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Minireview

Response, resistance, and recovery of gut bacteria to human-targeted drug exposure

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Survival strategies of human-associated microbes to drug exposure have been mainly studied in the context of bona fide pathogens exposed to antibiotics. Less well understood are the survival strategies of non-pathogenic microbes and host-associated commensal communities to the variety of drugs and xenobiotics to which humans are exposed. The lifestyle of microbial commensals within complex communities offers a variety of ways to adapt to different drug-induced stresses. Here, we review the responses and survival strategies employed by gut commensals when exposed to drugs—antibiotics and non-antibiotics—at the individual and community level. We also discuss the factors influencing the recovery and establishment of a new community structure following drug exposure. These survival strategies are key to the stability and resilience of the gut microbiome, ultimately influencing the overall health and well-being of the host.

INTRODUCTION

The gut microbiome plays a key role in maintaining human health by contributing to and responding to diverse physiological processes. Located at the interface between our body and the environment, the gut microbiome is exposed to a range of external stimuli. These include substances such as dietary compounds, pharmaceuticals, and xenobiotics in general, which can affect the host, the individual microbes, as well as the community as a whole. Compositional and functional changes in the gut microbiome have been linked to numerous health conditions,[1–3] highlighting the need to understand how external factors can affect host-associated microbial communities,[4] to investigate their ability to withstand stressors, and to assess the resulting impact on the host.

Historically, research into how drugs interact with host-associated microbes has been conducted in the context of infection, that is, how different compounds can be used to eliminate infectious agents. This has led to the distinction between pathogenic and commensal microbes and between antibiotics and non-antibiotic drugs. However, these distinctions are not always clear-cut. We now know that non-antibiotics can also affect the gut microbiome, that gut bacteria can metabolize drugs and thus alter the efficacy and safety of pharmaceutical interventions,[5] and that, under certain conditions, otherwise beneficial microbes can be detrimental to host health.[6] This has sparked interest in investigating drug-microbiome interactions beyond the antibiotic-pathogen paradigm. Drugs from several classes affect the human gut microbiome,[2,3] including not only traditional antibiotics but also drugs that are not classified as antibacterial, such as antifungals or antiprotozoals, and drugs that target human physiology, hereafter referred to as "human-targeted" drugs. Studies in large, well-characterized cohorts have consistently shown that medication use is associated with microbiome composition changes[2] and that the differences in the composition of the community are proportional to the number and type of medications consumed.[7] Moreover, statistical models assessing the medication-microbiome association show a higher explanatory power compared with those evaluating the host health-microbiome link, particularly when incorporating environmental and other host-related variables.[3] Cohort reports and subsequent reviews and meta-analyses have focused on the association of a few commonly used human-targeted medications, including statins, proton pump inhibitors (PPIs), beta-blockers, the antidiabetic metformin, and more recently, selective serotonin reuptake inhibitors (SSRIs).[4,5] For many other classes of drugs, the effect on the gut microbiome is still largely unknown.

Recognition of the potential impact of drugs on host-associated microbial communities is critical to the development of therapeutic strategies that take into account the broader ecological context of the human microbiome. Here, we provide an overview of the current understanding of how gut microbial communities respond to, resist, and recover from exposure to drugs, highlighting examples of studies focusing on human-targeted drugs and other non-antibacterials whenever possible.

HUMAN-TARGETED DRUGS AFFECT GUT MICROBIOME COMPOSITION AND FUNCTION

Similar to antibiotics, human-targeted drugs can directly or indirectly affect the gut microbiome (Figure 1). Drugs directly affect the microbes by altering their metabolic processes, the functioning of their molecular machinery, or the integrity of their cellular structures. Conversely, indirect effects arise from changes in the microbe's surroundings, thereby influencing the composition or function of the microbial community. Exposure to drugs can trigger complex ecological changes within the microbiome. These effects could potentially occur through direct interactions with multiple microbial species or through targeted

