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Article

Simulating single versus cocktail antibiotic effects on the human gut microbiome

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SUMMARY

The human microbiome is an ecosystem of diverse microorganisms that reside within the body. It includes several stable and abundant bacterial species that define an individual's gut enterotype. These bacteria play a critical role in regulating metabolism and immune function. Antibiotics are intended to treat pathogenic bacterial infections, but they often act non-selectively, killing commensal bacteria in the gut microbiome. Antibiotics exert a selective pressure that promotes the development of antibiotic resistance through random bacterial mutations. Combining multiple antibiotics into a cocktail increases the number of mutations required for bacteria to become resistant to the treatment. However, these cocktails may also eliminate commensal species in the microbiome. We hypothesized that antibiotic cocktails composed of broad-spectrum drugs would lead to a more substantial reduction in gut microbial diversity compared to single-drug treatments. We used SimulATe, a computational model of the gut microbiome, to simulate the effects of penicillins, quinolones, tetracyclines, and trimethoprims administered individually and in combination across three gut enterotypes. We found that most single antibiotics preserved a subset of bacterial species within the microbiome. In contrast, we identified that antibiotic cocktails rapidly eliminated nearly all bacterial species, demonstrating the strong consequence of combinatorial treatments on the gut microbiome. Our findings underscore the importance of considering gut microbiome dynamics in antibiotic therapy, suggesting that antibiotic cocktails should be optimized to balance effective infection control with the preservation of microbiome integrity. These insights could inform clinical strategies to enhance patient outcomes and combat the growing challenge of antibiotic resistance.

INTRODUCTION

The human microbiome is a collection of trillions of microorganisms, including bacteria, yeast, and viruses (1). Recent advances in microbiome research have highlighted the intricate relationships between these microbial communities and their host, influencing processes from digestion to immune responses (2). For example, germ-free mice lacking a gut microbiome exhibit distinct immune cell gene expression profiles compared to mice with an intact microbiome (3). These transcriptional differences contribute to systemic immune deficiencies, such as impaired defenses against viruses or bacteria (3). The community structure of the human microbiome is shaped by both genetic and environmental factors (1, 2). Although an individual's microbiome may be composed of unique bacterial species at varying abundances, there is a strong consensus that any disruption to an existing balance in the microbiome community can trigger a wide range of maladies, from infection to cancer (1, 2, 4).

Antibiotics can greatly reshape the composition of the microbiome (4). Classical antibiotics target bacterial-specific structures and processes of cell replication and survival, such as the 30S or 50S ribosomal subunits or the cell wall (5). While this selectivity inflicts minimal damage to animal cells, most bacterial pathogens are structurally indistinguishable from good commensal bacteria of the microbiome (6). An antibiotic will indiscriminately target both pathogenic and commensal bacteria, disrupting the balanced community of the microbiome (6).

In addition to commensal bacteria, the microbiome may harbor opportunistic pathogens at low levels (7). Opportunistic pathogens are dynamically reintroduced to the microbiome from the environment (7). Abundant commensal bacteria normally inhibit the growth of opportunistic pathogens by competing for space and nutrients, as well as via the secretion of inhibitory molecules (7). Antibacterial inhibitory molecules naturally select for opportunistic pathogens with additional defenses, such as drug resistance genes (7). These concealed defenses become problematic upon the transient reduction in commensal bacteria from antibiotic treatment, which reduces competition, allowing antibiotic-resistant opportunistic pathogens to thrive (7). One such infection, *Clostridioides difficile*, has an 8- to 10- fold increase in occurrence risk following antibiotic therapy (8). Gut inflammation induced by C. difficile infection manifests in diarrhea, fever, and potentially life-threatening colitis (8). Ultimately, a reduction of microbiome diversity by antibiotic treatment can have profound and lasting impacts on host health (9).

