequist, Caitlin M. Burk, and Kimberly G. Blumenthal. Vancomycin Infusion Reaction — Moving beyond "Red Man Syndrome". *The New England journal of medicine*, 384(14):1283, 4 2021. ISSN 0028-4793. doi: 10.1056/NEJMP2031891. URL https://pmc.ncbi.nlm.nih.gov/articles/PMC9235042/.

[434] Ishani Wickramage, Patrizia Spigaglia, and Xingmin Sun. Mechanisms of antibiotic resistance of Clostridioides difficile. *The Journal of Antimicrobial Chemotherapy*, 76(12):3077–3090, nov 2021. ISSN 1460-2091. doi: 10.1093/jac/dkab231.

[435] Taryn A. Eubank, Anne J. Gonzales-Luna, Julian G. Hurdle, and Kevin W. Garey. Genetic Mechanisms of Vancomycin Resistance in Clostridioides difficile: A Systematic Review. *Antibiotics (Basel, Switzerland)*, 11(2):258, feb 2022. ISSN 2079-6382. doi: 10.3390/antibiotics11020258.

[436] Vanessa W. Stevens, Karim Khader, Kelly Echevarria, Richard E. Nelson, Yue Zhang, Makoto Jones, Tristan T. Timbrook, Matthew H. Samore, and Michael A. Rubin. Use of Oral Vancomycin for Clostridioides difficile Infection and the Risk of Vancomycin-Resistant Enterococci. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 71(3): 645–651, jul 2020. ISSN 1537-6591. doi: 10.1093/cid/ciz871.

[437] Jinhee Jo, Chenlin Hu, Khurshida Begum, Weiqun Wang, Thanh M. Le, Samantha Agyapong, Blake M. Hanson, Hossaena Ayele, Chris Lancaster, M. Jahangir Alam, Anne J. Gonzales-Luna, and Kevin W. Garey. Fecal Pharmacokinetics and Gut Microbiome Effects of Oral Omadacycline Versus Vancomycin in Healthy Volunteers. *The Journal of Infectious Diseases*, 229(1):273–281, jan 2024. ISSN 1537-6613. doi: 10.1093/infdis/jiad537.

[438] Jee Hoon Park, Rachel E. Reviello, and Patrick J. Loll. Crystal structure of vancomycin bound to the resistance determinant d-alanine-d-serine. *IUCrJ*, 11(Pt 2):133–139, jan 2024. ISSN 2052-2525. doi: 10.1107/S2052252524000289. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10916290/.

[439] Chenlin Hu, Nicholas D. Beyda, and Kevin W. Garey. A Vancomycin HPLC Assay for Use in Gut Microbiome Research. *Microbiology Spectrum*, 10(3):e0168821, jun 2022. ISSN 2165-0497. doi: 10.1128/spectrum.01688-21.

[440] W. Wehrli, F. Knüsel, K. Schmid, and M. Staehelin. Interaction of rifamycin with bacterial RNA polymerase. *Proceedings of the National Academy of Sciences of the United States of America*, 61 (2):667–673, oct 1968. ISSN 0027-8424. doi: 10.1073/pnas.61.2.667.

[441] C. Coronelli, R. J. White, G. C. Lancini, and F. Parenti. Lipiarmycin, a new antibiotic from Actinoplanes. II. Isolation, chemical, biological and biochemical characterization. *The Journal of Antibiotics*, 28(4):253–259, apr 1975. ISSN 0021-8820. doi: 10.7164/antibiotics.28.253.

[442] F. Parenti, H. Pagani, and G. Beretta. Lipiarmycin, a new antibiotic from Actinoplanes. I. Description of the producer strain and fermentation studies. *The Journal of Antibiotics*, 28(4):247–252, apr 1975. ISSN 0021-8820. doi: 10.7164/antibiotics.28.247.

[443] S. Sergio, G. Pirali, R. White, and F. Parenti. Lipiarmycin, a new antibiotic from Actinoplanes III. Mechanism of action. *The Journal of Antibiotics*, 28(7):543–549, jul 1975. ISSN 0021-8820. doi: 10.7164/antibiotics.28.543.

[444] P. Sensi. History of the development of rifampin. *Reviews of Infectious Diseases*, 5 Suppl 3:S402–406, 1983. ISSN 0162-0886. doi: 10.1093/clinids/5.supplement_3.s402.

[445] D. J. Jin and C. A. Gross. Mapping and sequencing of mutations in the Escherichia coli rpoB gene that lead to rifampicin resistance. *Journal of Molecular Biology*, 202(1):45–58, jul 1988. ISSN 0022-2836. doi: 10.1016/0022-2836(88)90517-7.

[446] Heinz G. Floss and Tin-Wein Yu. Rifamycin-mode of action, resistance, and biosynthesis. *Chemical Reviews*, 105(2):621–632, feb 2005. ISSN 0009-2665. doi: 10.1021/cr030112j.

[447] T. Louie, M. Miller, C. Donskey, K. Mullane, and E. J. C. Goldstein. Clinical Outcomes, Safety, and Pharmacokinetics of OPT-80 in a Phase 2 Trial with Patients with Clostridium difficile Infection. *Antimicrobial Agents and Chemotherapy*, 53(1):223–228, jan 2009. doi: 10.1128/aac.01442-07. URL https://journals.asm.org/doi/full/10.1128/aac.01442-07.

[448] Thomas J. Louie, Judy Emery, Walter Krulicki, Brendan Byrne, and Manuel Mah. OPT-80 Eliminates Clostridium difficile and Is Sparing of Bacteroides Species during Treatment of C. difficile Infection. *Antimicrobial Agents and Chemotherapy*, 53(1):261–263, jan 2009. doi: 10.1128/aac.01443-07. URL https://journals.asm.org/doi/full/10.1128/aac.01443-07.

[449] Gerald W. Tannock, Karen Munro, Corinda Taylor, Blair Lawley, Wayne Young, Brendan Byrne, Judy Emery, and Thomas Louie. A new macrocyclic antibiotic, fidaxomicin (OPT-80), causes less alteration to the bowel microbiota of Clostridium difficile-infected patients than does vancomycin. *Microbiology (Reading, England)*, 156(Pt 11):3354–3359, nov 2010. ISSN 1465-2080. doi: 10.1099/mic.0.042010-0.

[450] Ellie J. C. Goldstein, Diane M. Citron, Pamela Sears, Farah Babakhani, Susan P. Sambol, and Dale N. Gerding. Comparative susceptibilities to fidaxomicin (OPT-80) of isolates collected at baseline, recurrence, and failure from patients in two phase III trials of fidaxomicin against Clostridium difficile infection. *Antimicrobial Agents and Chemotherapy*, 55(11):5194–5199, nov 2011. ISSN 1098-6596. doi: 10.1128/AAC.00625-11.

[451] Thomas J. Louie, Mark A. Miller, Kathleen M. Mullane, Karl Weiss, Arnold Lentnek, Yoav Golan, Sherwood Gorbach, Pamela Sears, Youe-Kong Shue, and OPT-80-003 Clinical Study Group. Fidaxomicin versus vancomycin for Clostridium difficile infection. *The New England Journal of Medicine*, 364(5):422–431, feb 2011. ISSN 1533-4406. doi: 10.1056/NEJMoa0910812.

[452] Oliver A. Cornely, Mark A. Miller, Thomas J. Louie, Derrick W. Crook, and Sherwood L. Gorbach. Treatment of first recurrence of Clostridium difficile infection: fidaxomicin versus vancomycin. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 55 Suppl 2(Suppl 2), aug 2012. ISSN 1537-6591. doi: 10.1093/CID/CIS462. URL https://pubmed.ncbi.nlm.nih.gov/22752865/. 248 citations (Crossref) [2022-10-28] 315 citations (Semantic Scholar/DOI) [2022-06-23] Publisher: Clin Infect Dis.

[453] Ellie J. C. Goldstein, Farah Babakhani, and Diane M. Citron. Antimicrobial activities of fidaxomicin. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 55 Suppl 2(Suppl 2):S143–148, aug 2012. ISSN 1537-6591. doi: 10.1093/cid/cis339.

[454] Thomas J. Louie, Kris Cannon, Brendan Byrne, Judy Emery, Linda Ward, Melissa Eyben, and Walter Krulicki. Fidaxomicin preserves the intestinal microbiome during and after treatment of clostridium difficile infection (CDI) and reduces both toxin reexpression and recurrence of CDI. *Clinical Infectious Diseases*, 55(SUPPL.2):S132–S142, aug 2012. ISSN 10584838. doi: 10.1093/cid/cis338. 207 citations (Crossref) [2022-10-28] 253 citations (Semantic Scholar/DOI) [2022-06-23].

[455] Pamela Sears, Derrick W. Crook, Thomas J. Louie, Mark A. Miller, and Karl Weiss. Fidaxomicin attains high fecal concentrations with minimal plasma concentrations following oral administration in patients with Clostridium difficile infection. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 55 Suppl 2(Suppl 2):S116–120, aug 2012. ISSN 1537-6591. doi: 10.1093/cid/cis337.

[456] Anilrudh A. Venugopal and Stuart Johnson. Fidaxomicin: a novel macrocyclic antibiotic approved for treatment of Clostridium difficile infection. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 54(4):568–574, feb 2012. ISSN 1537-6591. doi: 10.1093/cid/cir830.

[457] Sarah M. Bartsch, Craig A. Umscheid, Neil Fishman, and Bruce Y. Lee. Is Fidaxomicin Worth the Cost? An Economic Analysis. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 57(4):555–561, aug 2013. ISSN 1058-4838. doi: 10.1093/cid/cit346. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3719891/.

[458] Giancarlo Lancini. In memory of Piero Sensi (1920-2013). *The Journal of Antibiotics*, 67(9):609–611, sep 2014. ISSN 1881-1469. doi: 10.1038/ja.2014.104.

[459] Angelo Bedeschi, Piera Fonte, Giovanni Fronza, Claudio Fuganti, and Stefano Serra. The Co-identity of Lipiarmycin A3 and Tiacumicin B. *Natural Product Communications*, 11(5):569–572, may 2016. ISSN 1934-578X.

[460] Stefano Serra, Luciana Malpezzi, Angelo Bedeschi, Claudio Fuganti, and Piera Fonte. Final Demonstration of the Co-Identity of Lipiarmycin A3 and Tiacumicin B (Fidaxomicin) through Single Crystal X-ray Analysis. *Antibiotics (Basel, Switzerland)*, 6(1):7, feb 2017. ISSN 2079-6382. doi: 10.3390/antibiotics6010007.

[461] Benoit Guery, Francesco Menichetti, Veli-Jukka Anttila, Nicholas Adomakoh, Jose Maria Aguado, Karen Bisnauthsing, Areti Georgopali, Simon D. Goldenberg, Andreas Karas, Gbenga Kazeem, Chris Longshaw, Jose Alejandro Palacios-Fabrega, Oliver A. Cornely, Maria J. G. T. Vehreschild, and EXTEND Clinical Study Group. Extended-pulsed fidaxomicin versus vancomycin for Clostridium difficile infection in patients 60 years and older (EXTEND): a randomised, controlled, open-label, phase 3b/4 trial. *The Lancet. Infectious Diseases*, 18(3):296–307, mar 2018. ISSN 1474-4457. doi: 10.1016/S1473-3099(17)30751-X.

[462] Christoph Högenauer, Yashwant Mahida, Andreas Stallmach, Philippe Marteau, Grazyna Rydzewska, Vladimir Ivashkin, Panagiotis Gargalianos-Kakolyris, Ingrid Michon, Nicholas Adomakoh, Areti Georgopali, Reiner Tretter, Andreas Karas, and Walter Reinisch. Pharmacokinetics and safety of fidaxomicin in patients with inflammatory bowel disease and Clostridium difficile infection: an open-label Phase IIIb/IV study (PROFILE). *The Journal of Antimicrobial Chemotherapy*, 73(12):3430–3441, dec 2018. ISSN 1460-2091. doi: 10.1093/jac/dky368.

[463] Radha Rajasingham, Eva A. Enns, Alexander Khoruts, and Byron P. Vaughn. Cost-effectiveness of Treatment Regimens for Clostridioides difficile Infection: An Evaluation of the 2018 Infectious Diseases Society of America Guidelines. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 70(5):754–762, feb 2020. ISSN 1537-6591. doi: 10.1093/cid/ciz318.

[464] Stephen K. Burley, Charmi Bhikadiya, Chunxiao Bi, Sebastian Bittrich, Henry Chao, Li Chen, Paul A. Craig, Gregg V. Crichlow, Kenneth Dalenberg, Jose M. Duarte, Shuchismita Dutta, Maryam Fayazi, Zukang Feng, Justin W. Flatt, Sai Ganesan, Sutapa Ghosh, David S. Goodsell, Rachel Kramer Green, Vladimir Guranovic, Jeremy Henry, Brian P. Hudson, Igor Khokhriakov, Catherine L. Lawson, Yuhe Liang, Robert Lowe, Ezra Peisach, Irina Persikova, Dennis W. Piehl, Yana Rose, Andrej Sali, Joan Segura, Monica Sekharan, Chenghua Shao, Brinda Vallat, Maria Voigt, Ben Webb, John D. Westbrook, Shamara Whetstone, Jasmine Y. Young, Arthur Zalevsky, and Christine Zardecki. RCSB Protein Data Bank (RCSB.org): delivery of experimentally-determined PDB structures alongside one million computed structure models of proteins from artificial intelligence/machine learning. *Nucleic Acids Research*, 51(D1):D488–D508, jan 2023. ISSN 1362-4962. doi: 10.1093/nar/gkac1077.

[465] Sho Tashiro, Kazuaki Taguchi, Yuki Enoki, and Kazuaki Matsumoto. Fecal pharmacokinetics/pharmacodynamics characteristics of fidaxomicin and vancomycin against Clostridioides difficile infection elucidated by in vivo feces-based infectious evaluation models. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 29(5):616–622, may 2023. ISSN 1469-0691. doi: 10.1016/j.cmi.2022.12.015.

[466] Chetna Dureja, Abiola O. Olaitan, and Julian G. Hurdle. Mechanisms and impact of antimicrobial resistance in Clostridioides difficile. *Current Opinion in Microbiology*, 66:63–72, apr 2022. ISSN 1879-0364. doi: 10.1016/j.mib.2022.01.004.

[467] Anne J. Gonzales-Luna, Chetna Dureja, Taryn A. Eubank, Kevin W. Garey, and Julian G. Hurdle. Surveillance of Clostridioides difficile Antimicrobial Resistance in the United States. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 76(11): 2038–2039, jun 2023. ISSN 1537-6591. doi: 10.1093/cid/ciad135.

[468] Taryn A. Eubank, Chetna Dureja, Kevin W. Garey, Julian G. Hurdle, and Anne J. Gonzales-Luna. Reduced Vancomycin Susceptibility in Clostridioides difficile Is Associated With Lower Rates of Initial Cure and Sustained Clinical Response. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 79(1):15–21, jul 2024. ISSN 1537-6591. doi: 10.1093/cid/ciae087.

[469] W. J. Shen, A. Deshpande, K. E. Hevener, and et al. Constitutive expression of the cryptic vangcd operon promotes vancomycin resistance in clostridioides difficile clinical isolates. *Journal of Antimicrobial Chemotherapy*, 75:859–867, 2020. doi: 10.1093/jac/dkz550.

[470] Aditi Deshpande, Xiaoqian Wu, Wenwen Huo, Kelli L. Palmer, and Julian G. Hurdle. Chromosomal Resistance to Metronidazole in Clostridioides difficile Can Be Mediated by Epistasis between Iron Homeostasis and Oxidoreductases. *Antimicrobial Agents and Chemotherapy*, 64(8): 10.1128/aac.00415–20, jul 2020. doi: 10.1128/aac.00415-20. URL https://journals.asm.org/doi/full/10.1128/aac.00415-20.

[471] Simon D Baines, Rachael O'Connor, Jane Freeman, Warren N Fawley, Celine Harmanus, Paola Mastrantonio, Ed J Kuijper, and Mark H Wilcox. Emergence of reduced susceptibility to metronidazole in clostridium difficile. *J. Antimicrob. Chemother.*, 62(5):1046–1052, November 2008.