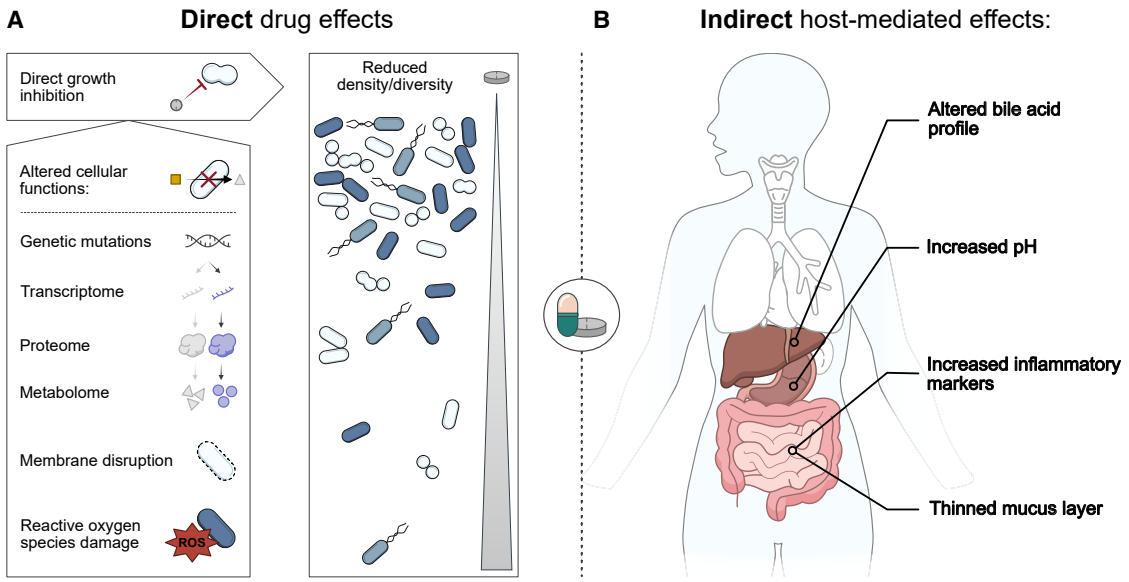

Figure 1. The effect of drugs on gut microbes can be direct or mediated through the host
(A) Drugs directly affect cellular functions and inhibit microbial growth. This, in turn, reduces the density and strain diversity of the gut microbiome.
(B) Indirect host-mediated drug effects arise from changes in the host physiology, which alter the microbial milieu.

modulation of keystone species, subsequently influencing the overall structure of the microbial community.

Direct effects

Drugs, including antibiotics and human-targeted drugs, can affect microbial physiology by directly inhibiting or promoting the growth of individual taxa. Consequently, shifts in the microbiome result from a combination of the elimination of certain species from the community (due to bactericidal effects) and the inhibition of the growth of others (due to bacteriostatic effects). Likewise, some species may benefit from the compound itself as a substrate.

Direct growth inhibition of gut microbes by human-targeted drugs is often less severe than with antibiotics, yet it is quite common. Our previous work, in which we systematically probed the direct fitness effects of more than 1,000 FDA-approved drugs on a panel of 40 representative species of the gut microbiome at physiologically relevant concentrations,[8] showed that almost a quarter of the tested human-targeted drugs inhibited the growth of commensal bacteria; this effect is dose dependent.[9] Work by other groups has confirmed these observations and further explored the responses of commensals to drug exposure. For example, Li and colleagues developed an *ex vivo* screening method to assess changes in the proteome of donor-derived communities and evaluated the effect of 35 human-targeted drugs. The majority of the human-targeted drugs were found to inhibit the growth of at least one microbial species in the communities. The authors showed that human-targeted drugs can modify both the overall functional profile and specific pathways of the microbiome. In some cases, changes in the microbial functional profile occurred without a concomitant change in microbial abundance.[10] This phenomenon was also observed by Ricaurte and colleagues, who showed that at low concentrations, human-targeted drugs can alter the transcriptional profile of multiple gut species without affecting their growth patterns.[11]