Bacteria rapidly mutate and adapt to the selective pressure of antibiotics (10). Antibiotic resistance mechanisms include target-site mutation, drug inactivation, drug out-competition, and drug efflux (10). Difficult bacterial infections may necessitate combining multiple antibiotics to effectively clear the pathogen (10-13). Drug combinations are often designed to inhibit bacterial growth by multiple mechanisms of action, for example, by targeting the cell wall and the bacterial ribosomes (10-13). Thus, a single drug will usually target a narrower range of bacteria, whereas antibiotic cocktails will act broadly and

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can cause more extensive microbiome disruptions (14, 15). An added benefit of combining antibiotics is that it can reduce the likelihood of resistance because the bacterium would need to acquire multiple resistance mechanisms (11, 12, 15). Antibiotic combinations may also yield synergistic effects by simultaneously inhibiting multiple essential bacterial cellular functions, such as protein synthesis and folic acid synthesis (16, 17). So too, one drug may enhance bacterial sensitivity to another drug (15, 17).

Antibiotic cocktails are clinically relevant for several high-impact bacterial infections, including *Mycobacterium tuberculosis* (Mtb), *Helicobacter pylori*, and cases of sepsis (13). For example, a combinatorial treatment of isoniazid, rifampin, pyrazinamide, and ethambutol is used for drug-susceptible Mtb because it improves cure rates and prevents the rapid emergence of resistance when compared to each of the single-drug treatments (18). Patients who underwent this combinatorial program had an immediate and prolonged (>1 year) decrease in microbial diversity compared to untreated age-matched individuals (19, 20). Consequences included an impaired inflammatory response and reinfection by Mtb (21). Given that combinatorial therapies may improve the elimination of challenging bacterial infections and limit the emergence of resistance, it would be valuable to systematically analyze how specific drug combinations disrupt the bacterial species of the gut microbiome.

In recent years, computational modeling has become a powerful tool for exploring the dynamics of the microbiome under different treatment scenarios (22-27). Simulation tools such as Simulator of Antibiotic Therapy Effects on the Dynamics of Bacterial Populations (SimulATe) enable researchers to model virtual microbiomes and antibiotic regimens (22, 23). Specifically, SimulATe applies ordinary differential equations (ODEs) to model the changes to a species of bacteria upon antibiotic treatment. Users can adjust key variables (bacterial species, antibiotic treatment type) and parameters (antibiotic concentration, initial bacterial densities, start time of treatment, etc.) of the ODEs. For example, SimulATe modeling has demonstrated that a delayed start to antibiotic treatment after infection results in a higher bacterial load compared to immediate antibiotic treatment (22). This elevates the risks of antibiotic resistance and infection relapse, particularly in immunosuppressed individuals (22). However, given that SimulATe was developed as an educational tool, its utility for microbiome research has yet to be fully explored. This computational model enables examination of the complex human gut microbiome and reveals some of the nuances of combinatorial antibiotic therapies.

SimulATe has an extensive range of eight antibiotic classes, categorized by their mechanism of action. Lincosamides, macrolides, streptogramins, and tetracyclines disrupt bacterial protein synthesis through interactions with bacterial ribosomes (5). Trimethoprims disrupt bacterial nucleic acid synthesis by inhibiting folic acid synthesis (5). Quinolones disrupt bacterial DNA synthesis by inhibiting DNA gyrase and topoisomerase IV (5). Penicillins, a class of beta-lactam antibiotics, disrupt bacterial cell wall synthesis by inhibiting penicillin-binding proteins (5). These mechanisms of action are an important consideration for the development of antibiotic cocktails because a close mechanistic overlap may allow a bacterium to become resistant through fewer mutations.

In this study, we used SimulATe to model changes in microbial diversity and community structure upon administering a cocktail of antibiotics versus a single antibiotic. We hypothesized that antibiotic cocktails, particularly those combining broad-spectrum drugs like tetracyclines and trimethoprims, would cause a more significant reduction in gut microbial diversity compared to single-drug treatments. Additionally, we expected that trios of antibiotics would exert a greater disruption of the microbiome composition than pairs, due to their broader collective range of activity. Our findings confirmed a greater loss of microbial diversity with combinatorial treatments compared to single-drug treatments. While individual antibiotics dynamically reshaped the surviving species of the microbiome, antibiotic cocktails frequently cleared all species of the gut microbiome. Although antibiotic cocktails could lower the risk of resistance and facilitate rapid pathogen clearance, we identified that it is likely to greatly disrupt the gut microbiome. This scale of dysregulation is a concern for patient health, underscoring the value of modeling the gut microbiome integrity under these combinatorial antibiotic treatments whether via *in silico* or *in vivo* approaches.