[472] Abiola O Olaitan, Chetna Dureja, Madison A Youngblom, Madeline A Topf, Wan-Jou Shen, Anne J Gonzales-Luna, Aditi Deshpande, Kirk E Hevener, Jane Freeman, Mark H Wilcox, Kelli L Palmer, Kevin W Garey, Caitlin S Pepperell, and Julian G Hurdle. Decoding a cryptic mechanism of metronidazole resistance among globally disseminated fluoroquinolone-resistant clostridioides difficile. *Nat. Commun.*, 14(1):4130, July 2023.

[473] T. Peláez, L. Alcalá, R. Alonso, M. Rodríguez-Créixems, J. M. García-Lechuz, and E. Bouza. Reassessment of Clostridium difficile susceptibility to metronidazole and vancomycin. *Antimicrobial Agents and Chemotherapy*, 46(6):1647–1650, jun 2002. ISSN 0066-4804. doi: 10.1128/AAC.46.6.1647-1650.2002.

[474] Joseph D. Lutgring, Susannah L. McKay, Amy S. Gargis, Alison L. Halpin, and L. Clifford McDonald. Are Vancomycin Non-Susceptible Clostridioides difficile Strains Emerging? *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 75(9): 1677–1678, oct 2022. ISSN 1537-6591. doi: 10.1093/cid/ciac375.

[475] Charles Darkoh, Kadiatou Keita, Chioma Odo, Micah Oyaro, Eric L. Brown, Cesar A. Arias, Blake M. Hanson, and Herbert L. DuPont. Emergence of Clinical Clostridioides difficile Isolates With Decreased Susceptibility to Vancomycin. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 74(1):120–126, jan 2022. ISSN 1537-6591. doi: 10.1093/cid/ciaa912.

[476] David Henry Greentree, Louis B. Rice, and Curtis J. Donskey. Houston, We Have a Problem: Reports of Clostridioides difficile Isolates With Reduced Vancomycin Susceptibility. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 75(9):1661–1664, oct 2022. ISSN 1537-6591. doi: 10.1093/cid/ciac444.

[477] Baban Kolte and Ulrich Nübel. Genetic determinants of resistance to antimicrobial therapeutics are rare in publicly available Clostridioides difficile genome sequences. *The Journal of Antimicrobial Chemotherapy*, 79(6):1320–1328, jun 2024. ISSN 1460-2091. doi: 10.1093/jac/dkae101.

[478] Sarah N. Redmond, Jennifer L. Cadnum, Annette L. Jencson, Claire E. Kaple, Brigid M. Wilson, Andrew M. Skinner, Amy S. Gargis, Munok Hwang, Hosoon Choi, Piyali Chatterjee, Chetan Jinadatha, and Curtis J. Donskey. Emergence and Spread of Clostridioides difficile Isolates With Reduced Fidaxomicin Susceptibility in an Acute Care Hospital. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 80(5):984–991, jun 2025. ISSN 1537-6591. doi: 10.1093/cid/ciaf028.

[479] Sarah A. Kuehne, Andrew W. Dempster, Mark M. Collery, Nimitray Joshi, Jamie Jowett, Michelle L. Kelly, Rory Cave, Chris M. Longshaw, and Nigel P. Minton. Characterization of the impact of rpoB mutations on the in vitro and in vivo competitive fitness of Clostridium difficile and susceptibility to fidaxomicin. *The Journal of Antimicrobial Chemotherapy*, 73(4):973–980, apr 2018. ISSN 1460-2091. doi: 10.1093/jac/dkx486.

[480] ThanhPhuong M. Le, Taryn A. Eubank, Ann M. McKelvey, Xinyun Cao, Julian G. Hurdle, and Kevin W. Garey. Fidaxomicin resistance in Clostridioides difficile: a systematic review and predictive modeling with RNA polymerase binding sites. *Antimicrobial Agents and Chemotherapy*, 68 (12):e0120624, dec 2024. ISSN 1098-6596. doi: 10.1128/aac.01206-24.

[481] C. M. Thorpe, L. A. McDermott, M. K. Tran, J. Chang, S. G. Jenkins, E. J. C. Goldstein, R. Patel, B. A. Forbes, S. Johnson, D. N. Gerding, and D. R. Snydman. U.S.-Based National Surveillance for Fidaxomicin Susceptibility of Clostridioides difficile-Associated Diarrheal Isolates from 2013 to 2016. *Antimicrobial Agents and Chemotherapy*, 63(7):e00391–19, jul 2019. ISSN 1098-6596. doi: 10.1128/AAC.00391-19.

[482] Papanin Putsathit, Stacey Hong, Narelle George, Christine Hemphill, Peter G. Huntington, Tony M. Korman, Despina Kotsanas, Monica Lahra, Rodney McDougall, Andrew McGlinchey, Casey V. Moore, Graeme R. Nimmo, Louise Prendergast, Jennifer Robson, Lynette Waring, Michael C. Wehrhahn, Gerhard F. Weldhagen, Richard M. Wilson, Thomas V. Riley, and Daniel R. Knight. Antimicrobial resistance surveillance of Clostridioides difficile in Australia, 2015-18. *The Journal of Antimicrobial Chemotherapy*, 76(7):1815–1821, jun 2021. ISSN 1460-2091. doi: 10.1093/jac/dkab099.

[483] D. R. Snydman, L. A. McDermott, C. M. Thorpe, E. J. C. Goldstein, A. N. Schuetz, S. Johnson, D. N. Gerding, L. Gluck, D. Bourdas, K. C. Carroll, C. K. Lancaster, K. W. Garey, Q. Wang, S. T. Walk, and E. Duperchy. A US-based national surveillance study for the susceptibility and epidemiology of Clostridioides difficile isolates with special reference to ridinilazole: 2020-2021. *Antimicrobial Agents and Chemotherapy*, 67(10):e0034923, oct 2023. ISSN 1098-6596. doi: 10.1128/aac.00349-23.

[484] Hélène Marchandin, Cyril Anjou, Gaëtan Poulen, Jane Freeman, Mark Wilcox, Hélène Jean-Pierre, and Frédéric Barbut. In vivo emergence of a still uncommon resistance to fidaxomicin in the urgent antimicrobial resistance threat Clostridioides difficile. *The Journal of Antimicrobial Chemotherapy*, 78(8):1992–1999, aug 2023. ISSN 1460-2091. doi: 10.1093/jac/dkad194.

[485] Liam S. Redgrave, Sam B. Sutton, Mark A. Webber, and Laura J. V. Piddock. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends in Microbiology*, 22(8):438–445, August 2014. ISSN 1878-4380. doi: 10.1016/j.tim.2014.04.007.

[486] Erika van Eijk, Bert Wittekoek, Ed J. Kuijper, and Wiep Klaas Smits. DNA replication proteins as potential targets for antimicrobials in drug-resistant bacterial pathogens. *The Journal of Antimicrobial Chemotherapy*, 72(5):1275–1284, may 2017. ISSN 1460-2091. doi: 10.1093/jac/dkw548.

[487] Wei-Chu Xu, Michael H. Silverman, Xiang Yang Yu, George Wright, and Neal Brown. Discovery and development of DNA polymerase IIIC inhibitors to treat Gram-positive infections. *Bioorganic & Medicinal Chemistry*, 27(15):3209–3217, aug 2019. ISSN 1464-3391. doi: 10.1016/j.bmc.2019.06.017. URL https://www.ncbi.nlm.nih.gov/pubmed/31221610.

[488] E. V. Koonin and P. Bork. Ancient duplication of dna polymerase inferred from analysis of complete bacterial genomes. *Trends Biochem Sci*, 21(4):128–9, 1996. ISSN 0968-0004 (Print) 0968-0004 (Linking). doi: 10.1016/S0968-0004(96)80165-4. URL https://www.ncbi.nlm.nih.gov/pubmed/8701468.

[489] K stutis Timinskas, Monika Balvočiute, Albertas Timinskas, and Česlovas Venclovas. Comprehensive analysis of DNA polymerase III α subunits and their homologs in bacterial genomes. *Nucleic Acids Research*, 42(3):1393–1413, 2 2014. ISSN 0305-1048. doi: 10.1093/NAR/GKT900. URL https://academic.oup.com/nar/article/42/3/1393/1062885.

[490] F. Griffith. The Significance of Pneumococcal Types. *The Journal of Hygiene*, 27(2):113–159, jan 1928. ISSN 0022-1724. doi: 10.1017/s0022172400031879.

[491] Oswald T Avery, Colin M MacLeod, and Maclyn McCarty. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type iii. *Die Entdeckung der Doppelhelix*, 97, 1944.

[492] M. Meselson and F. W. Stahl. THE REPLICATION OF DNA IN ESCHERICHIA COLI. *Proceedings of the National Academy of Sciences of the United States of America*, 44(7):671–682, jul 1958. ISSN 0027-8424. doi: 10.1073/pnas.44.7.671.

[493] F. Crick. Central dogma of molecular biology. *Nature*, 227(5258):561–563, aug 1970. ISSN 0028-0836. doi: 10.1038/227561a0.

[494] M. J. Bessman, A. Kornberg, I. R. Lehman, and E. S. Simms. Enzymic synthesis of deoxyribonucleic acid. *Biochimica Et Biophysica Acta*, 21(1):197–198, jul 1956. ISSN 0006-3002. doi: 10.1016/0006-3002(56)90127-5.

[495] I. R. Lehman, M. J. Bessman, E. S. Simms, and A. Kornberg. Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from Escherichia coli. *The Journal of Biological Chemistry*, 233(1):163–170, jul 1958. ISSN 0021-9258.

[496] M. J. Bessman, I. R. Lehman, E. S. Simms, and A. Kornberg. Enzymatic synthesis of deoxyribonucleic acid. II. General properties of the reaction. *The Journal of Biological Chemistry*, 233(1): 171–177, jul 1958. ISSN 0021-9258.

[497] Errol C. Friedberg. The eureka enzyme: the discovery of DNA polymerase. *Nature Reviews. Molecular Cell Biology*, 7(2):143–147, feb 2006. ISSN 1471-0072. doi: 10.1038/nrm1787.

[498] T. Kornberg and M. L. Gefter. DNA synthesis in cell-free extracts of a DNA polymerase-defective mutant. *Biochemical and Biophysical Research Communications*, 40(6):1348–1355, sep 1970. ISSN 0006-291X. doi: 10.1016/0006-291x(70)90014-8.

[499] T. Kornberg and M. L. Gefter. Purification and DNA synthesis in cell-free extracts: properties of DNA polymerase II. *Proceedings of the National Academy of Sciences of the United States of America*, 68(4):761–764, apr 1971. ISSN 0027-8424. doi: 10.1073/pnas.68.4.761.

[500] T. Kornberg, A. Lockwood, and A. Worcel. Replication of the Escherichia coli chromosome with a soluble enzyme system. *Proceedings of the National Academy of Sciences of the United States of America*, 71(8):3189–3193, aug 1974. ISSN 0027-8424. doi: 10.1073/pnas.71.8.3189.

[501] Arthur Kornberg and Tania A Baker. *DNA replication*. University Science Books, 2005.

[502] K. B. Gass and N. R. Cozzarelli. Further genetic and enzymological characterization of the three Bacillus subtilis deoxyribonucleic acid polymerases. *The Journal of Biological Chemistry*, 248(22): 7688–7700, nov 1973. ISSN 0021-9258.

[503] J. Ito and D. K. Braithwaite. Compilation and alignment of DNA polymerase sequences. *Nucleic Acids Research*, 19(15):4045–4057, aug 1991. ISSN 0305-1048. doi: 10.1093/nar/19.15.4045.

[504] D. K. Braithwaite and J. Ito. Compilation, alignment, and phylogenetic relationships of DNA polymerases. *Nucleic Acids Research*, 21(4):787–802, feb 1993. ISSN 0305-1048. doi: 10.1093/nar/21.4.787.

[505] Jonathan Filée, Patrick Forterre, Tang Sen-Lin, and Jacqueline Laurent. Evolution of DNA polymerase families: evidences for multiple gene exchange between cellular and viral proteins. *Journal of Molecular Evolution*, 54(6):763–773, jun 2002. ISSN 0022-2844. doi: 10.1007/s00239-001-0078-x.

[506] Mike O'Donnell. Replisome architecture and dynamics in Escherichia coli. *The Journal of Biological Chemistry*, 281(16):10653–10656, apr 2006. ISSN 0021-9258. doi: 10.1074/jbc.R500028200.

[507] Charles S. McHenry. Bacterial replicases and related polymerases. *Current opinion in chemical biology*, 15(5):587, oct 2011. ISSN 13675931. doi: 10.1016/J.CBPA.2011.07.018. URL /pmc/articles/PMC3190588/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3190588/. 52 citations (Semantic Scholar/DOI) [2022-06-23] Publisher: NIH Public Access.

[508] Irina Bruck, Roxana E. Georgescu, and Mike O'Donnell. Conserved interactions in the Staphylococcus aureus DNA PolC chromosome replication machine. *The Journal of Biological Chemistry*, 280(18):18152–18162, may 2005. ISSN 0021-9258. doi: 10.1074/jbc.M413595200.

[509] Wen-Jin Wu, Wei Yang, and Ming-Daw Tsai. How DNA polymerases catalyse replication and repair with contrasting fidelity. *Nature Reviews Chemistry*, 1(9):0068, sep 2017. ISSN 2397-3358. doi: 10.1038/s41570-017-0068. URL http://www.nature.com/articles/s41570-017-0068. 35 citations (Semantic Scholar/DOI) [2022-09-12] 38 citations (Crossref) [2022-09-12].

[510] Glenn M. Sanders, H. Garry Dallmann, and Charles S. McHenry. Reconstitution of the B. subtilis replisome with 13 proteins including two distinct replicases. *Molecular Cell*, 37(2):273–281, jan 2010. ISSN 1097-4164. doi: 10.1016/j.molcel.2009.12.025.

[511] Yang Gao, Yanxiang Cui, Tara Fox, Shiqiang Lin, Huaibin Wang, Natalia de Val, Z. Hong Zhou, and Wei Yang. Structures and operating principles of the replisome. *Science (New York, N.Y.)*, 363 (6429):eaav7003, feb 2019. ISSN 1095-9203. doi: 10.1126/science.aav7003.

[512] Arkadiusz W. Kulczyk, Arne Moeller, Peter Meyer, Piotr Sliz, and Charles C. Richardson. Cryo-EM structure of the replisome reveals multiple interactions coordinating DNA synthesis. *Proceedings of the National Academy of Sciences*, 114(10):E1848–E1856, mar 2017. doi: 10.1073/pnas.1701252114. URL https://www.pnas.org/doi/abs/10.1073/pnas.1701252114.

[513] Morgan L Jones, Yasemin Baris, Martin R G Taylor, and Joseph T P Yeeles. Structure of a human replisome shows the organisation and interactions of a DNA replication machine. *The EMBO Journal*, 40(23):e108819, dec 2021. ISSN 0261-4189. doi: 10.15252/embj.2021108819. URL https://www.embopress.org/doi/full/10.15252/embj.2021108819.

[514] Michael O'Donnell, Lance Langston, and Bruce Stillman. Principles and concepts of DNA replication in bacteria, archaea, and eukarya. *Cold Spring Harbor Perspectives in Biology*, 5(7):a010108, jul 2013. ISSN 1943-0264. doi: 10.1101/cshperspect.a010108.

[515] R. Okazaki, T. Okazaki, K. Sakabe, K. Sugimoto, and A. Sugino. Mechanism of DNA chain growth. I. Possible discontinuity and unusual secondary structure of newly synthesized chains. *Proceedings of the National Academy of Sciences of the United States of America*, 59(2):598–605, feb 1968. ISSN 0027-8424. doi: 10.1073/pnas.59.2.598.