Human-targeted drugs can directly impact microbial physiology in multiple ways.[12] Wang and colleagues exposed *Escherichia coli* and *Pseudomonas putida* to non-steroidal anti-inflammatory drugs (NSAIDs), beta-blockers, and lipid-lowering drugs *in vitro*. The authors observed disruptions in the cell membrane, causing increased permeability and leakage of the cytoplasmic contents. They also noted an increased response to reactive oxygen species (ROS) damage, suggesting that drug-induced oxidative stress leads to impaired microbial growth.[13] Human-targeted drugs can also target specific macromolecules within bacterial cells. For instance, the ovulation stimulant clomiphene inhibits a conserved bacterial enzyme involved in the synthesis of an essential precursor for cell wall carbohydrate polymers, as shown by Farha and colleagues.[14] Alternatively, human-targeted drugs can interact with microbial analogs of their human targets: Fung and colleagues reported that *Turicibacter sanguinis* encodes a protein homologous to the mammalian serotonin transporter, which is used by the bacterium to import serotonin.[15] This transporter can be blocked by fluoxetine, an SSRI. Treatment with fluoxetine had a cytostatic effect on the bacterium, induced changes in the expression of genes involved in energy metabolism, sporulation, metal homeostasis, and stress response *in vitro*, and reduced the ability of the microbe to colonize the mouse gut.

Indirect effects

Microbes constantly interact with their environment, and in the case of the gut microbiome, this environment includes the host itself. Therefore, drug-induced changes in host physiology can impact the composition of the community. A classic example is the widely reported increase in gastrointestinal pH caused by PPIs, which is linked to an overrepresentation of certain

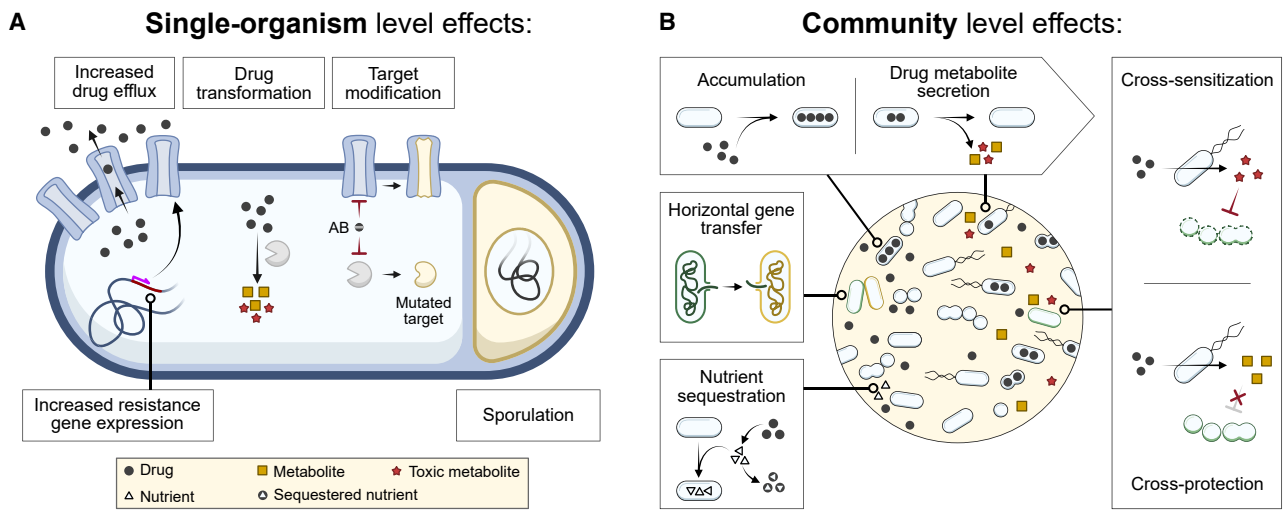

Figure 2. Microbial response and resistance to drugs occur at the single-organism and community level

(A) Survival strategies at the single-organism level include three main mechanisms: reduction of intracellular xenobiotic concentration by decreased uptake, increased efflux, or sequestration; degradation of the compounds; and, as is known in the case of antibiotics, modification of their target to prevent inhibition. Persistence and sporulation are also used by bacteria to withstand environmental stress.