RESULTS

Because antibiotic therapies may concurrently eliminate pathogenic and commensal bacteria, our experiment sought to identify the disruption of the gut microbiome community structure in response to mono- versus combinatorial antibiotic treatment. We hypothesized that combinatorial antibiotics would exert greater, and thus faster, disruption to the microbiome than their single-drug constituents. To investigate this hypothesis, we conducted a series of *in silico* experiments using the microbiome simulator SimulATe (22). Given the large number of available antibiotics, we limited our analysis to the most potent representatives from each mechanism of action. We defined potency as the number of days required to eliminate all bacterial species in the modeled gut microbiome. We were interested in this metric because high-potency antibiotics may induce dysbiosis, manifesting as gastrointestinal issues, gut inflammation, opportunistic infections, or potential systemic issues (4). We assessed potency by simulating gut microbiome responses to the eight antibiotic classes included in SimulATe – lincosamides, macrolides, penicillins, quinolones, streptogramins, sulfonamides, tetracyclines, and trimethoprims – using the maximum input concentration (120 mg/L).

Through these simulations, we identified tetracyclines (protein synthesis), quinolones (DNA synthesis), and penicillins (cell wall) as our most potent candidates with non-overlapping mechanisms of action for further analysis in cocktails and as individual antibiotics **(Figure 1A)**. Combining antibiotics with the same mechanism of action could promote pathogenic bacteria to develop resistance via mutations to a single target, whereas combining antibiotics with unique mechanisms of action necessitates mutating multiple targets (17). Although streptogramins and lincosamides demonstrated complete elimination of the modeled bacterial species, we selected

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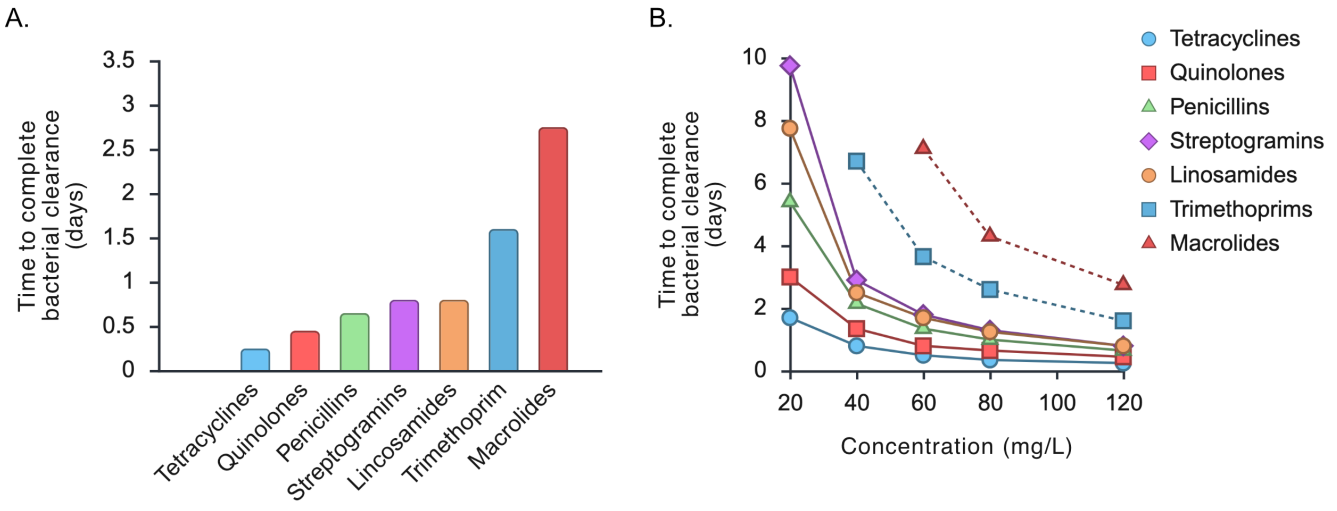