[516] Malcolm L. Gefter, Yukinori Hirota, Thomas Kornberg, James A. Wechsler, and C. Barnoux. Analysis of DNA Polymerases II and III in Mutants of Escherichia coli Thermosensitive for DNA Synthesis. *Proceedings of the National Academy of Sciences*, 68(12):3150–3153, dec 1971. doi: 10.1073/pnas.68.12.3150. URL https://www.pnas.org/doi/abs/10.1073/pnas.68.12.3150. 242 citations (Crossref) [2022-09-08] 214 citations (Semantic Scholar/DOI) [2022-07-05] Publisher: Proceedings of the National Academy of Sciences.

[517] C. McHenry and A. Kornberg. DNA polymerase III holoenzyme of Escherichia coli. Purification and resolution into subunits. *The Journal of Biological Chemistry*, 252(18):6478–6484, sep 1977. ISSN 0021-9258.

[518] H. Maki and A. Kornberg. The polymerase subunit of DNA polymerase III of Escherichia coli. II. Purification of the alpha subunit, devoid of nuclease activities. *The Journal of Biological Chemistry*, 260(24):12987–12992, oct 1985. ISSN 0021-9258.

[519] Emmanuelle Le Chatelier, Olivier J. Bécherel, Emmanuelle d'Alençon, Danielle Canceill, S. Dusko Ehrlich, Robert P. P. Fuchs, and Laurent Jannière. Involvement of DnaE, the second replicative

DNA polymerase from Bacillus subtilis, in DNA mutagenesis. *The Journal of Biological Chemistry*, 279(3):1757–1767, jan 2004. ISSN 0021-9258. doi: 10.1074/jbc.M310719200.

[520] B Sanjanwala and A T Ganesan. DNA polymerase III gene of Bacillus subtilis. *Proceedings of the National Academy of Sciences*, 86(12):4421–4424, jun 1989. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.86.12.4421. URL https://pnas.org/doi/full/10.1073/pnas.86.12.4421. 12 citations (Crossref) [2022-09-12] 18 citations (Semantic Scholar/DOI) [2022-09-08].

[521] B. Sanjanwala and A. T. Ganesan. Genetic structure and domains of DNA polymerase III of Bacillus subtilis. *Molecular and General Genetics MGG*, 226(3):467–472, may 1991. ISSN 1432-1874. doi: 10.1007/BF00260660. URL https://doi.org/10.1007/BF00260660. 18 citations (Crossref) [2022-09-12] 12 citations (Semantic Scholar/DOI) [2022-09-08].

[522] R. A. Hammond, M. H. Barnes, S. L. Mack, J. A. Mitchener, and N. C. Brown. Bacillus subtilis DNA polymerase III: complete sequence, overexpression, and characterization of the polC gene. *Gene*, 98(1):29–36, feb 1991. ISSN 0378-1119. doi: 10.1016/0378-1119(91)90100-p. URL https://www.ncbi.nlm.nih.gov/pubmed/1901559.

[523] M. H. Barnes, R. A. Hammond, C. C. Kennedy, S. L. Mack, and N. C. Brown. Localization of the exonuclease and polymerase domains of Bacillus subtilis DNA polymerase III. *Gene*, 111(1):43–49, feb 1992. ISSN 0378-1119. doi: 10.1016/0378-1119(92)90601-k.

[524] E. Dervyn, C. Suski, R. Daniel, C. Bruand, J. Chapuis, J. Errington, L. Jannière, and S. D. Ehrlich. Two essential DNA polymerases at the bacterial replication fork. *Science (New York, N.Y.)*, 294 (5547):1716–1719, nov 2001. ISSN 0036-8075. doi: 10.1126/science.1066351. URL https://www.ncbi.nlm.nih.gov/pubmed/11721055.

[525] R. Inoue, C. Kaito, M. Tanabe, K. Kamura, N. Akimitsu, and K. Sekimizu. Genetic identification of two distinct DNA polymerases, DnaE and PolC, that are essential for chromosomal DNA replication in Staphylococcus aureus. *Molecular genetics and genomics: MGG*, 266(4):564–571, dec 2001. ISSN 1617-4615. doi: 10.1007/s004380100564.

[526] Kimberly A. Foster, Marjorie H. Barnes, Ryan O. Stephenson, Michelle M. Butler, Donna J. Skow, William A. LaMarr, and Neal C. Brown. DNA polymerase III of Enterococcus faecalis: expression and characterization of recombinant enzymes encoded by the polC and dnaE genes. *Protein Expression and Purification*, 27(1):90–97, jan 2003. ISSN 1046-5928. doi: 10.1016/s1046-5928(02)00577-6. URL https://www.ncbi.nlm.nih.gov/pubmed/12509989.

[527] I. Bruck and M. O'Donnell. The DNA replication machine of a gram-positive organism. *The Journal of Biological Chemistry*, 275(37):28971–28983, sep 2000. ISSN 0021-9258. doi: 10.1074/jbc.M003565200. URL https://www.ncbi.nlm.nih.gov/pubmed/10878011.

[528] Marjorie H. Barnes, Shelley D. Miller, and Neal C. Brown. DNA polymerases of low-GC gram-positive eubacteria: identification of the replication-specific enzyme encoded by dnaE. *Journal of Bacteriology*, 184(14):3834–3838, jul 2002. ISSN 0021-9193. doi: 10.1128/JB.184.14.3834-3838.2002. URL https://www.ncbi.nlm.nih.gov/pubmed/12081953.

[529] D. Karamata and J. D. Gross. Isolation and genetic analysis of temperature-sensitive mutants of B. subtilis defective in DNA synthesis. *Molecular & general genetics: MGG*, 108(3):277–287, 1970. ISSN 0026-8925. doi: 10.1007/BF00283358.

[530] Silvano Riva, Cornelis van Sluis, Giorgio Mastromei, Carmen Attolini, Giorgio Mazza, Mario Polsinelli, and Arturo Falaschi. A new mutant of Bacillus subtilis altered in the initiation of chromosome replication. *Molecular and General Genetics MGG*, 137(3):185–202, sep 1975. ISSN 1432-1874. doi: 10.1007/BF00333015. URL https://doi.org/10.1007/BF00333015. 18 citations (Crossref) [2022-09-12] 6 citations (Semantic Scholar/DOI) [2022-09-08].

[531] M. J. Vrooman, M. H. Barnes, and N. C. Brown. Bacillus subtilis dnaF: a mutation of the gene specifying the structure of DNA polymerase III. *Molecular & general genetics: MGG*, 164(3):335–339, sep 1978. ISSN 0026-8925. doi: 10.1007/BF00333165. URL https://www.ncbi.nlm.nih.gov/pubmed/101768.

[532] G. W. Bazill and J. D. Gross. Effect of 6-(p-hydroxyphenyl)-azouracil on B. subtilis DNA polymerases. *Nature: New Biology*, 240(98):82–83, nov 1972. ISSN 0090-0028. doi: 10.1038/newbio240082a0. URL https://www.ncbi.nlm.nih.gov/pubmed/4628970.

[533] Kenneth B. Gass, Robert L. Low, and Nicholas R. Cozzarelli. Inhibition of a DNA Polymerase from Bacillus subtilis by Hydroxyphenylazopyrimidines. *Proceedings of the National Academy of Sciences of the United States of America*, 70(1):103–107, jan 1973. ISSN 0027-8424. doi: 10.1073/pnas.70.1.103. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC433193/.

[534] N. R. Cozzarelli and R. L. Low. Mutational alteration of Bacillus subtilis DNA polymerase 3 to hydroxyphenylazopyrimidine resistance: polymerase 3 is necessary for DNA replication. *Biochemical and Biophysical Research Communications*, 51(1):151–157, mar 1973. ISSN 0006-291X. doi: 10.1016/0006-291x(73)90521-4.

[535] J. M. Mackenzie, M. M. Neville, G. E. Wright, and N. C. Brown. Hydroxyphenylazopyrimidines: characterization of the active forms and their inhibitory action on a DNA polymerase from Bacillus subtilis. *Proceedings of the National Academy of Sciences of the United States of America*, 70(2):512–516, feb 1973. ISSN 0027-8424. doi: 10.1073/pnas.70.2.512.

[536] Robert L. Low, Stephan A. Rashbaum, and Nicholas R. Cozzarelli. Mechanism of Inhibition of Bacillus subtilis DNA Polymerase III by the Arylhydrazinopyrimidine Antimicrobial Agents. *Proceedings of the National Academy of Sciences of the United States of America*, 71(8):2973–2977, aug 1974. ISSN 0027-8424. doi: 10.1073/pnas.71.8.2973. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC388601/.

[537] G. E. Wright and N. C. Brown. Inhibitors of Bacillus subtilis DNA polymerase III. Structure-activity relationships of 6-(phenylhydrazino)uracils. *Journal of Medicinal Chemistry*, 20(9):1181–1185, sep 1977. ISSN 0022-2623. doi: 10.1021/jm00219a014.

[538] N. C. Brown, J. Gambino, and G. E. Wright. Inhibitors of Bacillus subtilis DNA polymerase III. 6-(arylalkylamino)uracils and 6-anilinouracils. *Journal of Medicinal Chemistry*, 20(9):1186–1189, sep 1977. ISSN 0022-2623. doi: 10.1021/jm00219a015.

[539] George E. Wright and Neal C. Brown. Inhibitors of Bacillus Subtilis DNA Polymerase III. 6-Anilinouracils and 6-(Alkylamino) Uracils. *Journal of Medicinal Chemistry*, 23(1):34–38, jan 1980. ISSN 15204804. doi: 10.1021/JM00175A007/ASSET/JM00175A007.FP.PNG_V03. URL https://pubs.acs.org/doi/abs/10.1021/jm00175a007.

[540] Carmen Attolini, Giorgio Mazza, Adriana Fortunato, Giovanni Ciarrocchi, Giorgio Mastromei, Silvano Riva, and Arturo Falaschi. On the identity of dnaP and dnaF genes of Bacillus subtilis. *Molecular and General Genetics MGG*, 148(1):9–17, jan 1976. ISSN 1432-1874. doi: 10.1007/BF00268540. URL https://doi.org/10.1007/BF00268540. 13 citations (Crossref) [2022-09-12] 6 citations (Semantic Scholar/DOI) [2022-09-08].

[541] E. Love, D. D'Ambrosio, and N. C. Brown. Mapping of the gene specifying DNA polymerase III of Bacillus subtilis. *Molecular & general genetics: MGG*, 144(3):313–321, mar 1976. ISSN 0026-8925. doi: 10.1007/BF00341730. URL https://www.ncbi.nlm.nih.gov/pubmed/818505.

[542] S. D. Rowley and N. C. Brown. Bacillus subtilis DNA polymerase III is required for the replication of DNA of bacteriophages SPP-1 and phi 105. *Journal of Virology*, 21(2):493–496, feb 1977. ISSN 0022-538X. doi: 10.1128/JVI.21.2.493-496.1977.

[543] R W Ott, M H Barnes, N C Brown, and A T Ganesan. Cloning and characterization of the polC region of Bacillus subtilis. *Journal of Bacteriology*, 165(3):951–957, mar 1986. ISSN 0021-9193. doi: 10.1128/jb.165.3.951-957.1986. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC214521/.

[544] Xiao-Qian Zhao, Jian-Fei Hu, and Jun Yu. Comparative analysis of eubacterial DNA polymerase III alpha subunits. *Genomics, Proteomics & Bioinformatics*, 4(4):203–211, nov 2006. ISSN 1672-0229. doi: 10.1016/S1672-0229(07)60001-1.

[545] Josh Abramson, Jonas Adler, Jack Dunger, Richard Evans, Tim Green, Alexander Pritzel, Olaf Ronneberger, Lindsay Willmore, Andrew J. Ballard, Joshua Bambrick, Sebastian W. Bodenstein, David A. Evans, Chia-Chun Hung, Michael O'Neill, David Reiman, Kathryn Tunyasuvunakool, Zachary Wu, Akvilė Žemgulytė, Eirini Arvaniti, Charles Beattie, Ottavia Bertolli, Alex Bridgland, Alexey Cherepanov, Miles Congreve, Alexander I. Cowen-Rivers, Andrew Cowie, Michael Figurnov, Fabian B. Fuchs, Hannah Gladman, Rishub Jain, Yousuf A. Khan, Caroline M. R. Low, Kuba Perlin, Anna Potapenko, Pascal Savy, Sukhdeep Singh, Adrian Stecula, Ashok Thillaisundaram, Catherine Tong, Sergei Yakneen, Ellen D. Zhong, Michal Zielinski, Augustin Žídek, Victor Bapst, Pushmeet Kohli, Max Jaderberg, Demis Hassabis, and John M. Jumper. Accurate structure prediction of biomolecular interactions with alphafold 3. *Nature*, 630(8016):493–500, May 2024. ISSN 1476-4687. doi: 10.1038/s41586-024-07487-w. URL http://dx.doi.org/10.1038/s41586-024-07487-w.

[546] Oleg Trott and Arthur J. Olson. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2):455–461, jan 2010. ISSN 1096-987X. doi: 10.1002/jcc.21334.

[547] Thomas D. Goddard, Conrad C. Huang, Elaine C. Meng, Eric F. Pettersen, Gregory S. Couch, John H. Morris, and Thomas E. Ferrin. UCSF ChimeraX: Meeting modern challenges in visualization and analysis. *Protein Science: A Publication of the Protein Society*, 27(1):14–25, jan 2018. ISSN 1469-896X. doi: 10.1002/pro.3235. URL https://www.ncbi.nlm.nih.gov/pubmed/28710774.

[548] E. F. Pettersen, T. D. Goddard, C. C. Huang, E. C. Meng, G. S. Couch, T. I. Croll, J. H. Morris, and T. E. Ferrin. Ucsf chimerax: Structure visualization for researchers, educators, and developers. *Pro-*

tein Sci, 30(1):70–82, 2021. ISSN 1469-896X (Electronic) 0961-8368 (Print) 0961-8368 (Linking). doi: 10.1002/pro.3943. URL https://www.ncbi.nlm.nih.gov/pubmed/32881101.

[549] Yang Liu, Xiaocong Yang, Jianhong Gan, Shuang Chen, Zhi-Xiong Xiao, and Yang Cao. CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. *Nucleic Acids Research*, 50(W1):W159–W164, jul 2022. ISSN 1362-4962. doi: 10.1093/nar/gkac394.

[550] Rachel Nelson-Rigg, Sean P. Fagan, William J. Jaremko, and Janice D. Pata. Pre-Steady-State Kinetic Characterization of an Antibiotic-Resistant Mutant of Staphylococcus aureus DNA Polymerase PolC. *Antimicrobial Agents and Chemotherapy*, 67(6):e0157122, jun 2023. ISSN 1098-6596. doi: 10.1128/aac.01571-22. URL https://www.ncbi.nlm.nih.gov/pubmed/37222615.

[551] M. H. Barnes, R. A. Hammond, K. A. Foster, J. A. Mitchener, and N. C. Brown. The cloned polC gene of Bacillus subtilis: characterization of the azp12 mutation and controlled in vitro synthesis of active DNA polymerase III. *Gene*, 85(1):177–186, dec 1989. ISSN 0378-1119. doi: 10.1016/0378-1119(89)90478-2. URL https://www.ncbi.nlm.nih.gov/pubmed/2515995. Barnes, M H Hammond, R A Foster, K A Mitchener, J A Brown, N C eng GM28775/GM/NIGMS NIH HHS/ Research Support, U.S. Gov't, P.H.S. Netherlands 1989/12/21 Gene. 1989 Dec 21;85(1):177-86. doi: 10.1016/0378-1119(89)90478-2.

[552] Ronald J. Evans, Douglas R. Davies, James M. Bullard, Jeffrey Christensen, Louis S. Green, Joseph W. Guiles, Janice D. Pata, Wendy K. Ribble, Nebojsa Janjic, and Thale C. Jarvis. Structure of PolC reveals unique DNA binding and fidelity determinants. *Proceedings of the National Academy of Sciences of the United States of America*, 105(52):20695–20700, dec 2008. ISSN 1091-6490. doi: 10.1073/pnas.0809989106. URL https://www.ncbi.nlm.nih.gov/pubmed/19106298.