(B) Community response to drug exposure emerges from the response of individual microbes and community behavior, such as cross-sensitization or cross-protection. Other context-dependent strategies include the acquisition of resistance genes by horizontal gene transfer and the alteration of nutrient availability.

members of the upper gastrointestinal tract in the gut, namely, the genera *Rothia*, *Haemophilus*, *Veillonella*, and *Streptococcus*.[16]

Less studied is the impact of host bile acid metabolism on microbiome composition. Bile acids are secreted by the host to facilitate the absorption of lipids in the small intestine. They also act as hormones that regulate their own synthesis and the homeostasis of lipids and glucose.[17] In the gut, there is a bidirectional relationship between them and the microbiome. Bile acids have antibacterial effects that shape the structure of the microbiome. Conversely, certain microbes can metabolize bile acids, and the resulting pool of secondary bile acids can influence host physiology.[18] Nolan and colleagues evaluated the impact of rosuvastatin on the bile acid profile and microbiome composition of mice.[19] They reported changes in the overall bile acid profile of treated animals and changes in the expression levels of inflammation markers associated with community structure. The authors observed changes in the composition of the caecal microbiome of treated mice, but rosuvastatin treatment did not affect the *in vitro* growth of microbes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase, the enzyme targeted by statins. This suggests that changes in the microbiome after rosuvastatin treatment are mediated by alterations in host bile acid metabolism rather than by the drug itself.

More complex interactions have also been reported, such as those between drugs, host physiology, and its nutritional status. Ng and colleagues found that antibiotic-induced thinning of the protective mucus layer in mice leads to increased competition for mucin-derived polysaccharides between taxa that can use them as a carbohydrate source, such as *Bacteroides* and *Akkermansia*.[20] Changes in the mucus layer are also linked to an expansion of enteric pathogens that benefit from the removal of the barrier between the microbiome and the epithelium, such as *Citrobacter rodentium*,[21] and the proliferation of pathogens that exploit the mucosal glycans released by the microbiome, such as *Salmonella enterica* serovar Typhimurium or *Clostridioides difficile*.[20]

Finally, drugs can alter the levels of nutrients available to microbes without necessarily altering host physiology. Pereira and colleagues used an *ex vivo* system to evaluate the effect of entacapone, a drug used to treat Parkinson's disease, on donor-derived microbial communities.[22] They observed that the drug induced large shifts in the composition of the microbial community that were driven by the depletion of available iron. Entacapone chelates ferric iron, which is an essential nutrient for most microbes; reduced levels of this element resulted in the inhibition of several commensal taxa, while potentially pathogenic species able to thrive in such conditions showed an increased abundance. The effects of entacapone exposure were reversed by iron supplementation.

MICROBIAL DRUG RESPONSE AND RESISTANCE AT CELLULAR AND ECOLOGICAL LEVELS

Survival strategies at the strain and species level

Despite the extensive impact of drugs on human gut bacteria, microorganisms have effective ways of protecting themselves against these compounds (Figure 2). At the cellular level, resistance to xenobiotics is typically achieved through three main mechanisms: (1) reduction of intracellular xenobiotic concentration through decreased uptake, increased efflux, or sequestration; (2) degradation of the compound; and (3) microbe modifying the drug target to avoid inhibition. While it is reasonable to assume that all three mechanisms apply to human-targeted drugs, we currently lack information about the molecular targets of most non-antibiotics in bacteria. However, there is experimental evidence for the first two of the mechanisms noted above.