Figure 1. Potency of antibiotics measured as the number of days to clear of all bacterial species modeled in the gut microbiome. The time (in days) needed for various antibiotics to eliminate all modeled bacterial species of the gut microbiota in enterotype 1. (A.) The number of days to complete bacterial clearance measured at a high concentration of 120 mg/L. This value is referred to as the antibiotic "potency" in this study. A rapid time to complete bacterial clearance is considered a high potency. (B.) Dose response curves of the time to complete bacterial clearance for each antibiotic. Concentrations that did not achieve complete clearance by 20 days were not plotted. Antibiotic groups that required a minimum concentration of >20 mg/L to achieve complete clearance were represented with a dashed line. Graphical representation made with BioRender.

penicillins as the representative antibiotic that inhibits the cell wall because it had the highest potency. We excluded sulfonamides from further investigation because they failed to clear all the modeled species of bacteria within 20 days at the maximum input concentration. We also chose to include trimethoprims, despite its slower clearance time, due to its unique antibacterial activity from our other candidates via the inhibition of folic acid synthesis. All together, we surveyed the antibiotic classes: tetracyclines, quinolones, penicillins, and trimethoprims.

We observed that each class of antibiotic exhibited a non-linear dose-response curve **(Figure 1B)**. Tetracyclines rapidly clear the gut microbiota at low doses, whereas trimethoprims retained at least one of the modeled species of the gut microbiome until a minimum dose of 40 mg/L. Penicillins displayed a steeper slope than tetracyclines or quinolones between the doses of 20 mg/L and 40 mg/L. Under penicillin treatment at 20 mg/L, we found that *Acidaminococcus* and *Bifidobacterium* were retained the longest among the modeled species until 5.5 days, whereas a dose of 40 mg/L shortened their retention to 2.5 days (not shown). Beyond 40 mg/L, we found the toxicity was sufficient to rapidly eliminate all the modeled species. In contrast, we saw that quinolones and tetracyclines rapidly eliminated all species at concentrations below 20 mg/L, resulting in a shallower dose-response curve slope than penicillins in the range of 20-120 mg/L. Overall, each antibiotic resulted in a differential elimination of some species of bacteria yet retention of other species.

To evaluate the efficiency of single antibiotics in reducing bacterial diversity in the gut microbiome, we simulated the four highly potent drugs across concentrations of 5.5 to 11.5 mg/L. We selected this range because it is clinically relevant for treating pathogenic bacteria (28-30). The classes of antibiotics surveyed in this study are not selective to a pathogenic bacterial target and therefore affect commensal bacteria as well. Meanwhile, it has been reported that the composition of bacteria in the human gut microbiome fits into several common gut enterotypes (31). The three most common enterotypes are best distinguished by the abundance of *Bacteroides* (Enterotype 1), *Prevotella* (Enterotype 2), or *Ruminococcus* (Enterotype 3) (31). We modeled select bacterial genera for each enterotype **(Table 1)**. Since antibiotic sensitivity varies by its mechanisms of action and the bacterial species present, we tested all three enterotypes to detect differential effects.

In gut enterotype 1, penicillins (P), quinolones (Q), and trimethoprims (Tr) consistently failed to reach complete bacterial clearance within 20 days, indicating that some microbiome diversity could still be preserved after treatment **(Figure 2A)**. For example, a 7 mg/L dose of trimethoprims, the relevant steady-state serum level for the treatment of *Pneumocystis* carinii pneumonia (32), eliminated *Roseburia*, *Bifidobacterium*, and *Alistipes*, among other species of bacteria in the gut enterotype 1, whereas *Bacteroides* and *Collinsella* were preserved over the clinically relevant six day time course for treatment. Tetracyclines (Te) displayed more potent results, achieving complete bacterial clearance within 5.25 to 15.60 days. We observed that high concentrations from 11.5 to 9 mg/L (5.25 to 6.55 days) more rapidly reduced the bacterial diversity than low concentrations (7.45 to 20+ days). Similar trends were observed in gut enterotype 2 **(Figure 2B)**. We found that penicillins, quinolones, and trimethoprims preserved at least one species of commensal bacterial species for 20+ days, emphasizing their capacity to spare key members of this enterotype, thereby minimizing the consequences of dysbiosis. Unlike gut enterotype 1,