[553] Meindert H. Lamers and Mike O'Donnell. A consensus view of DNA binding by the C family of replicative DNA polymerases. *Proceedings of the National Academy of Sciences of the United States of America*, 105(52):20565–20566, dec 2008. ISSN 1091-6490. doi: 10.1073/pnas.0811279106. URL https://www.ncbi.nlm.nih.gov/pubmed/19106294.

[554] Kęstutis Timinskas and Česlovas Venclovas. The N-terminal region of the bacterial DNA polymerase PolC features a pair of domains, both distantly related to domain V of the DNA polymerase III τ subunit. *The FEBS journal*, 278(17):3109–3118, sep 2011. ISSN 1742-4658. doi: 10.1111/j.1742-4658.2011.08236.x.

[555] T. A. Steitz, S. J. Smerdon, J. Jäger, and C. M. Joyce. A unified polymerase mechanism for nonhomologous DNA and RNA polymerases. *Science (New York, N.Y.)*, 266(5193):2022–2025, dec 1994. ISSN 0036-8075. doi: 10.1126/science.7528445.

[556] T. A. Steitz. A mechanism for all polymerases. *Nature*, 391(6664):231–232, jan 1998. ISSN 0028-0836. doi: 10.1038/34542.

[557] E. Chargaff. Chemical specificity of nucleic acids and mechanism of their enzymatic degradation. *Experientia*, 6(6):201–209, jun 1950. ISSN 0014-4754. doi: 10.1007/BF02173653.

[558] A. E. Pritchard and C. S. McHenry. Identification of the acidic residues in the active site of DNA polymerase III. *Journal of Molecular Biology*, 285(3):1067–1080, jan 1999. ISSN 0022-2836. doi: 10.1006/jmbi.1998.2352.

[559] T. A. Steitz. DNA polymerases: structural diversity and common mechanisms. *The Journal of Biological Chemistry*, 274(25):17395–17398, jun 1999. ISSN 0021-9258. doi: 10.1074/jbc.274.25.17395.

[560] Catherine M. Joyce and Stephen J. Benkovic. DNA polymerase fidelity: kinetics, structure, and checkpoints. *Biochemistry*, 43(45):14317–14324, nov 2004. ISSN 0006-2960. doi: 10.1021/bi048422z.

[561] William A. Beard and Samuel H. Wilson. Structural insights into the origins of DNA polymerase fidelity. *Structure (London, England: 1993)*, 11(5):489–496, may 2003. ISSN 0969-2126. doi: 10.1016/s0969-2126(03)00051-0.

[562] Thomas A. Kunkel. DNA replication fidelity. *The Journal of Biological Chemistry*, 279(17):16895–16898, apr 2004. ISSN 0021-9258. doi: 10.1074/jbc.R400006200.

[563] Michael Lynch, Matthew S. Ackerman, Jean-Francois Gout, Hongan Long, Way Sung, W. Kelley Thomas, and Patricia L. Foster. Genetic drift, selection and the evolution of the mutation rate. *Nature Reviews. Genetics*, 17(11):704–714, oct 2016. ISSN 1471-0064. doi: 10.1038/nrg.2016.104.

[564] C. M. Joyce and T. A. Steitz. Function and structure relationships in DNA polymerases. *Annual Review of Biochemistry*, 63:777–822, 1994. ISSN 0066-4154. doi: 10.1146/annurev.bi.63.070194.004021.

[565] C. M. Joyce. Choosing the right sugar: how polymerases select a nucleotide substrate. *Proceedings of the National Academy of Sciences of the United States of America*, 94(5):1619–1622, mar 1997. ISSN 0027-8424. doi: 10.1073/pnas.94.5.1619.

[566] G. Gao, M. Orlova, M. M. Georgiadis, W. A. Hendrickson, and S. P. Goff. Conferring RNA polymerase activity to a DNA polymerase: a single residue in reverse transcriptase controls substrate selection. *Proceedings of the National Academy of Sciences of the United States of America*, 94(2):407–411, jan 1997. ISSN 0027-8424. doi: 10.1073/pnas.94.2.407.

[567] Rajesh Kasiviswanathan and William C. Copeland. Ribonucleotide discrimination and reverse transcription by the human mitochondrial DNA polymerase. *The Journal of Biological Chemistry*, 286(36):31490–31500, sep 2011. ISSN 1083-351X. doi: 10.1074/jbc.M111.252460.

[568] Janne J. Mäkinen, Yeonoh Shin, Eeva Vieras, Pasi Virta, Mikko Metsä-Ketelä, Katsuhiko S. Murakami, and Georgiy A. Belogurov. The mechanism of the nucleo-sugar selection by multisubunit RNA polymerases. *Nature Communications*, 12(1):796, feb 2021. ISSN 2041-1723. doi: 10.1038/s41467-021-21005-w.

[569] Nisha A. Cavanaugh, William A. Beard, and Samuel H. Wilson. DNA polymerase beta ribonucleotide discrimination: insertion, misinsertion, extension, and coding. *The Journal of Biological Chemistry*, 285(32):24457–24465, aug 2010. ISSN 1083-351X. doi: 10.1074/jbc.M110.132407.

[570] Bret D. Freudenthal, William A. Beard, David D. Shock, and Samuel H. Wilson. Observing a DNA polymerase choose right from wrong. *Cell*, 154(1):157–168, jul 2013. ISSN 1097-4172. doi: 10.1016/j.cell.2013.05.048.

[571] Olivier Rannou, Emmanuelle Le Chatelier, Marilynn A. Larson, Hamid Nouri, Bérengère Dalmais, Charles Laughton, Laurent Jannière, and Panos Soultanas. Functional interplay of DnaE polymerase, DnaG primase and DnaC helicase within a ternary complex, and primase to polymerase hand-off during lagging strand DNA replication in Bacillus subtilis. *Nucleic Acids Research*, 41(10): 5303–5320, may 2013. ISSN 1362-4962. doi: 10.1093/nar/gkt207.

[572] Elena M. Seco and Silvia Ayora. Bacillus subtilis DNA polymerases, PolC and DnaE, are required for both leading and lagging strand synthesis in SPP1 origin-dependent DNA replication. *Nucleic Acids Research*, 45(14):8302–8313, aug 2017. ISSN 1362-4962. doi: 10.1093/nar/gkx493.

[573] Vasileios Paschalis, Emmanuelle Le Chatelier, Matthew Green, Hamid Nouri, François Képès, Panos Soultanas, and Laurent Janniere. Interactions of the Bacillus subtilis DnaE polymerase with replisomal proteins modulate its activity and fidelity. *Open Biology*, 7(9):170146, sep 2017. ISSN 2046-2441. doi: 10.1098/rsob.170146.

[574] Paul R. Dohrmann and Charles S. McHenry. A bipartite polymerase-processivity factor interaction: only the internal beta binding site of the alpha subunit is required for processive replication by the DNA polymerase III holoenzyme. *Journal of Molecular Biology*, 350(2):228–239, jul 2005. ISSN 0022-2836. doi: 10.1016/j.jmb.2005.04.065.

[575] Luke G. O'Neal, Madeline N. Drucker, Ngoc Khanh Lai, Ashley F. Clemente, Alyssa P. Campbell, Lindsey E. Way, Sinwoo Hong, Emily E. Holmes, Sarah J. Rancic, Nicholas Sawyer, Xindan Wang, and Elizabeth S. Thrall. "The B. subtilis replicative polymerases bind the sliding clamp with different strengths to tune replication processivity and fidelity". *bioRxiv: The Preprint Server for Biology*, page 2025.03.10.642433, mar 2025. ISSN 2692-8205. doi: 10.1101/2025.03.10.642433.

[576] K. A. Johnson. Rapid quench kinetic analysis of polymerases, adenosinetriphosphatases, and enzyme intermediates. *Methods in Enzymology*, 249:38–61, 1995. ISSN 0076-6879. doi: 10.1016/0076-6879(95)49030-2.

[577] Indrajit Lahiri, Purba Mukherjee, and Janice D. Pata. Kinetic characterization of exonuclease-deficient Staphylococcus aureus PolC, a C-family replicative DNA polymerase. *PloS One*, 8(5): e63489, 2013. ISSN 1932-6203. doi: 10.1371/journal.pone.0063489.

[578] Sean P Fagan, Purba Mukherjee, William J Jaremko, Rachel Nelson-Rigg, Ryan C Wilson, Tyler L Dangerfield, Kenneth A Johnson, Indrajit Lahiri, and Janice D Pata. Pyrophosphate release acts as a kinetic checkpoint during high-fidelity DNA replication by the Staphylococcus aureus replicative polymerase PolC. *Nucleic Acids Research*, 49(14):8324–8338, aug 2021. ISSN 0305-1048. doi: 10.1093/nar/gkab613. URL https://doi.org/10.1093/nar/gkab613. 8 citations (Crossref) [2025-02-20].

[579] Teruya Nakamura, Ye Zhao, Yuriko Yamagata, Yue-jin Hua, and Wei Yang. Watching DNA polymerase η make a phosphodiester bond. *Nature*, 487(7406):196–201, jul 2012. ISSN 1476-4687. doi: 10.1038/nature11181.

[580] Motoharu Ono, Shunsaku Koga, and Hisao Ohtsuki. Japan's superconducting maglev train. *IEEE Instrumentation & Measurement Magazine*, 5(1):9–15, 2002.

[581] N. C. Brown. 6(p-Hydroxyphenylazo)-uracil: a reversible, selective inhibitor of the replication of deoxyribonucleic acid of staphylococcal bacteriophage P11-M15. *Journal of Virology*, 8(5):759–765, nov 1971. ISSN 0022-538X. doi: 10.1128/JVI.8.5.759-765.1971.

[582] Michelle M. Butler, Donna J. Skow, Ryan O. Stephenson, Patrick T. Lyden, William A. LaMarr, and Kimberly A. Foster. Low frequencies of resistance among Staphylococcus and Enterococcus species to the bactericidal DNA polymerase inhibitor N(3)-hydroxybutyl 6-(3'-ethyl-4'-methylanilino) uracil. *Antimicrobial Agents and Chemotherapy*, 46(12):3770–3775, dec 2002. ISSN 0066-4804. doi: 10.1128/AAC.46.12.3770-3775.2002. URL https://www.ncbi.nlm.nih.gov/pubmed/12435675.

[583] Geoffrey S. Briggs, Wiep Klaas Smits, and Panos Soultanas. Chromosomal replication initiation machinery of low-G+C-content Firmicutes. *Journal of Bacteriology*, 194(19):5162–5170, oct 2012. ISSN 1098-5530. doi: 10.1128/JB.00865-12.

[584] Lawrence A. David, Corinne F. Maurice, Rachel N. Carmody, David B. Gootenberg, Julie E. Button, Benjamin E. Wolfe, Alisha V. Ling, A. Sloan Devlin, Yug Varma, Michael A. Fischbach, Sudha B. Biddinger, Rachel J. Dutton, and Peter J. Turnbaugh. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, 505(7484):559–563, dec 2014. ISSN 00280836. doi: 10.1038/nature12820. URL https://www.nature.com/articles/nature12820.pdf. 6178 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nature Publishing Group.

[585] Ilseung Cho, Shingo Yamanishi, Laura Cox, Barbara A. Methé, Jiri Zavadil, Kelvin Li, Zhan Gao, Douglas Mahana, Kartik Raju, Isabel Teitler, Huilin Li, Alexander V. Alekseyenko, and Martin J. Blaser. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature*, 488(7413):621–626, aug 2012. ISSN 00280836. doi: 10.1038/nature11400. URL http://www.https://www.nature.com/articles/nature11400. 1263 citations (Semantic Scholar/DOI) [2022-06-23] Publisher: Nature Publishing Group.

[586] Raphaela Joos, Katy Boucher, Aonghus Lavelle, Manimozhiyan Arumugam, Martin J. Blaser, Marcus J. Claesson, Gerard Clarke, Paul D. Cotter, Luisa De Sordi, Maria G. Dominguez-Bello, Bas E. Dutilh, Stanislav D. Ehrlich, Tarini Shankar Ghosh, Colin Hill, Christophe Junot, Leo Lahti, Trevor D. Lawley, Tine R. Licht, Emmanuelle Maguin, Thulani P. Makhalanyane, Julian R. Marchesi, Jelle Matthijnssens, Jeroen Raes, Jacques Ravel, Anne Salonen, Pauline D. Scanlan, Andrey Shkoporov, Catherine Stanton, Ines Thiele, Igor Tolstoy, Jens Walter, Bo Yang, Natalia Yutin, Alexandra Zhernakova, Hub Zwart, Human Microbiome Action Consortium, Joël Doré, and R. Paul Ross. Examining the healthy human microbiome concept. *Nature Reviews. Microbiology*, 23(3): 192–205, mar 2025. ISSN 1740-1534. doi: 10.1038/s41579-024-01107-0.

[587] Hilary P. Browne, Samuel C. Forster, Blessing O. Anonye, Nitin Kumar, B. Anne Neville, Mark D. Stares, David Goulding, and Trevor D. Lawley. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature*, 533(7604):543–546, may 2016. ISSN 1476-4687. doi: 10.1038/nature17645.

[588] Jacob McPherson, Chenlin Hu, Khurshida Begum, Weiqun Wang, Chris Lancaster, Anne J. Gonzales-Luna, Caroline Loveall, Michael H. Silverman, M. Jahangir Alam, and Kevin W. Garey.

Functional and Metagenomic Evaluation of Ibezapolstat for Early Evaluation of Anti-Recurrence Effects in Clostridioides difficile Infection. *Antimicrobial Agents and Chemotherapy*, 66(8): e0224421, aug 2022. ISSN 1098-6596. doi: 10.1128/aac.02244-21. URL https://www.ncbi.nlm.nih.gov/pubmed/35862742.

[589] Kevin W. Garey, Jacob McPherson, An Q. Dinh, Chenlin Hu, Jinhee Jo, Weiqun Wang, Chris K. Lancaster, Anne J. Gonzales-Luna, Caroline Loveall, Khurshida Begum, M. Jahangir Alam, Michael H. Silverman, and Blake M. Hanson. Efficacy, Safety, Pharmacokinetics, and Microbiome Changes of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 75(7):1164–1170, sep 2022. ISSN 1537-6591. doi: 10.1093/cid/ciac096. URL https://www.ncbi.nlm.nih.gov/pubmed/35134880.

[590] Jacob K. McPherson, Julian G. Hurdle, Matthew L. Baker, Tahir Hussain, Ashok Kumar, and Kevin W. Garey. The microbiome-restorative potential of ibezapolstat for the treatment of Clostridioides difficile infection is predicted through variant PolC-type DNA polymerase III in Lachnospiraceae and Oscillospiraceae. *Antimicrobial Agents and Chemotherapy*, 69(4):e0167924, apr 2025. ISSN 1098-6596. doi: 10.1128/aac.01679-24.

[591] Kevin W. Garey, Khurshida Begum, Chris Lancaster, Anne Gonzales-Luna, Dinh Bui, Julie Mercier, Corinne Seng Yue, Murray P. Ducharme, Ming Hu, Bradley Vince, Michael H. Silverman, M. Jahangir Alam, and Martin Kankam. A randomized, double-blind, placebo-controlled, single and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal microbiome effects of ibezapolstat administered orally to healthy subjects. *The Journal of Antimicrobial Chemotherapy*, 75(12):3635–3643, dec 2020. ISSN 1460-2091. doi: 10.1093/jac/dkaa364. URL https://www.ncbi.nlm.nih.gov/pubmed/32892222.

[592] Taryn A. Eubank, Jinhee Jo, M. Jahangir Alam, Khurshida Begum, Jacob K. McPherson, Thanh-Phuong M. Le, Thomas D. Horvath, Sigmund J. Haidacher, Eugene C. Poggio, Rong Lin, Corinne Seng Yue, Murray P. Ducharme, Georges Koudssi, Julie Mercier, Jeffrey D. Alder, Michael H. Silverman, Kevin W. Garey, and Ibezapolstat Phase 2 Investigator Group. Efficacy, safety, pharmacokinetics, and associated microbiome changes of ibezapolstat compared with vancomycin in adults with Clostridioides difficile infection: a phase 2b, randomised, double-blind, active-controlled, multicentre study. *The Lancet. Microbe*, page 101126, jun 2025. ISSN 2666-5247. doi: 10.1016/j.lanmic.2025.101126.