Microbial cells have sophisticated cell envelopes, providing inherent resistance to certain xenobiotics. While larger, hydrophobic compounds can target gram-positive commensals, gram-negatives are usually protected due to their selective outer membrane barrier.[23] In addition to having a barrier function, the cell membrane acts as the anchoring point of the transport machinery used by microbes to modulate the intracellular concentration of drugs. Certain antibiotics enter the cell through protein channels; changes in the transporter proteins can alter the efficacy of the drugs by preventing them from causing damage within the cell. An example of such a compound is fosfomycin. It uses two nutrient transport systems to access the cytosol: the hexose phosphate transporter and the glycerol-3-phosphate transferase system. Changes in the presence or regulation of these transporters can alter the susceptibility of *E. coli* to fosfomycin, as shown by Ballestero-Téllez and colleagues.[24] The exact microbial uptake mechanisms of human-targeted drugs and the impact of alterations in the uptake machinery remain to be elucidated. Likewise, efflux pumps are recognized as contributors to antimicrobial resistance, and recent studies have shown that they are also involved in the response of various bacteria to human-targeted drugs. For example, Escalante and colleagues assessed how simvastatin affects 39 members of the microbiome and analyzed changes in the transcriptional profile of two species, *Eggerthella lenta* and *Bacteroides thetaiotaomicron*.[25] Three efflux systems of the resistance-nodulation-division (RND)-type homologous to the AcrAB-TolC efflux pump of *E. coli* were up-regulated in *B. thetaiotaomicron*. Further evaluation of the role of these genes in simvastatin resistance showed that knocking them out resulted in an increased susceptibility to the drug. The upregulation of the AcrAB-TolC efflux system in *Bacteroidales* species has also been confirmed by Ricaurte and colleagues; however, this increase in expression comes with the drawback of increased susceptibility to other compounds, such as vitamin A and secondary bile acids.[11] Other studies have shown that the expression of RND-type pumps is also increased in *B. thetaiotaomicron* in response to the antipsychotic chlorpromazine.[26] It remains to be systematically tested whether the expression of efflux pumps strongly correlates with resistance to the pump-inducing compound, as is known for classic multidrug efflux pumps in pathogens.

Gut microbes also possess an enzymatic repertoire capable of degrading numerous compounds. Zimmermann and colleagues assessed the ability of 76 representative members of the human gut microbiome to metabolize 271 medications under anaerobic conditions *in vitro*.[27] They found that nearly two-thirds of the compounds were metabolized by at least one microbe. All species were able to metabolize more than one compound, albeit with varied efficiency. The study also identified genes involved in the transformation of the different drugs; species capable of metabolizing a particular compound had a higher abundance of the corresponding genes in their genomes. Moreover, in complex microbial communities, the abundance of these genes correlated with the drug-metabolizing capacity of the communities. However, the degradation of a drug may be independent of the actual mechanism of resistance to the compound. Thus, it still needs to be tested on a case-by-case basis whether the transformation of human-targeted drugs confers resistance to those drugs in the strains capable of such degradation, similar to what is known for antibiotics, or if the two phenomena are unrelated.

Another survival strategy, persistence, involves a subset of microbial cells entering a metabolically dormant state, making them less susceptible to xenobiotics.[28] These persistent cells can resume normal growth once the drug concentration decreases. Importantly, persistence is a non-genetic and reversible state. Persistence has been extensively studied in pathogenic bacteria in response to antibiotics, less so in commensal bacteria. Wang and colleagues showed that commonly used antidepressants can enhance the persistence of *E. coli* to subsequent antibiotic exposure,[29] although the extent to which other commensals employ this mechanism remains largely unknown.