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Gut Enterotype 1	Gut Enterotype 2	Gut Enterotype 3
Acidaminococcus	*Alistipes*	*Akkermansia*
Alistipes	*Bacteroides*	*Alistipes*
Anaerostipes	*Bifidobacterium*	*Bacteroides*
Bacteroides	*Collinsella*	*Bifidobacterium*
Bifidobacterium	*Coprococcus*	*Blautia*
Collinsella	*Faecalibacterium*	*Collinsella*
Faecalibacterium	*Lachnospiraceae*	*Faecalibacterium*
Lachnospiraceae	***Prevotella***	*Lachnospiraceae*
Parabacteroides	*Roseburia*	*Roseburia*
Roseburia	*Streptococcus*	***Ruminococcus***

Table 1. Distribution of bacterial genera across three gut enterotypes. The human gut microbiome has been found to commonly be composed of one of three sets of bacteria (enterotypes). Though this is not an exhaustive list of all bacteria that can be found in an individual's gut microbiome, this table lays out the genera that comprised the three enterotypes we investigated in the SimulATe model. Genera are listed in alphabetical order. The genera that best distinguish the three enterotypes because of their relative abundance are bolded.

tetracyclines in gut enterotype 2 demonstrated no substantial impact after 20 days and did not eliminate all the species comprising the normal gut microbiome. In gut enterotype 3, penicillins, quinolones, trimethoprims, and tetracyclines all similarly preserved at least one commensal bacterial species, as in enterotype 2 **(Figure 2C)**. Single antibiotic treatment appeared to decrease the retention of microbiome species from enterotype 1 more than enterotypes 2 and 3.

To examine the combinatorial effect of antibiotics in cocktails on maintaining bacterial diversity, we arranged 11 additional treatments using all possible combinations of our selected highly potent drugs. We tested the following pairs: penicillins and quinolones (PQ), penicillins and tetracyclines (PTe), penicillins and trimethoprims (PTr), quinolones and tetracyclines (QTe), quinolones and trimethoprims (QTr), and tetracyclines and trimethoprims (TeTr). We also tested the possible combinations of these four drugs in trios and as a quartet (PQTr, PQTe, QTeTr, PTeTr, PQTeTr). Pairs, trios, or quartets of drugs were introduced using the same dose for all drugs in a cocktail to standardize the comparisons.

In gut enterotype 1, we found that the combination PQ yielded the strongest off-target potency among the penicillin pair combinations, resulting in complete bacterial clearance within the rapid timeframe of 3.45 to 5.37 days **(Figure 2A)**. Additionally, combinations such as PTe and QTe exhibited similar reductions in clearance times. In gut enterotype 2, cocktails like PQTe and QTeTr displayed remarkable potency against the microbiome, reducing clearance times to 2.95 and 3.25 days, respectively, outperforming all tested single antibiotics **(Figure 2B)**. Furthermore, in gut enterotype 3, combinations such as QTe and PQTeTr illustrated the superior performance of antibiotic cocktails, achieving complete clearance within 2.96 to 4.20 days across various concentrations **(Figure 2C)**. Each gut enterotype followed the trend that drug combinations are less likely to preserve microbiome diversity. Particularly, drug combinations with tetracyclines or quinolones (i.e. broad-spectrum antibiotics) exacerbated the complete and rapid bacterial clearance for all the enterotypes. However, these simulations also reveal that the enterotypes will have differential responses to various combinations of the antibiotics.

DISCUSSION

Although antibiotic cocktails may reduce the risk of developing antibiotic resistance and enhance pathogen elimination (10-13), we found that they tend to decrease the diversity of the gut microbiome compared to single antibiotic treatments. Both single drugs and cocktails exerted enterotype-specific effects. Tetracyclines showed the most consistent potency, eliminating all the modeled species of bacteria from each enterotype at low dosages, whereas other antibiotics may spare some diversity dependent on the gut enterotype. These findings highlight how antibiotic cocktails substantially reshape the gut microbiome. Clinical reports suggest that an imbalance of a single species, or even a larger community structure, of the gut microbiome can be sufficient to promote opportunistic infections and other severe health complications (1, 2, 4, 7, 8).