[593] Liam P. Shaw, Hassan Bassam, Chris P. Barnes, A. Sarah Walker, Nigel Klein, and Francois Balloux. Modelling microbiome recovery after antibiotics using a stability landscape framework. *The ISME Journal*, 13(7):1845–1856, jul 2019. ISSN 1751-7370. doi: 10.1038/s41396-019-0392-1. URL https://www.nature.com/articles/s41396-019-0392-1. 93 citations (Crossref) [2025-02-20] Number: 7 Publisher: Nature Publishing Group.

[594] Hyunmin Koo, Joseph A. Hakim, David K. Crossman, Ranjit Kumar, Elliot J. Lefkowitz, and Casey D. Morrow. Individualized recovery of gut microbial strains post antibiotics. *npj Biofilms and Microbiomes*, 5(1):30, dec 2019. ISSN 2055-5008. doi: 10.1038/s41522-019-0103-8. URL http://www.nature.com/articles/s41522-019-0103-8. 28 citations (Crossref) [2025-02-20] 9 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: NLM (Medline).

[595] Katharine Michelle Ng, Andrés Aranda-Díaz, Carolina Tropini, Matthew Ryan Frankel, William Van Treuren, Colleen T. O'Loughlin, Bryan Douglas Merrill, Feiqiao Brian Yu, Kali M. Pruss, Rita Almeida Oliveira, Steven Kyle Higginbottom, Norma F. Neff, Michael Andrew Fischbach, Karina Bivar Xavier, Justin Laine Sonnenburg, and Kerwyn Casey Huang. Recovery of the Gut Microbiota after Antibiotics Depends on Host Diet, Community Context, and Environmental Reservoirs. *Cell Host & Microbe*, 26(5):650–665.e4, nov 2019. ISSN 1934-6069. doi: 10.1016/j.chom.2019.10.011.

[596] Neal C. Brown. Inhibition of bacterial DNA replication by 6-(p-Hydroxyphenylazo)-uracil: Differential effect on repair and semi-conservative synthesis in Bacillus subtilis. *Journal of Molecular Biology*, 59(1):1–16, jul 1971. ISSN 0022-2836. doi: 10.1016/0022-2836(71)90409-8. URL https://www.sciencedirect.com/science/article/pii/0022283671904098. 102 citations (Crossref) [2022-09-24].

[597] M. M. Neville and N. C. Brown. Inhibition of a discrete bacterial DNA polymerase by 6-(p-hydroxyphenylazo)-uracil and 6-(p-hydroxyphenylazo-)-isocytosine. *Nature: New Biology*, 240(98):80–82, nov 1972. ISSN 0090-0028. doi: 10.1038/newbio240080a0. URL https://www.ncbi.nlm.nih.gov/pubmed/4628969.

[598] J. S. Daly, T. J. Giehl, N. C. Brown, C. Zhi, G. E. Wright, and R. T. Ellison. In vitro antimicrobial activities of novel anilinouracils which selectively inhibit DNA polymerase III of gram-positive bacteria. *Antimicrobial Agents and Chemotherapy*, 44(8):2217–2221, aug 2000. ISSN 0066-4804. doi: 10.1128/AAC.44.8.2217-2221.2000.

[599] Chengxin Zhi, Zheng-Yu Long, Joseph Gambino, Wei-Chu Xu, Neal C. Brown, Marjorie Barnes, Michelle Butler, William LaMarr, and George E. Wright. Synthesis of substituted 6-anilinouracils and their inhibition of DNA polymerase IIIC and Gram-positive bacterial growth. *Journal of Medicinal Chemistry*, 46(13):2731–2739, jun 2003. ISSN 0022-2623. doi: 10.1021/jm020591z.

[600] Chengxin Zhi, Zheng-Yu Long, Andrzej Manikowski, Jeanne Comstock, Wei-Chu Xu, Neal C. Brown, Paul M. Tarantino, Karsten A. Holm, Edward J. Dix, George E. Wright, Marjorie H. Barnes, Michelle M. Butler, Kimberly A. Foster, William A. LaMarr, Benoit Bachand, Richard Bethell, Caroline Cadilhac, Sylvie Charron, Serge Lamothe, Irina Motorina, and Richard Storer. Hybrid antibacterials. DNA polymerase-topoisomerase inhibitors. *Journal of Medicinal Chemistry*, 49(4): 1455–1465, feb 2006. ISSN 0022-2623. doi: 10.1021/jm0510023.

[601] Michelle M. Butler, William A. LaMarr, Kimberly A. Foster, Marjorie H. Barnes, Donna J. Skow, Patrick T. Lyden, Lauren M. Kustigian, Chengxin Zhi, Neal C. Brown, George E. Wright, and Terry L. Bowlin. Antibacterial Activity and Mechanism of Action of a Novel Anilinouracil-Fluoroquinolone Hybrid Compound. *Antimicrobial Agents and Chemotherapy*, 51(1):119–127, jan 2007. ISSN 0066-4804. doi: 10.1128/AAC.01311-05. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1797695/.

[602] Craig Knox, Mike Wilson, Christen M. Klinger, Mark Franklin, Eponine Oler, Alex Wilson, Allison Pon, Jordan Cox, Na Eun (Lucy) Chin, Seth A. Strawbridge, Marysol Garcia-Patino, Ray Kruger, Aadhavya Sivakumaran, Selena Sanford, Rahil Doshi, Nitya Khetarpal, Omolola Fatokun, Daphnee Doucet, Ashley Zubkowski, Dorsa Yahya Rayat, Hayley Jackson, Karxena Harford, Afia Anjum, Mahi Zakir, Fei Wang, Siyang Tian, Brian Lee, Jaanus Liigand, Harrison Peters, Ruo Qi (Rachel)

Wang, Tue Nguyen, Denise So, Matthew Sharp, Rodolfo da Silva, Cyrella Gabriel, Joshua Scantlebury, Marissa Jasinski, David Ackerman, Timothy Jewison, Tanvir Sajed, Vasuk Gautam, and David S. Wishart. DrugBank 6.0: the DrugBank Knowledgebase for 2024. *Nucleic Acids Research*, 52(D1):D1265–D1275, jan 2024. ISSN 0305-1048. doi: 10.1093/nar/gkad976. URL https://dx.doi.org/10.1093/nar/gkad976.

[603] Antoine Daina, Olivier Michielin, and Vincent Zoete. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7:42717, mar 2017. ISSN 2045-2322. doi: 10.1038/srep42717.

[604] Igor V. Tetko and Vsevolod Yu Tanchuk. Application of associative neural networks for prediction of lipophilicity in ALOGPS 2.1 program. *Journal of Chemical Information and Computer Sciences*, 42(5):1136–1145, 2002. ISSN 0095-2338. doi: 10.1021/ci025515j.

[605] Sunghwan Kim, Paul A. Thiessen, Evan E. Bolton, Jie Chen, Gang Fu, Asta Gindulyte, Lianyi Han, Jane He, Siqian He, Benjamin A. Shoemaker, Jiyao Wang, Bo Yu, Jian Zhang, and Stephen H. Bryant. PubChem Substance and Compound databases. *Nucleic Acids Research*, 44(D1):D1202–1213, jan 2016. ISSN 1362-4962. doi: 10.1093/nar/gkv951.

[606] Sunghwan Kim, Jie Chen, Tiejun Cheng, Asta Gindulyte, Jia He, Siqian He, Qingliang Li, Benjamin A. Shoemaker, Paul A. Thiessen, Bo Yu, Leonid Zaslavsky, Jian Zhang, and Evan E. Bolton. PubChem 2023 update. *Nucleic Acids Research*, 51(D1):D1373–D1380, jan 2023. ISSN 1362-4962. doi: 10.1093/nar/gkac956.

[607] S. Prasanna and R. J. Doerksen. Topological polar surface area: a useful descriptor in 2D-QSAR. *Current Medicinal Chemistry*, 16(1):21–41, 2009. ISSN 0929-8673. doi: 10.2174/092986709787002817.

[608] Ina Hubatsch, Eva G. E. Ragnarsson, and Per Artursson. Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nature Protocols*, 2(9):2111–2119, 2007. ISSN 1750-2799. doi: 10.1038/nprot.2007.303.

[609] Hans Lineweaver and Dean Burk. The determination of enzyme dissociation constants. *Journal of the American chemical society*, 56(3):658–666, 1934.

[610] Leonor Michaelis, Maud L Menten, et al. Die kinetik der invertinwirkung. *Biochem. z*, 49(333-369): 352, 1913.

[611] Leonor Michaelis, Maud Leonora Menten, Kenneth A. Johnson, and Roger S. Goody. The original Michaelis constant: translation of the 1913 Michaelis-Menten paper. *Biochemistry*, 50(39):8264–8269, October 2011. ISSN 1520-4995. doi: 10.1021/bi201284u.

[612] Michelle M. Butler and George E. Wright. A Method to Assay Inhibitors of DNA Polymerase IIIC Activity. In W. Scott Champney, editor, *New Antibiotic Targets*, Methods In Molecular Medicine™, pages 25–36. Humana Press, Totowa, NJ, 2008. ISBN 978-1-59745-246-5. URL https://doi.org/10.1007/978-1-59745-246-5_3.

[613] Andrea Torti, Andrea Lossani, Lida Savi, Federico Focher, George Edward Wright, Neal Curtis Brown, and Wei-Chu Xu. Clostridium difficile DNA polymerase IIIC: basis for activity of antibacterial compounds. *Current Enzyme Inhibition*, 7(3):147–153, oct 2011. ISSN 1573-4080. doi: 10.2174/157340811798807597. URL https://www.ncbi.nlm.nih.gov/pubmed/22844265.

[614] E. van Eijk, I. M. Boekhoud, E. J. Kuijper, I. Bos-Sanders, G. Wright, and W. K. Smits. Genome location dictates the transcriptional response to polc inhibition in clostridium difficile. *Antimicrobial Agents and Chemotherapy*, 63:e01263–18, 2019. doi: 10.1128/AAC.01263-18.

[615] Beverly Murray, Cindy Wolfe, Andrea Marra, Chris Pillar, and Dean Shinabarger. In vitro activity of the novel antibacterial agent ibezapolstat (ACX-362E) against Clostridioides difficile. *The Journal of Antimicrobial Chemotherapy*, 75(8):2149–2155, aug 2020. ISSN 1460-2091. doi: 10.1093/jac/dkaa134.

[616] Orna Schwartz, Maya Azrad, and Avi Peretz. In vitro susceptibility of clinical Clostridioides difficile isolates in Israel to metronidazole, vancomycin, fidaxomicin, ridinilazole and ibezapolstat. *BMC gastroenterology*, 25(1):209, mar 2025. ISSN 1471-230X. doi: 10.1186/s12876-025-03800-7.

[617] Eugénie Bassères, Taryn A. Eubank, Khurshida Begum, M. Jahangir Alam, Jinhee Jo, Thanh M. Le, Chris K. Lancaster, Anne J. Gonzales-Luna, and Kevin W. Garey. Antibacterial activity of ibezapolstat against antimicrobial-resistant clinical strains of Clostridioides difficile. *Antimicrobial Agents and Chemotherapy*, 68(3):e0162123, mar 2024. ISSN 1098-6596. doi: 10.1128/aac.01621-23.

[618] Sofya Dvoskin, Wei-Chu Xu, Neal C. Brown, Ivan B. Yanachkov, Milka Yanachkova, and George E. Wright. A novel agent effective against Clostridium difficile infection. *Antimicrobial Agents and Chemotherapy*, 56(3):1624–1626, mar 2012. ISSN 1098-6596. doi: 10.1128/AAC.06097-11.

[619] Trenton M. Wolfe, Jinhee Jo, Nick V. Pinkham, Kevin W. Garey, and Seth T. Walk. The impact of ibezapolstat and other Clostridioides difficile infection-relevant antibiotics on the microbiome of humanized mice. *Antimicrobial Agents and Chemotherapy*, 69(4):e0160424, apr 2025. ISSN 1098-6596. doi: 10.1128/aac.01604-24.

[620] M. J. Alam, S. T. Walk, B. T. Endres, and et al. Community environmental contamination of toxigenic clostridium difficile. *Open Forum Infectious Diseases*, 4(1):ofx018, 2017. doi: 10.1093/ofid/ofx018.

[621] E. Bassères, B. T. Endres, K. M. Dotson, M. J. Alam, and K. W. Garey. Novel antibiotics in development to treat clostridium difficile infection. *Current Opinion in Gastroenterology*, 33:1–7, 2017. doi: 10.1097/MOG.0000000000000332.

[622] B. T. Endres, E. Bassères, M. J. Alam, and K. W. Garey. Cadazolid for the treatment of clostridium difficile. *Expert Opinion on Investigational Drugs*, 26:509–514, 2017. doi: 10.1080/13543784.2017.1304538.

[623] D. N. Gerding, O. A. Cornely, S. Grill, H. Kracker, A. C. Marrast, C. E. Nord, G. H. Talbot, M. Buitrago, I. Gheorghe Diaconescu, C. Murta de Oliveira, L. Preotescu, J. Pullman, T. J. Louie, and M. H. Wilcox. Cadazolid for the treatment of clostridium difficile infection: results of two double-blind, placebo-controlled, non-inferiority, randomised phase 3 trials. *The Lancet Infectious Diseases*, 19:265–274, 2019. doi: 10.1016/S1473-3099(18)30614-5.

[624] B. T. Endres, E. Bassères, M. Khaleduzzaman, M. J. Alam, L. Chesnel, and K. W. Garey. Evaluating the effects of surotomycin treatment on clostridium difficile toxin a and b production, immune response, and morphological changes. *Antimicrobial Agents and Chemotherapy*, 60:3519–3523, 2016. doi: 10.1128/AAC.00211-16.

[625] C. H. Lee, H. Patino, C. Stevens, S. Rege, L. Chesnel, T. Louie, and K. M. Mullane. Surotomycin versus vancomycin for clostridium difficile infection: phase 2, randomized, controlled, double-blind, non-inferiority, multicentre trial. *Journal of Antimicrobial Chemotherapy*, 71:2964–2971, 2016. doi: 10.1093/jac/dkw246.

[626] V. Boix, R. N. Fedorak, K. M. Mullane, Y. Pesant, U. Stoutenburgh, M. Jin, A. Adedoyin, L. Chesnel, D. Guris, K. B. Larson, and Y. Murata. Primary outcomes from a phase 3, randomized, double-blind, active-controlled trial of surotomycin in subjects with clostridium difficile infection. *Open Forum Infectious Diseases*, 4:ofw275, 2017. doi: 10.1093/ofid/ofw275.

[627] P. Daley, T. Louie, J. E. Lutz, S. Khanna, U. Stoutenburgh, M. Jin, A. Adedoyin, L. Chesnel, D. Guris, K. B. Larson, and Y. Murata. Surotomycin versus vancomycin in adults with clostridium difficile infection: primary clinical outcomes from the second pivotal, randomized, double-blind, phase 3 trial. *Journal of Antimicrobial Chemotherapy*, 72:3462–3470, 2017. doi: 10.1093/jac/dkx299.

[628] Max Scherer, Carsten Gnewuch, Gerd Schmitz, and Gerhard Liebisch. Rapid quantification of bile acids and their conjugates in serum by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 877(30):3920–3925, nov 2009. ISSN 1873-376X. doi: 10.1016/j.jchromb.2009.09.038.

[629] Philipp Solbach, Patrick Chhatwal, Sabrina Woltemate, Evelina Tacconelli, Michael Buhl, Markus Gerhard, Christoph K. Thoeringer, Maria J. G. T. Vehreschild, Nathalie Jazmati, Jan Rupp, Michael P. Manns, Oliver Bachmann, and Sebastian Suerbaum. BaiCD gene cluster abundance is negatively correlated with Clostridium difficile infection. *PloS One*, 13(5):e0196977, 2018. ISSN 1932-6203. doi: 10.1371/journal.pone.0196977.