Certain bacteria use spores, highly resilient dormant structures, as a form of persistence to survive environmental stressors. An example is *C. difficile*, which forms spores that are able to endure adverse environmental conditions in the gut. Once the stressor is removed, the spores can revert to an active form, allowing *C. difficile* to proliferate and potentially cause infection,[30] for example, in a drug-perturbed microbiome. Spore formation is not limited to *C. difficile*. Browne and colleagues showed that a diverse set of gut species, primarily from the phylum *Bacillota*, are spore formers.[31] These species tolerate environmental challenges better, surviving up to 21 days under aerobic conditions, compared with 2–6 days for non-spore formers. Although the impact of human-targeted drugs on sporulation is not well understood, its prevalence in gut species could play a role in microbial resilience and recovery from challenges with human-targeted drugs.

Exposure to drugs is a recent event in microbial evolution, and the benefits of evolving and encoding survival strategies against rare stressors are intriguing. Two non-exclusive explanations have been suggested.[5,32] First, a limited set of gene products, including enzymes or efflux pumps, may provide resistance to a wide range of substances, albeit with low efficiency. This approach would allow microbes to metabolize diverse, albeit infrequently encountered, substances such as human-targeted drugs. Second, the machinery for xenobiotic metabolism and resistance might have evolved in other environments and later been horizontally transferred to the gut microbiome.[33] Frequent exposure to chemical stressors acts as a selective pressure favoring the development, acquisition, or maintenance of resistance systems that enable gut bacteria to cope with different types of stressors. This is evidenced by the observation that gut microbes resistant to antibiotics also show increased resistance to human-targeted drugs.[9] This is also supported by the work of Wang and colleagues, who showed that exposing *E. coli* to five commonly used antidepressants can lead to the development of resistance against antibiotics by promoting the expression of efflux pumps and inducing mutations in genes involved in detoxification and drug accumulation.[29] We speculate that pathogenic *Gammaproteobacteria* species, which are more resistant to non-antibiotic drugs than commensal gut bacteria of different phyla,[9] could act as a genetic reservoir conferring resistance to human-targeted drugs.

Survival strategies at the microbial community level

Microbes possess a large repertoire of tools to respond to drug exposure. However, the sensitivity of a species measured in pure

culture does not necessarily correlate with the actual response of the microbe as part of a complex microbial community, whether *in vitro* or *in vivo*. This discrepancy is an emergent property of complex communities in which two opposite phenomena can be distinguished: cross-sensitization and cross-protection. Cross-sensitization occurs when exposure to a drug in a community context makes the microbe more sensitive than it would be in isolation. Cross-protection, on the other hand, involves a collective defense in which a microbe is less susceptible to a drug challenge in a community than when grown in pure culture. Using *in vitro* screening assays and 16S rRNA gene sequencing, García-Santamarina[34] and colleagues and Grießhammer[9] and colleagues compared the growth of 20 and 32 human gut bacterial species, respectively, in pure culture and as part of a synthetic community after exposure to dozens of human-targeted drugs. Although the majority of the drug-species interactions in the community recapitulated those in pure culture, between 20% and 26% resulted in cross-protection. Cross-sensitization, conversely, was between 4 and 6 times less common, yet it became more frequent with increasing drug concentrations.[34] This is consistent with perturbations observed in human cohorts[3] and underlines that the ability of the microbiome to protect its members is based on and extends beyond the capacity of its members to cope with drug challenges.[34]