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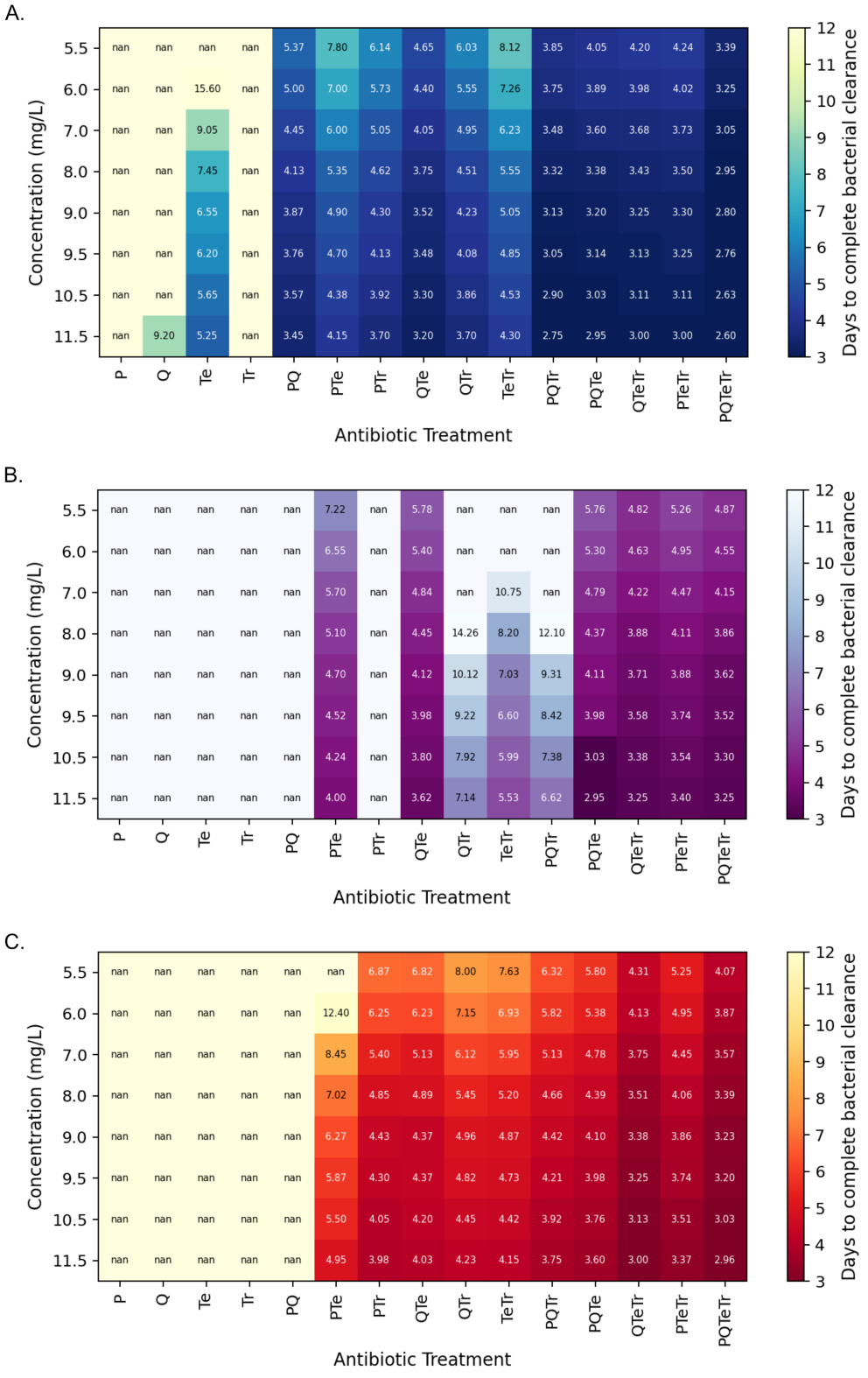

Figure 2. Duration (in days) for various single drugs and drug cocktails to achieve complete bacterial clearance in each of the gut enterotypes. Entries marked as "nan" indicate that some bacteria survived after 20 days of treatment, highlighting the variable efficacy of different antibiotic combinations. Drug abbreviations are “P” for penicillins, “Q” for quinolones, “Te” for tetracyclines, and “Tr” for trimethoprims. Combinations of these abbreviations represent different cocktails. Each section represents either a singular antibiotic, a bipartite cocktail, a tripartite cocktail, or a quadripartite cocktail, accordingly. The concentration on the y-axis represents the concentration used for each single antibiotic as well as the concentration of each antibiotic within a particular cocktail. (A.) Gut enterotype 1, (B.) Gut enterotype 2, (C.) Gut enterotype 3. Heat maps created using Python.