[630] Nicola Segata, Jacques Izard, Levi Waldron, Dirk Gevers, Larisa Miropolsky, Wendy S. Garrett, and Curtis Huttenhower. Metagenomic biomarker discovery and explanation. *Genome Biology*, 12(6): R60, 6 2011. ISSN 14747596. doi: 10.1186/gb-2011-12-6-r60.

[631] K. Korthauer, P. K. Kimes, C. Duvallet, A. Reyes, A. Subramanian, M. Teng, C. Shukla, E. J. Alm, and S. C. Hicks. A practical guide to methods controlling false discoveries in computational biology. *Genome Biology*, 20:118, 2019. doi: 10.1186/s13059-019-1716-1.

[632] Alyxandria M. Schubert, Hamide Sinani, and Patrick D. Schloss. Antibiotic-Induced Alterations of the Murine Gut Microbiota and Subsequent Effects on Colonization Resistance against Clostridium difficile. *mBio*, 6(4):e00974, jul 2015. ISSN 2150-7511. doi: 10.1128/mBio.00974-15.

[633] X. Qian, K. Yanagi, A. V. Kane, N. Alden, M. Lei, D. R. Snydman, R. J. Vickers, K. Lee, and C. M. Thorpe. Ridinilazole, a narrow spectrum antibiotic for treatment of clostridioides difficile infection, enhances preservation of microbiota-dependent bile acids. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 319:G227–G237, 2020. doi: 10.1152/ajpgi.00046.2020.

[634] Cheleste M. Thorpe, Anne V. Kane, Justin Chang, Albert Tai, Richard J. Vickers, and David R. Snydman. Enhanced preservation of the human intestinal microbiota by ridinilazole, a novel Clostridium difficile-targeting antibacterial, compared to vancomycin. *PloS One*, 13(8):e0199810, 2018. ISSN 1932-6203. doi: 10.1371/journal.pone.0199810.

[635] Ying Taur and Eric G. Pamer. Harnessing microbiota to kill a pathogen: Fixing the microbiota to treat Clostridium difficile infections. *Nature Medicine*, 20(3):246–247, mar 2014. ISSN 1546-170X. doi: 10.1038/nm.3492.

[636] Benjamin H. Mullish and Jessica R. Allegretti. The contribution of bile acid metabolism to the pathogenesis of Clostridioides difficile infection. *Therapeutic Advances in Gastroenterology*, 14: 17562848211017725, 2021. ISSN 1756-283X. doi: 10.1177/17562848211017725.

[637] L. Martinez-Gili, J. A. K. McDonald, Z. Liu, D. Kao, J. R. Allegretti, T. M. Monaghan, G. F. Barker, J. Miguéns Blanco, H. R. T. Williams, E. Holmes, M. R. Thursz, J. R. Marchesi, and B. H. Mullish. Understanding the mechanisms of efficacy of fecal microbiota transplant in treating recurrent clostridioides difficile infection and beyond: the contribution of gut microbial-derived metabolites. *Gut Microbes*, 12:1810531, 2020. doi: 10.1080/19490976.2020.1810531.

[638] J. R. Allegretti, S. Kearney, N. Li, E. Bogart, K. Bullock, G. K. Gerber, L. Bry, C. B. Clish, E. Alm, and J. R. Korzenik. Recurrent clostridium difficile infection associates with distinct bile acid and microbiome profiles. *Alimentary Pharmacology & Therapeutics*, 43:1142–1153, 2016. doi: 10.1111/apt.13616.

[639] J. M. Ridlon, S. Devendran, J. M. Alves, H. Doden, P. G. Wolf, G. V. Pereira, L. Ly, A. Volland, H. Takei, H. Nittono, T. Murai, T. Kurosawa, G. E. Chlipala, S. J. Green, A. G. Hernandez, C. J. Fields, C. L. Wright, G. Kakiyama, I. Cann, P. Kashyap, V. McCracken, and H. R. Gaskins. The 'in vivo lifestyle' of bile acid 7alpha-dehydroxylating bacteria: comparative genomics, metatranscriptomic, and bile acid metabolomics analysis of a defined microbial community in gnotobiotic mice. *Gut Microbes*, 11:381–404, 2020. doi: 10.1080/19490976.2019.1618173.

[640] M. Vital, T. Rud, S. Rath, D. H. Pieper, and D. Schlüter. Diversity of bacteria exhibiting bile acid-inducible 7alpha-dehydroxylation genes in the human gut. *Computational and Structural Biotechnology Journal*, 17:1016–1019, 2019. doi: 10.1016/j.csbj.2019.07.012.

[641] A. Heinken, D. A. Ravcheev, F. Baldini, L. Heirendt, R. M. T. Fleming, and I. Thiele. Systematic assessment of secondary bile acid metabolism in gut microbes reveals distinct metabolic capabilities in inflammatory bowel disease. *Microbiome*, 7:75, 2019. doi: 10.1186/s40168-019-0689-3.

[642] C. J. Stewart, N. J. Ajami, J. L. O'Brien, D. S. Hutchinson, D. P. Smith, M. C. Wong, M. C. Ross, R. E. Lloyd, H. Doddapaneni, G. A. Metcalf, D. Muzny, R. A. Gibbs, T. Vatanen, C. Huttenhower, R. J. Xavier, M. Rewers, W. Hagopian, J. Toppari, A.-G. Ziegler, J.-X. She, B. Akolkar, Å. Lernmark, H. Hyöty, K. Vehik, J. P. Krischer, and J. F. Petrosino. Temporal development of the gut microbiome in early childhood from the teddy study. *Nature*, 562:583–588, 2018. doi: 10.1038/s41586-018-0617-x.

[643] A. J. Gonzales-Luna, J. K. Spinler, N. Oezguen, and et al. Systems biology evaluation of refractory clostridioides difficile infection including multiple failures of fecal microbiota transplantation. *Anaerobe*, 70:102387, 2021. doi: 10.1016/j.anaerobe.2021.102387.

[644] D. Oka, N. Yamaya, T. Kuno, and et al. In vitro and in vivo antibacterial activities of a novel quinolone compound, ops-2071, against clostridioides difficile. *Antimicrobial Agents and Chemotherapy*, 65:e01170–20, 2021. doi: 10.1128/AAC.01170-20.

[645] A. Khoruts, J. Dicksved, J. K. Jansson, and M. J. Sadowsky. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent clostridium difficile-associated diarrhea. *Journal of Clinical Gastroenterology*, 44:354–360, 2010. doi: 10.1097/MCG.0b013e3181c87e02.

[646] A. J. Gonzales-Luna, T. J. Carlson, K. M. Dotson, and et al. Pcr ribotypes of clostridioides difficile across texas from 2011 to 2018 including emergence of ribotype 255. *Emerging Microbes & Infections*, 9:341–347, 2020. doi: 10.1080/22221751.2020.1716906.

[647] K. Begum, E. Bassères, J. Miranda, and et al. In vitro activity of omadacycline, a new tetracycline analog, and comparators against clostridioides difficile. *Antimicrobial Agents and Chemotherapy*, 64:e00522–20, 2020. doi: 10.1128/AAC.00522-20.

[648] D. W. Fadrosh, B. Ma, P. Gajer, and et al. An improved dual-indexing approach for multiplexed 16s rrna gene sequencing on the illumina miseq platform. *Microbiome*, 2:6, 2014. doi: 10.1186/2049-2618-2-6.

[649] J. N. Walker, B. M. Hanson, C. L. Pinkner, and et al. Insights into the microbiome of breast implants and periprosthetic tissue in breast implant-associated anaplastic large cell lymphoma. *Scientific Reports*, 9:10393, 2019. doi: 10.1038/s41598-019-46752-3.

[650] R Core Team. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria, 2013. URL http://www.R-project.org/. Accessed 22 February 2022.

[651] J. M. Ridlon, D. J. Kang, and P. B. Hylemon. Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research*, 47:241–259, 2006. doi: 10.1194/jlr.R500013-JLR200.

[652] R. Kullar, M. N. Tran, and E. J. C. Goldstein. Investigational treatment agents for recurrent clostridioides difficile infection (rcdi). *Journal of Experimental Pharmacology*, 12:371–384, 2020. doi: 10.2147/JEP.S226264.

[653] C. Rubio-Terrés, J. M. Aguado, B. Almirante, J. Cobo, S. Grau, M. Salavert, E. González Antona Sánchez, C. López Gutiérrez, and D. Rubio-Rodríguez. Extended-pulsed fidaxomicin versus vancomycin in patients 60 years and older with clostridium difficile infection: cost-effectiveness analysis in spain. *European Journal of Clinical Microbiology & Infectious Diseases*, 38:1105–1111, 2019. doi: 10.1007/s10096-019-03503-4.

[654] R. K. R. Marreddy, X. Wu, M. Sapkota, A. M. Prior, J. A. Jones, D. Sun, K. E. Hevener, and J. G. Hurdle. The fatty acid synthesis protein enoyl-acp reductase ii (fabk) is a target for narrow-spectrum antibacterials for clostridium difficile infection. *ACS Infectious Diseases*, 5:208–217, 2019. doi: 10.1021/acsinfecdis.8b00205.

[655] Daniel H. Haft, Michael DiCuccio, Azat Badretdin, Vyacheslav Brover, Vyacheslav Chetvernin, Kathleen O'Neill, Wenjun Li, Farideh Chitsaz, Myra K. Derbyshire, Noreen R. Gonzales, Marc Gwadz, Fu Lu, Gabriele H. Marchler, James S. Song, Narmada Thanki, Roxanne A. Yamashita, Chanjuan Zheng, Françoise Thibaud-Nissen, Lewis Y. Geer, Aron Marchler-Bauer, and Kim D. Pruitt. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Research*, 46(D1):D851–D860, jan 2018. ISSN 1362-4962. doi: 10.1093/nar/gkx1068. URL https://www.ncbi.nlm.nih.gov/pubmed/29112715.

[656] Wenjun Li, Kathleen R O'Neill, Daniel H. Haft, Michael DiCuccio, Vyacheslav Chetvernin, Azat Badretdin, George Coulouris, Farideh Chitsaz, Myra K Derbyshire, A. Scott Durkin, Noreen R. Gonzales, Marc Gwadz, Christopher J Lanczycki, James S. Song, Narmada Thanki, Jiyao Wang, Roxanne A Yamashita, Mingzhang Yang, Chanjuan Zheng, Aron Marchler-Bauer, and Françoise Thibaud-Nissen. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. *Nucleic Acids Research*, 49(D1):D1020–D1028, jan 2021. ISSN 0305-1048. doi: 10.1093/nar/gkaa1105. URL https://pubmed.ncbi.nlm.nih.gov/33270901/https://academic.oup.com/nar/article/49/D1/D1020/6018440. 645 citations (Crossref) [2025-02-20] 156 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Nucleic Acids Res.

[657] T. Tatusova, M. DiCuccio, A. Badretdin, V. Chetvernin, E. P. Nawrocki, L. Zaslavsky, A. Lomsadze, K. D. Pruitt, M. Borodovsky, and J. Ostell. Ncbi prokaryotic genome annotation pipeline. *Nucleic Acids Res*, 44(14):6614–24, 2016. ISSN 1362-4962 (Electronic) 0305-1048 (Print) 0305-1048 (Linking). doi: 10.1093/nar/gkw569. URL https://www.ncbi.nlm.nih.gov/pubmed/27342282.

[658] John Jumper, Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger, Kathryn Tunyasuvunakool, Russ Bates, Augustin Žídek, Anna Potapenko, Alex Bridgland, Clemens Meyer, Simon A. A. Kohl, Andrew J. Ballard, Andrew Cowie, Bernardino Romera-Paredes, Stanislav Nikolov, Rishub Jain, Jonas Adler, Trevor Back, Stig Petersen, David Reiman, Ellen Clancy, Michal Zielinski, Martin Steinegger, Michalina Pacholska, Tamas Berghammer, Sebastian Bodenstein, David Silver, Oriol Vinyals, Andrew W. Senior, Koray Kavukcuoglu, Pushmeet Kohli, and Demis Hassabis. Highly accurate protein structure prediction with AlphaFold. *Nature*, 596(7873):583–589, aug 2021. ISSN 1476-4687. doi: 10.1038/s41586-021-03819-2. URL https://www.nature.com/articles/s41586-021-03819-2https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8371605/pdf/41586_2021_Article_3819.pdf.

[659] M. Mirdita, K. Schütze, Y. Moriwaki, L. Heo, S. Ovchinnikov, and M. Steinegger. Colabfold: making protein folding accessible to all. *Nature Methods*, 19:679–682, 2022. doi: 10.1038/s41592-022-01488-1.

[660] M. Steinegger and J. Söding. Mmseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. *Nature Biotechnology*, 35:1026–1028, 2017. doi: 10.1038/nbt.3988.

[661] Y. Liu, M. Grimm, W.-T. Dai, M.-C. Hou, Z.-X. Xiao, and Y. Cao. Cb-dock: a web server for cavity detection-guided protein-ligand blind docking. *Acta Pharmacologica Sinica*, 41:138–144, 2020. doi: 10.1038/s41401-019-0228-6.

[662] J. Eberhardt, D. Santos-Martins, A. F. Tillack, and S. Forli. Autodock vina 1.2.0: new docking methods, expanded force field, and python bindings. *Journal of Chemical Information and Modeling*, 61:3891–3898, 2021. doi: 10.1021/acs.jcim.1c00203.

[663] D. Seeliger and B. L. de Groot. Ligand docking and binding site analysis with pymol and autodock/vina. *Journal of Computer-Aided Molecular Design*, 24:417–422, 2010. doi: 10.1007/s10822-010-9352-6.

[664] M. A. Lill and M. L. Danielson. Computer-aided drug design platform using pymol. *Journal of Computer-Aided Molecular Design*, 25:13–19, 2011. doi: 10.1007/s10822-010-9395-8.

[665] S. Salentin, S. Schreiber, V. J. Haupt, M. F. Adasme, and M. Schroeder. Plip: fully automated protein-ligand interaction profiler. *Nucleic Acids Research*, 43:W443–W447, 2015. doi: 10.1093/nar/gkv315.

[666] G. E. Crooks, G. Hon, J.-M. Chandonia, and S. E. Brenner. Weblogo: a sequence logo generator. *Genome Research*, 14:1188–1190, 2004. doi: 10.1101/gr.849004.

[667] G. E. Wright and J. J. Gambino. Quantitative structure-activity relationships of 6-anilinouracils as inhibitors of bacillus subtilis dna polymerase iii. *J Med Chem*, 27(2):181–5, 1984. ISSN 0022-2623 (Print) 0022-2623 (Linking). doi: 10.1021/jm00368a013. URL https://www.ncbi.nlm.nih.gov/pubmed/6420570.

[668] Wiep Klaas Smits, Mia Urem, Annemieke Friggen, Nina Musch, Michael Silverman, Christopher Swain, Michael Barbachyn, Lawrence Mortin, Xiang Yu, Robert DeLuccia, and Meindert Lamers. A unique inhibitor conformation selectively targets the DNA polymerase PolC of Gram-positive priority pathogens, jun 2025. URL https://www.researchsquare.com/article/rs-6470547/v1.

[669] C. E. Shannon. A mathematical theory of communication. *The Bell System Technical Journal*, 27 (3):379–423, jul 1948. ISSN 0005-8580. doi: 10.1002/j.1538-7305.1948.tb01338.x. URL https://ieeexplore.ieee.org/document/6773024.

[670] E. H. Simpson. Measurement of Diversity. *Nature*, 163(4148):688–688, apr 1949. ISSN 1476-4687. doi: 10.1038/163688a0. URL https://www.nature.com/articles/163688a0.

[671] J. Roger Bray and John T. Curtis. An ordination of the upland forest communities of southern wisconsin. *Ecological Monographs*, 27(4):325–349, 1957. doi: 10.2307/1942268.

[672] Andy Trotti, A. Dimitrios Colevas, Ann Setser, Valerie Rusch, David Jaques, Volker Budach, Corey Langer, Barbara Murphy, Richard Cumberlin, C. Norman Coleman, and Philip Rubin. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Seminars in Radiation Oncology*, 13(3):176–181, jul 2003. ISSN 1053-4296. doi: 10.1016/S1053-4296(03)00031-6.

[673] James W. Bartholomew and Tod Mittwer. THE GRAM STAIN. *Bacteriological Reviews*, 16(1): 1–29, mar 1952. ISSN 0005-3678. doi: 10.1128/br.16.1.1-29.1952. URL https://journals.asm.org/doi/10.1128/br.16.1.1-29.1952.

[674] IVAN C. HALL and ELIZABETH O'TOOLE. INTESTINAL FLORA IN NEW-BORN INFANTS: WITH A DESCRIPTION OF A NEW PATHOGENIC ANAEROBE, BACILLUS DIFFICILIS. *American Journal of Diseases of Children*, 49(2):390–402, feb 1935. ISSN 0096-8994. doi: 10.1001/archpedi.1935.01970020105010. URL https://doi.org/10.1001/archpedi.1935.01970020105010. 520 citations (Crossref) [2022-10-26].

[675] A. R. Prévot. Études de systématique bactérienne. iv. critique de la conception actuelle du genre *Clostridium*. *Annales de l'Institut Pasteur*, 61:72–91, 1938.

[676] Paul A. Lawson, Diane M. Citron, Kerin L. Tyrrell, and Sydney M. Finegold. Reclassification of Clostridium difficile as Clostridioides difficile (Hall and O'Toole 1935) Prévot 1938. *Anaerobe*, 40:95–99, 8 2016. ISSN 10759964. doi: 10.1016/j.anaerobe.2016.06.008. URL https://linkinghub.elsevier.com/retrieve/pii/S1075996416300762.

[677] R. E. Hungate. Studies on cellulose fermentation. i. the culture and physiology of an anaerobic cellulose-digesting bacterium. *Journal of Bacteriology*, 48(5):499–511, 1944.

[678] R. E. Hungate. The anaerobic mesophilic cellulolytic bacteria. *Bacteriological Reviews*, 14(1):1–49, mar 1950. ISSN 0005-3678. doi: 10.1128/br.14.1.1-49.1950.

[679] A. Arank, S. A. Syed, E. B. Kenney, and R. Freter. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. *Applied Microbiology*, 17 (4):568–576, apr 1969. ISSN 0003-6919. doi: 10.1128/am.17.4.568-576.1969.

[680] Jean-Christophe Lagier, Grégory Dubourg, Matthieu Million, Frédéric Cadoret, Melhem Bilen, Florence Fenollar, Anthony Levasseur, Jean-Marc Rolain, Pierre-Edouard Fournier, and Didier Raoult. Culturing the human microbiota and culturomics. *Nature Reviews. Microbiology*, 16:540–550, may 2018. ISSN 1740-1534. doi: 10.1038/s41579-018-0041-0.

[681] B. Halliwell. Biochemical mechanisms accounting for the toxic action of oxygen on living organisms: the key role of superoxide dismutase. *Cell Biology International Reports*, 2(2):113–128, mar 1978. ISSN 0309-1651. doi: 10.1016/0309-1651(78)90032-2.

[682] F. P. Tally, B. R. Goldin, N. V. Jacobus, and S. L. Gorbach. Superoxide dismutase in anaerobic bacteria of clinical significance. *Infection and Immunity*, 16(1):20–25, apr 1977. ISSN 0019-9567. doi: 10.1128/iai.16.1.20-25.1977.

[683] F. E. Jenney, M. F. Verhagen, X. Cui, and M. W. Adams. Anaerobic microbes: oxygen detoxification without superoxide dismutase. *Science (New York, N.Y.)*, 286(5438):306–309, oct 1999. ISSN 0036-8075. doi: 10.1126/science.286.5438.306.

[684] Hualiang Pi, Rong Sun, James R. McBride, Angela R. S. Kruse, Katherine N. Gibson-Corley, Evan S. Krystofiak, Maribeth R. Nicholson, Jeffrey M. Spraggins, Qiangjun Zhou, and Eric P. Skaar. Clostridioides difficile ferrosome organelles combat nutritional immunity. *Nature*, 623(7989):1009–1016, nov 2023. ISSN 1476-4687. doi: 10.1038/s41586-023-06719-9. URL https://www.nature.com/articles/s41586-023-06719-9.

[685] Gary Sisson, Avery Goodwin, Ausra Raudonikiene, Nicky J. Hughes, Asish K. Mukhopadhyay, Douglas E. Berg, and Paul S. Hoffman. Enzymes associated with reductive activation and action of nitazoxanide, nitrofurans, and metronidazole in Helicobacter pylori. *Antimicrobial Agents and Chemotherapy*, 46(7):2116–2123, jul 2002. ISSN 0066-4804. doi: 10.1128/AAC.46.7.2116-2123.2002.

[686] David Leitsch, Anita G. Burgess, Linda A. Dunn, Kenia G. Krauer, Kevin Tan, Michael Duchêne, Peter Upcroft, Lars Eckmann, and Jacqueline A. Upcroft. Pyruvate:ferredoxin oxidoreductase and thioredoxin reductase are involved in 5-nitroimidazole activation while flavin metabolism is linked to 5-nitroimidazole resistance in Giardia lamblia. *The Journal of Antimicrobial Chemotherapy*, 66 (8):1756–1765, aug 2011. ISSN 1460-2091. doi: 10.1093/jac/dkr192.

[687] C. Wenisch, B. Parschalk, M. Hasenhündl, A. M. Hirschl, and W. Graninger. Comparison of vancomycin, teicoplanin, metronidazole, and fusidic acid for the treatment of Clostridium difficile-associated diarrhea. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 22(5):813–818, may 1996. ISSN 1058-4838. doi: 10.1093/clinids/22.5.813.

[688] Emma Jane Bishop and Ravindranath Tiruvoipati. Management of Clostridioides difficile infection in adults and challenges in clinical practice: review and comparison of current IDSA/SHEA, ESCMID and ASID guidelines. *Journal of Antimicrobial Chemotherapy*, 78(1):21–30, jan 2023. ISSN 0305-7453. doi: 10.1093/jac/dkac404. URL https://doi.org/10.1093/jac/dkac404. 28 citations (Crossref) [2025-02-20].

[689] Jacques Pepin, Marie-Eve Alary, Louis Valiquette, Evelyne Raiche, Joannie Ruel, Katalin Fulop, Dominique Godin, and Claude Bourassa. Increasing risk of relapse after treatment of Clostridium difficile colitis in Quebec, Canada. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 40(11):1591–1597, jun 2005. ISSN 1537-6591. doi: 10.1086/430315.

[690] Anne J Gonzales-Luna, Abiola O Olaitan, Wan-Jou Shen, Aditi Deshpande, Travis J Carlson, Kierra M Dotson, Chris Lancaster, Khurshida Begum, M Jahangir Alam, Julian G Hurdle, and Kevin W Garey. Reduced Susceptibility to Metronidazole Is Associated With Initial Clinical Failure in Clostridioides difficile Infection. *Open Forum Infectious Diseases*, 8(8):ofab365, aug 2021. ISSN 2328-8957. doi: 10.1093/ofid/ofab365. URL https://doi.org/10.1093/ofid/ofab365.

[691] Ilse M Boekhoud, Igor Sidorov, Sam Nooij, Céline Harmanus, Ingrid M J G Bos-Sanders, Virginie Viprey, William Spittal, Emma Clark, Kerrie Davies, Jane Freeman, Ed J Kuijper, Wiep Klaas Smits, and the COMBACTE-CDI Consortium. Haem is crucial for medium-dependent metronidazole resistance in clinical isolates of Clostridioides difficile. *Journal of Antimicrobial Chemotherapy*, 76(7):1731–1740, jul 2021. ISSN 0305-7453. doi: 10.1093/jac/dkab097. URL https://doi.org/10.1093/jac/dkab097.

[692] K. H. Wilson, M. J. Kennedy, and F. R. Fekety. Use of sodium taurocholate to enhance spore recovery on a medium selective for Clostridium difficile. *Journal of Clinical Microbiology*, 15(3):443–446, mar 1982. ISSN 0095-1137. doi: 10.1128/jcm.15.3.443-446.1982.

[693] W. L. George, V. L. Sutter, D. Citron, and S. M. Finegold. Selective and differential medium for isolation of Clostridium difficile. *Journal of Clinical Microbiology*, 9(2):214–219, feb 1979. ISSN 0095-1137. doi: 10.1128/jcm.9.2.214-219.1979.

[694] T. Karasawa, S. Ikoma, K. Yamakawa, and S. Nakamura. A defined growth medium for Clostridium difficile. *Microbiology (Reading, England)*, 141 (Pt 2):371–375, feb 1995. ISSN 1350-0872. doi: 10.1099/13500872-141-2-371.

[695] David S. Wishart, Craig Knox, An Chi Guo, Dean Cheng, Savita Shrivastava, Dan Tzur, Bijaya Gautam, and Murtaza Hassanali. DrugBank: A knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Research*, 2006. doi: 10.1093/nar/gkm958.

[696] Zhengyu Feng and Raúl G. Barletta. Roles of Mycobacterium smegmatis D-alanine:D-alanine ligase and D-alanine racemase in the mechanisms of action of and resistance to the peptidoglycan inhibitor D-cycloserine. *Antimicrobial Agents and Chemotherapy*, 47(1):283–291, jan 2003. ISSN 0066-4804. doi: 10.1128/AAC.47.1.283-291.2003.

[697] A. E. Belanger, J. C. Porter, and G. F. Hatfull. Genetic analysis of peptidoglycan biosynthesis in mycobacteria: characterization of a ddlA mutant of Mycobacterium smegmatis. *Journal of Bacteriology*, 182(23):6854–6856, dec 2000. ISSN 0021-9193. doi: 10.1128/JB.182.23.6854-6856.2000.

[698] Hongbaek Cho, Tsuyoshi Uehara, and Thomas G. Bernhardt. Beta-Lactam Antibiotics Induce a Lethal Malfunctioning of the Bacterial Cell Wall Synthesis Machinery. *Cell*, 159(6):1300–1311, dec 2014. ISSN 0092-8674, 1097-4172. doi: 10.1016/j.cell.2014.11.017. URL https://www.cell.com/cell/abstract/S0092-8674(14)01448-2.

[699] M. Matsuhashi and S. Tamaki. Enzymatic studies on the mechanism of action of cefoxitin. Correlation between the affinities of cefoxitin to penicillin-binding proteins and its rates of inhibition of the respective penicillin-sensitive reactions in E. coli. *The Journal of Antibiotics*, 31(12):1292–1295, dec 1978. ISSN 0021-8820. doi: 10.7164/antibiotics.31.1292.

[700] R. Williamson, R. Hakenbeck, and A. Tomasz. In vivo interaction of beta-lactam antibiotics with the penicillin-binding proteins of Streptococcus pneumoniae. *Antimicrobial Agents and Chemotherapy*, 18(4):629–637, oct 1980. ISSN 0066-4804. doi: 10.1128/AAC.18.4.629.

[701] Marta Toth, Nichole K. Stewart, Clyde Smith, and Sergei B. Vakulenko. Intrinsic Class D β-Lactamases of Clostridium difficile. *mBio*, 9(6):e01803–18, dec 2018. ISSN 2150-7511. doi: 10.1128/mBio.01803-18.

[702] Marta Toth, Nuno Tiago Antunes, Nichole K. Stewart, Hilary Frase, Monolekha Bhattacharya, Clyde A. Smith, and Sergei B. Vakulenko. Class D β-lactamases do exist in Gram-positive bacteria. *Nature Chemical Biology*, 12(1):9–14, 1 2016. ISSN 15524469. doi: 10.1038/nchembio.1950.

[703] Alexander Fleming. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. *British journal of experimental pathology*, 10 (3):226–236, jun 1929. ISSN 0007-1021. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2048009/.

[704] Miao He, Mohammed Sebaihia, Trevor D. Lawley, Richard A. Stabler, Lisa F. Dawson, Melissa J. Martin, Kathryn E. Holt, Helena M.B. Seth-Smith, Michael A. Quail, Richard Rance, Karen Brooks, Carol Churcher, David Harris, Stephen D. Bentley, Christine Burrows, Louise Clark, Craig Corton, Vicky Murray, Graham Rose, Scott Thurston, Andries van Tonder, Danielle Walker, Brendan W. Wren, Gordon Dougan, and Julian Parkhill. Evolutionary dynamics of Clostridium difficile over short and long time scales. *Proceedings of the National Academy of Sciences of the United States of America*, 107(16):7527–7532, apr 2010. ISSN 0027-8424. doi: 10.1073/pnas.0914322107. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2867753/.

[705] Daniel R. Knight, Briony Elliott, Barbara J. Chang, Timothy T. Perkins, and Thomas V. Riley. Diversity and Evolution in the Genome of Clostridium difficile. *Clinical Microbiology Reviews*, 28(3): 721–741, jul 2015. ISSN 1098-6618. doi: 10.1128/CMR.00127-14.

[706] Salvador Castañeda-Barba, Eva M. Top, and Thibault Stalder. Plasmids, a molecular cornerstone of antimicrobial resistance in the One Health era. *Nature Reviews. Microbiology*, 22(1):18–32, jan 2024. ISSN 1740-1534. doi: 10.1038/s41579-023-00926-x.

[707] Mohammed Sebaihia, Brendan W. Wren, Peter Mullany, Neil F. Fairweather, Nigel Minton, Richard Stabler, Nicholas R. Thomson, Adam P. Roberts, Ana M. Cerdeño-Tárraga, Hongmei Wang, Matthew TG Holden, Anne Wright, Carol Churcher, Michael A. Quail, Stephen Baker, Nathalie Bason, Karen Brooks, Tracey Chillingworth, Ann Cronin, Paul Davis, Linda Dowd, Audrey Fraser, Theresa Feltwell, Zahra Hance, Simon Holroyd, Kay Jagels, Sharon Moule, Karen

Mungall, Claire Price, Ester Rabbinowitsch, Sarah Sharp, Mark Simmonds, Kim Stevens, Louise Unwin, Sally Whithead, Bruno Dupuy, Gordon Dougan, Bart Barrell, and Julian Parkhill. The multidrug-resistant human pathogen Clostridium difficile has a highly mobile, mosaic genome. *Nature Genetics*, 38(7):779–786, jul 2006. ISSN 1546-1718. doi: 10.1038/ng1830. URL https://www.nature.com/articles/ng1830.

[708] Thomas Hundsberger, Veil Braun, Manfred Weidmann, Petra Leukel, Markus Sauerborn, and Christoph Von Eichel-Streiber. Transcription Analysis of the Genes tcdA-E of the Pathogenicity Locus of Clostridium Difficile. *European Journal of Biochemistry*, 244(3):735–742, 1997. ISSN 1432-1033. doi: 10.1111/j.1432-1033.1997.t01-1-00735.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1432-1033.1997.t01-1-00735.x.

[709] Enhui Shen, Kangli Zhu, Danyang Li, Zhenrui Pan, Yun Luo, Qiao Bian, Liuqing He, Xiaojun Song, Ying Zhen, Dazhi Jin, and Liang Tao. Subtyping analysis reveals new variants and accelerated evolution of Clostridioides difficile toxin B. *Communications Biology*, 3(1):347, jul 2020. ISSN 2399-3642. doi: 10.1038/s42003-020-1078-y.

[710] Charles J. Norsigian, Heather A. Danhof, Colleen K. Brand, Numan Oezguen, Firas S. Midani, Bernhard O. Palsson, Tor C. Savidge, Robert A. Britton, Jennifer K. Spinler, and Jonathan M. Monk. Systems biology analysis of the Clostridioides difficile core-genome contextualizes microenvironmental evolutionary pressures leading to genotypic and phenotypic divergence. *npj Systems Biology and Applications*, 6(1):31, oct 2020. ISSN 2056-7189. doi: 10.1038/s41540-020-00151-9. URL https://www.nature.com/articles/s41540-020-00151-9.