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Consistent with prior research (33-36), we observed that monotherapies commonly resulted in partial clearance of the gut microbiome. We found that at least one microbiome species was preserved within a realistic therapeutic window (<20 days) using narrow-spectrum antibiotics, such as penicillins and trimethoprims. We found that broad-spectrum antibiotics, such as tetracyclines and quinolones, more consistently cleared all species of the gut microbiome. Tetracyclines stood out as the most potent among single antibiotics. However, the performance of tetracyclines in gut enterotypes 2 and 3 was substantially diminished compared to enterotype 1. This variability underscores that single antibiotics may preserve bacterial diversity, but the effects are enterotype dependent.

Antibiotic monotherapies are prone to resistance, often requiring escalation to stronger drugs which increases the risk of multidrug-resistant infections and greater microbiome disruption (10-13). Alternatively, antibiotic cocktails may rapidly eliminate the pathogenic bacteria and prevent the development of resistance (11, 12). However, in our simulations, we found that antibiotic cocktails coincide with widespread disruption the gut microbiome across all enterotypes, achieving faster and more comprehensive bacterial clearance. For instance, combinations such as penicillins and tetracyclines (PTe), quinolones and tetracyclines (QTe), and all trio cocktails significantly reduced treatment durations. Specifically, the combination of penicillins, quinolones, tetracyclines, and trimethoprims (PQTeTr) proved highly potent, achieving complete clearance within 2.96 to 4.20 days in gut enterotype 3. We observed that trio cocktails were generally more potent than pairs. This met our expectation that a broader range of commensal bacteria will be cleared as the number of drugs with non-overlapping mechanisms of action is increased. Thus, while antibiotic cocktails may reduce the likelihood of resistance, they invariably damage the microbiome community structure.

Our observations on the loss of microbial diversity from antibiotic cocktails raise concerns about the emergence of dysbiosis. Dysbiosis has been associated with inflammatory bowel disease, metabolic disorders, diabetes, and increased susceptibility to infections (9, 23, 37). The disease outcomes from dysbiosis are known to relate to the composition of the microbiome, and thus the enterotype (31, 38). Enterotype 1 has been reported to have a significantly higher abundance of *C. difficile* than the other two enterotypes, which could reflect a predisposition to *C. difficile* infection upon antibiotic-induced dysbiosis. Enterotype 2 has a greater risk of developing Type 2 diabetes mellitus following gut microbiome disruption (39). The common denominator across enterotypes is that a loss of bacterial diversity can negatively influence overall health.

Computational models like SimulATe provide valuable insights into the community structure of the gut microbiome under different antibiotic treatments (22). These simulations are a powerful tool for optimizing treatment regimens and minimizing adverse effects. However, a key limitation of this tool is the reductionistic representation of biochemical interactions between antibiotics in combination. The ordinary differential equations behind the SimulATe model assume antibiotics have additive effects in combination, whereas synergistic or antagonistic effects of drug cocktails have been reported *in vitro*, *in vivo*, and clinically (11, 17, 34). The predictive accuracy of computational models could be improved by tuning the ODE algorithms to reflect synergistic effects, as well as by incorporating clinical data and patient-specific microbiome profiles for more personalized treatment approaches (23, 40, 41). So too, simulations could be validated by profiling gut microbiome responses to antibiotics via 16S rDNA sequencing of human stool samples or murine stool samples from mice with a reconstituted human gut microbiome (42, 43). This could further inform synergistic effects that are missing from our *in silico* approach.

Future research should focus on several key areas to further enhance our understanding and management of antibiotic treatments. First, studies should identify optimal antibiotic combinations that maximize efficacy against pathogens while minimizing disruption to the microbiome, including appropriate drug selection, dosing, and treatment duration (34). Second, more personalized approaches to antibiotic therapy are needed. Advancements in microbiome sequencing and computational modeling enable clinicians to tailor treatment plans to the specific composition and dynamics of an individual's microbiome (40, 44). Third, sequencing the whole genome of pathogen isolates is valuable for identifying antibiotic resistance, informing treatment strategies, and determining emerging threats (45). Finally, research should explore adjunctive therapies that support microbiome health during antibiotic treatment. Probiotics, prebiotics, and fecal microbiota transplantation are potential strategies to restore and maintain microbial balance in patients undergoing antibiotic therapy (4, 9, 36, 46). These approaches can help mitigate the adverse effects of antibiotics and promote overall gut health.