[711] Volker Gürtler and Vilma A. Stanisich. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. *Microbiology (Reading, England)*, 142 (Pt 1):3–16, jan 1996. ISSN 1350-0872. doi: 10.1099/13500872-142-1-3.

[712] V. Gürtler. The role of recombination and mutation in 16S-23S rDNA spacer rearrangements. *Gene*, 238(1):241–252, sep 1999. ISSN 0378-1119. doi: 10.1016/s0378-1119(99)00224-3.

[713] V. Gürtler and B. C. Mayall. Genomic approaches to typing, taxonomy and evolution of bacterial isolates. *International Journal of Systematic and Evolutionary Microbiology*, 51(Pt 1):3–16, jan 2001. ISSN 1466-5026. doi: 10.1099/00207713-51-1-3.

[714] V. Gurtler. Typing of Clostridium difficile strains by PCR-amplification of variable length 16S-23S rDNA spacer regions. *Journal of General Microbiology*, 139(12):3089–3097, dec 1993. ISSN 00221287. doi: 10.1099/00221287-139-12-3089/CITE/REFWORKS. URL https://www.microbiologyresearch.org/content/journal/micro/10.1099/00221287-139-12-3089. Publisher: Microbiology Society.

[715] V. Gurtler and H. D. Barrie. Typing of Staphylococcus aureus strains by PCR-amplification of variable-length 16S-23S rDNA spacer regions: Characterization of spacer sequences. *Microbiology*, 141(5):1255–1265, may 1995. ISSN 13500872. doi: 10.1099/13500872-141-5-1255/CITE/REFWORKS. URL https://www.microbiologyresearch.org/content/journal/micro/10.1099/13500872-141-5-1255. Publisher: Microbiology Society.

[716] Philippe Bidet, FrÃ©dÃ©ric Barbut, ValÃ©rie Lalande, BÃ©atrice Burghoffer, and Jean-Claude Petit. Development of a new PCR-ribotyping method for Clostridium difficile based on ribosomal

RNA gene sequencing. *FEMS Microbiology Letters*, 175(2):261–266, jun 1999. ISSN 0378-1097. doi: 10.1111/J.1574-6968.1999.TB13629.X. URL https://academic.oup.com/femsle/article/175/2/261/470918. 373 citations (Semantic Scholar/DOI) [2022-06-23] Publisher: Oxford Academic.

[717] Philippe Bidet, Valérie Lalande, Béatrice Salauze, Béatrice Burghoffer, Véronique Avesani, Michel Delmée, Anne Rossier, Frédéric Barbut, and Jean Claude Petit. Comparison of PCR-ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for typing Clostridium difficile. *Journal of Clinical Microbiology*, 38(7):2484–2487, 2000. ISSN 00951137. doi: 10.1128/JCM.38.7.2484-2487.2000/FORMAT/EPUB. URL https://journals.asm.org/journal/jcm.

[718] Charlotte A. Huber, Niki F. Foster, Thomas V. Riley, and David L. Paterson. Challenges for Standardization of Clostridium difficile Typing Methods. *Journal of Clinical Microbiology*, 51(9):2810–2814, sep 2013. doi: 10.1128/jcm.00143-13. URL https://journals.asm.org/doi/full/10.1128/jcm.00143-13.

[719] Warren N Fawley, C W Knetsch, Duncan R MacCannell, Celine Harmanus, Tim Du, Michael R Mulvey, Ashley Paulick, Lydia Anderson, E J Kuijper, and Mark H Wilcox. Development and validation of an internationally-standardized, high-resolution capillary gel-based electrophoresis PCR-ribotyping protocol for Clostridium difficile. *PloS one*, 10(2):e0118150, feb 2015. ISSN 1932-6203. doi: 10.1371/journal.pone.0118150. URL http://www.ncbi.nlm.nih.gov/pubmed/25679978http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4332677https://dx.plos.org/10.1371/journal.pone.0118150. 157 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: Public Library of Science.

[720] Jonathan N.V. Martinson, Susan Broadaway, Egan Lohman, Christina Johnson, M. Jahangir Alam, Mohammed Khaleduzzaman, Kevin W. Garey, Jessica Schlackman, Vincent B. Young, Kavitha Santhosh, Krishna Raod, Robert H. Lyons, and Seth T. Walk. Evaluation of portability and cost of a fluorescent PCR ribotyping protocol for Clostridium difficile epidemiology. *Journal of Clinical Microbiology*, 53(4):1192–1238, apr 2015. ISSN 1098660X. doi: 10.1128/JCM.03591-14. URL https://pubmed.ncbi.nlm.nih.gov/25631804/http://dx.doi.org/10.1128/JCM.03591-14. 40 citations (Semantic Scholar/DOI) [2022-09-12] Publisher: J Clin Microbiol.

[721] Josué Flores-Kim, Genevieve S Dobihal, Andrew Fenton, David Z Rudner, and Thomas G Bernhardt. A switch in surface polymer biogenesis triggers growth-phase-dependent and antibiotic-induced bacteriolysis. *eLife*, 8:e44912, apr 2019. ISSN 2050-084X. doi: 10.7554/eLife.44912. URL https://doi.org/10.7554/eLife.44912.

[722] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, and P. E. Bourne. The Protein Data Bank. *Nucleic Acids Research*, 28(1):235–242, jan 2000. ISSN 0305-1048. doi: 10.1093/nar/28.1.235.

[723] Helen M. Berman and Stephen K. Burley. Protein Data Bank (PDB): Fifty-three years young and having a transformative impact on science and society. *Quarterly Reviews of Biophysics*, 58:e9, feb 2025. ISSN 1469-8994. doi: 10.1017/S0033583525000034.

[724] B. M. Duggar. Aureomycin; a product of the continuing search for new antibiotics. *Annals of the New York Academy of Sciences*, 51(Art. 2):177–181, nov 1948. ISSN 0077-8923. doi: 10.1111/j.1749-6632.1948.tb27262.x.

[725] Donald P Levine. Vancomycin: A History. *Clinical Infectious Diseases*, 2006. doi: 10.1086/491709. URL https://academic.oup.com/cid/article-abstract/42/Supplement_1/S5/275962. 634 citations (Semantic Scholar/DOI) [2022-06-24].

[726] Robert C. Moellering Jr. Vancomycin: a 50-year reassessment, 2006. URL https://academic.oup.com/cid/article-abstract/42/Supplement_1/S3/275483.

[727] G. Cammarota, L. Masucci, G. Ianiro, S. Bibbò, G. Dinoi, G. Costamagna, M. Sanguinetti, and A. Gasbarrini. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. *Alimentary Pharmacology & Therapeutics*, 41(9):835–843, may 2015. ISSN 1365-2036. doi: 10.1111/apt.13144.

[728] Susy S. Hota, Valerie Sales, George Tomlinson, Mary Jane Salpeter, Allison McGeer, Bryan Coburn, David S. Guttman, Donald E. Low, and Susan M. Poutanen. Oral Vancomycin Followed by Fecal Transplantation Versus Tapering Oral Vancomycin Treatment for Recurrent Clostridium difficile Infection: An Open-Label, Randomized Controlled Trial. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 64(3):265–271, feb 2017. ISSN 1537-6591. doi: 10.1093/cid/ciw731.

[729] G. Ianiro, L. Masucci, G. Quaranta, C. Simonelli, L. R. Lopetuso, M. Sanguinetti, A. Gasbarrini, and G. Cammarota. Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory Clostridium difficile infection-single versus multiple infusions. *Alimentary Pharmacology & Therapeutics*, 48(2):152–159, jul 2018. ISSN 1365-2036. doi: 10.1111/apt.14816.

[730] Iris Figueroa, Stuart Johnson, Susan P. Sambol, Ellie J. C. Goldstein, Diane M. Citron, and Dale N. Gerding. Relapse versus reinfection: recurrent Clostridium difficile infection following treatment with fidaxomicin or vancomycin. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 55 Suppl 2(Suppl 2):S104–109, aug 2012. ISSN 1537-6591. doi: 10.1093/cid/cis357.

[731] Derrick W. Crook, A. Sarah Walker, Yin Kean, Karl Weiss, Oliver A. Cornely, Mark A. Miller, Roberto Esposito, Thomas J. Louie, Nicole E. Stoesser, Bernadette C. Young, Brian J. Angus, Sherwood L. Gorbach, Timothy E. A. Peto, and Study 003/004 Teams. Fidaxomicin versus vancomycin for Clostridium difficile infection: meta-analysis of pivotal randomized controlled trials. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 55 Suppl 2(Suppl 2):S93–103, aug 2012. ISSN 1537-6591. doi: 10.1093/cid/cis499.

[732] Kathleen M. Mullane, Mark A. Miller, Karl Weiss, Arnold Lentnek, Yoav Golan, Pamela S. Sears, Youe-Kong Shue, Thomas J. Louie, and Sherwood L. Gorbach. Efficacy of fidaxomicin versus vancomycin as therapy for Clostridium difficile infection in individuals taking concomitant antibiotics for other concurrent infections. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 53(5):440–447, sep 2011. ISSN 1537-6591. doi: 10.1093/cid/cir404.

[733] Harry F. Noller. RNA Structure: Reading the Ribosome. *Science*, 309(5740):1508–1514, sep 2005. doi: 10.1126/science.1111771. URL https://www.science.org/doi/full/10.1126/science.1111771.

[734] Gary J. Quigley and Alexander Rich. Structural Domains of Transfer RNA Molecules. *Science*, 194 (4267):796–806, nov 1976. doi: 10.1126/science.790568. URL https://www.science.org/doi/abs/10.1126/science.790568.

[735] Jerard Hurwitz. The discovery of RNA polymerase. *The Journal of Biological Chemistry*, 280(52): 42477–42485, dec 2005. ISSN 0021-9258. doi: 10.1074/jbc.X500006200.

[736] Dmitry G. Vassylyev, Shun-ichi Sekine, Oleg Laptenko, Jookyung Lee, Marina N. Vassylyeva, Sergei Borukhov, and Shigeyuki Yokoyama. Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6 A resolution. *Nature*, 417(6890):712–719, jun 2002. ISSN 0028-0836. doi: 10.1038/nature752.

[737] Finn Werner. Structure and function of archaeal RNA polymerases. *Molecular Microbiology*, 65(6): 1395–1404, sep 2007. ISSN 0950-382X. doi: 10.1111/j.1365-2958.2007.05876.x.

[738] R. G. Roeder and W. J. Rutter. Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. *Nature*, 224(5216):234–237, oct 1969. ISSN 0028-0836. doi: 10.1038/224234a0.

[739] James Chen, Hande Boyaci, and Elizabeth A. Campbell. Diverse and Unified Mechanisms of Transcription Initiation in Bacteria. *Nature reviews. Microbiology*, 19(2):95–109, feb 2021. ISSN 1740-1526. doi: 10.1038/s41579-020-00450-2. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7855538/.

[740] R. R. Burgess, A. A. Travers, J. J. Dunn, and E. K. Bautz. Factor stimulating transcription by RNA polymerase. *Nature*, 221(5175):43–46, jan 1969. ISSN 0028-0836. doi: 10.1038/221043a0.

[741] Tomoya Baba, Takeshi Ara, Miki Hasegawa, Yuki Takai, Yoshiko Okumura, Miki Baba, Kirill A. Datsenko, Masaru Tomita, Barry L. Wanner, and Hirotada Mori. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular Systems Biology*, 2: 2006.0008, 2006. ISSN 1744-4292. doi: 10.1038/msb4100050.

[742] K. Kobayashi, S. D. Ehrlich, A. Albertini, G. Amati, K. K. Andersen, M. Arnaud, K. Asai, S. Ashikaga, S. Aymerich, P. Bessieres, F. Boland, S. C. Brignell, S. Bron, K. Bunai, J. Chapuis, L. C. Christiansen, A. Danchin, M. Débarbouille, E. Dervyn, E. Deuerling, K. Devine, S. K. Devine, O. Dreesen, J. Errington, S. Fillinger, S. J. Foster, Y. Fujita, A. Galizzi, R. Gardan, C. Eschevins, T. Fukushima, K. Haga, C. R. Harwood, M. Hecker, D. Hosoya, M. F. Hullo, H. Kakeshita, D. Karamata, Y. Kasahara, F. Kawamura, K. Koga, P. Koski, R. Kuwana, D. Imamura, M. Ishimaru, S. Ishikawa, I. Ishio, D. Le Coq, A. Masson, C. Mauël, R. Meima, R. P. Mellado, A. Moir, S. Moriya, E. Nagakawa, H. Nanamiya, S. Nakai, P. Nygaard, M. Ogura, T. Ohanan, M. O'Reilly, M. O'Rourke, Z. Pragai, H. M. Pooley, G. Rapoport, J. P. Rawlins, L. A. Rivas, C. Rivolta, A. Sadaie, Y. Sadaie, M. Sarvas, T. Sato, H. H. Saxild, E. Scanlan, W. Schumann, J. F. M. L. Seegers, J. Sekiguchi, A. Sekowska, S. J. Séror, M. Simon, P. Stragier, R. Studer, H. Takamatsu, T. Tanaka, M. Takeuchi, H. B. Thomaides, V. Vagner, J. M. van Dijl, K. Watabe, A. Wipat, H. Yamamoto, M. Yamamoto, Y. Yamamoto, K. Yamane, K. Yata, K. Yoshida, H. Yoshikawa, U. Zuber, and N. Ogasawara. Essential Bacillus subtilis genes. *Proceedings of the National Academy of Sciences of the United States of America*, 100(8):4678–4683, apr 2003. ISSN 0027-8424. doi: 10.1073/pnas.0730515100.

[743] E. A. Campbell, N. Korzheva, A. Mustaev, K. Murakami, S. Nair, A. Goldfarb, and S. A. Darst. Structural mechanism for rifampicin inhibition of bacterial rna polymerase. *Cell*, 104(6):901–912, mar 2001. ISSN 0092-8674. doi: 10.1016/s0092-8674(01)00286-0.

[744] Michelle M. Nerandzic, Kathleen Mullane, Mark A. Miller, Farah Babakhani, and Curtis J. Donskey. Reduced acquisition and overgrowth of vancomycin-resistant enterococci and Candida species in patients treated with fidaxomicin versus vancomycin for Clostridium difficile infection. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 55 Suppl 2(Suppl 2):S121–126, aug 2012. ISSN 1537-6591. doi: 10.1093/cid/cis440.

[745] Louis Pasteur. Mémoire sur les corpuscules organisés qui existent dans l'atmosphère: examen de la doctrine des générations spontanées. *Annales des Sciences Naturelles, 4e série*, 16:5–98, 1861.

[746] A. Veillon and M. Zuber. Recherches sur la flore intestinale normale et pathologique du tube digestif de l'homme et de quelques animaux. *Comptes rendus des séances de la Société de Biologie*, 50: 189–192, 1898.

[747] Johann Friedrich Miescher. *Ueber die chemische Zusammensetzung der Eiterzellen*. 1871.

[748] Ralf Dahm. Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. *Human Genetics*, 122(6):565–581, jan 2008. ISSN 1432-1203. doi: 10.1007/s00439-007-0433-0.

[749] A Kossel and Albert Neumann. Ueber das thymin, ein spaltungsproduct der nucleinsäure. *Berichte der deutschen chemischen Gesellschaft*, 26(3):2753–2756, 1893.

[750] PA Levene. The structure of yeast nucleic acid. iii. ammonia hydrolysis. *Studies from the Rockefeller Institute for Medical Research: Reprints*, 30:231, 1919.

[751] R. E. Franklin and R. G. Gosling. Molecular configuration in sodium thymonucleate. *Nature*, 171 (4356):740–741, apr 1953. ISSN 0028-0836. doi: 10.1038/171740a0.

[752] J. D. Watson and F. H. Crick. Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature*, 171(4356):737–738, apr 1953. ISSN 0028-0836. doi: 10.1038/171737a0.

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