Overall, our simulations demonstrated that antibiotic cocktails eliminated key members of the bacterial communities more rapidly and completely than antibiotic monotherapies. The use of a broad-spectrum antibiotic as part of a combinatorial therapy exacerbated these effects. Because a balanced microbiome plays a critical role in immune regulation, metabolic health, and protection against opportunistic pathogens, widespread microbial loss may carry significant long-term health consequences. These findings emphasize the importance of considering microbiome integrity in antibiotic treatment design. By leveraging computation models alongside experimental and clinical data, future strategies may optimize antibiotic regimens to effectively control infections while minimizing collateral damage to the gut microbiome.

MATERIALS AND METHODS

Selection of the computational tool

To model the response of bacterial communities to single versus cocktail antibiotics, this study was conducted using SimulATe. This open-access computational framework was ideal for investigating antibiotic effects on the gut microbiome due to its quantitative outputs, customizable antibiotic treatments, and representation of multiple gut enterotypes. While alternative *in silico* tools may have similarly quantified bacterial abundance upon antibiotic therapy, they typically lack one or more of the features provided by SimulATe (24, 25).

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Single antibiotic selection

Simulations were conducted for each monotherapy antibiotic using default Antibiotic Inhibition parameters and the highest possible concentration (120 mg/L). The effectiveness of each antibiotic was determined by the number of days required to clear all the modeled bacterial populations in the gut microbiome. This metric provided a measure of the antibiotic's potency.

Cocktails selection

Cocktails were created from antibiotics with unique mechanisms of action. The following combination pairs were tested: penicillins and quinolones (PQ), penicillins and tetracyclines (PTe), penicillins and trimethoprims (PTr), quinolones and tetracyclines (QTe), quinolones and trimethoprims (QTr), and tetracyclines and trimethoprims (TeTr). These combinations were selected to explore additive effects that could enhance bactericidal effects while maintaining a balanced microbiome. This study also used tri- or quad- part cocktail combinations, including penicillins, quinolones, and tetracyclines (PQTe); penicillins, quinolones, and trimethoprims (PQTr); penicillins, tetracyclines, and trimethoprims (PTeTr); quinolones, tetracyclines, and trimethoprims (QTeTr); and, penicillins, quinolones, tetracyclines, and trimethoprims (PQTeTr).

SimulATe parameters

All Antibiotic Inhibition parameters in the SimulATe v1.0 model were set to default values, which simulate the variability observed in clinical settings. The simulation speed was also set to default to maintain consistency. The study tested eight different antibiotic concentrations: 5.5 mg/L, 6 mg/L, 7 mg/L, 8 mg/L, 9 mg/L, 9.5 mg/L, 10.5 mg/L, and 11.5 mg/L. In the case of antibiotic cocktails, each constituent drug was administered at an equal concentration to evaluate their combined effects. We modeled three distinct gut enterotypes in SimulATe **(Table 1)**.

Running the simulation

Simulations were conducted for each singular antibiotic and each cocktail across the eight different concentrations. Antibiotic administration began on Day 2 to facilitate comprehensive data interpretation and extrapolation. Single or cocktail treatments were administered as a continuous concentration over the entire time course, mimicking the steady-state concentration that a patient achieves when taking their antibiotic prescription at the proper time intervals and ignoring local peaks and troughs in plasma concentration that would naturally occur from repeated dosing. The antibiotic treatment was stopped if all bacteria were not eliminated within 18 to 20 days of administration.

Generating the heat maps

Each simulation outputs the number of days to complete bacterial clearance for the set antibiotic monotherapy or combinatorial therapy. Outputs were recorded across a range of antibiotic concentrations (5.5–11.5 mg/L) for each treatment. If at least one bacterial species persisted after 20 days of simulated treatment, the result was labeled as “nan”. Heat maps were created in Python version 3.11 using pandas and matplotlib.pypolot packages.

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