Cross-sensitization occurs due to the formation of toxic byproducts, and cross-protection is explained by detoxification and changes in drug levels resulting from the transformation and accumulation of compounds by community members. As drug susceptibility is largely concentration-dependent,[9,34] a reduction in drug levels through drug transformation by strains encoding enzymes to degrade the drug may decrease the toxic effects of the drug on sensitive strains. Similarly, cytoplasmic sequestration of drugs by accumulator species may decrease drug levels in the extracellular environment and thereby mitigate potentially harmful effects on the community. Klünemann and colleagues exposed 25 bacterial species to 15 different compounds and measured the drug concentrations in the supernatant or total culture.[35] This allowed them to determine whether a drug was biotransformed or bioaccumulated. The authors observed that 4 drugs were bioaccumulated by 14 bacterial species, with concentration reductions ranging from 35% to 80%. Although bioaccumulation of a drug did not correlate with its effect on microbial growth, it altered the overall metabolism of the accumulator species as well as their syntrophic interactions. When grown in communities with an accumulator species and exposed to a specific compound, sensitive species became more abundant. This was attributed to changes in metabolites secreted by the accumulator species, which were unrelated to the accumulated compound.[35] A similar reduction in drug concentration could result from adsorption of the compound onto microbial surfaces. This phenomenon has been poorly studied, although Niehues and Hensel reported that levodopa, a drug used in the treatment of Parkinson's disease, can interact with adhesion proteins on the surface of *Helicobacter pylori*, leading to a decrease in the uptake of the compound by the host and impaired adhesion of the bacterium to host cells.[36]

Cross-sensitization and cross-protection depend on the intricate interactions between drugs and microbes, as well as among microbes themselves. In other words, the survival of sensitive community members relies on the presence of microbes capable of altering the levels of a specific compound and the efficiency with which they can accomplish this task. This is demonstrated in the work of Blaustein and colleagues, who assessed the effect of the chemotherapeutic drug doxorubicin on microbes in synthetic communities.[37] The authors observed that the level of cross-protection for sensitive species depended on the presence and efficiency of transforming taxa in the community. Similar observations have been made for nifurtimox, a drug used to treat Chagas disease, showing that in synthetic communities, the protection provided to nifurtimox-sensitive taxa varied based on the efficiency of two different biotransformer species.[34]

Context-dependency extends beyond synthetic communities. Javdan and colleagues developed an *ex vivo* culture system using microbial communities derived from human fecal samples.[38] Using this setup, they investigated the microbial metabolism of more than 500 clinically used drugs. Among them, 57 compounds from 28 pharmacological classes were identified as being transformed by community members. Similar to other microbiome responses, the likelihood of a compound being metabolized varied between donors. However, this variability was not entirely explained by the taxonomic composition of the donor-derived communities. This is consistent with other observations where the response to various human-targeted drugs and subsequent loss of resistance to *Salmonella* colonization varied between donor-derived communities.[9]

The community context also influences the acquisition of resistance mechanisms through horizontal gene transfer. While extensively studied for antibiotic resistance in clinical settings,[39] there is growing evidence that human-targeted drugs from various classes also contribute to the acquisition of antimicrobial resistance genes in gut bacteria, as recently reviewed by Alav and Buckner[12]: briefly, the proposed mechanisms include alterations in the cell envelope, namely membrane permeability, expression of outer membrane proteins and synthesis of lipopolysaccharide, response to oxidative stress, and reduced cell distance. Smillie and colleagues showed that pairs of genomes from bacteria isolated from humans were up to 25 times more likely to share transferred DNA than microbes from different environments,[33] irrespective of their phylogenetic relatedness. The higher frequency of horizontal gene transfer in the human gut emphasizes that the genetic pool available to bacteria is shaped by their ecological context, even though the ability to obtain novel resistance mechanisms in such a way might vary between individual taxa. The acquisition of foreign DNA can promote the dissemination of resistance to antibiotics among commensals and opportunistic pathogens, as shown by Wang and colleagues, after exposing complex microbial communities to multiple, commonly used human-targeted drugs.[40] Likewise, metabolite exchange between members of a microbial community increases the collective resistance against drugs. Auxotroph organisms lack the ability to produce substances they require for their growth; they are, therefore, forced to obtain them from their environment, frequently exchanging nutrients with other microbes. Yu and colleagues showed that in communities with a high proportion of auxotrophs, such as the gut microbiome, microbes alter their metabolism, favoring nutrient exchange over self-synthesis of specific essential substances, thus promoting

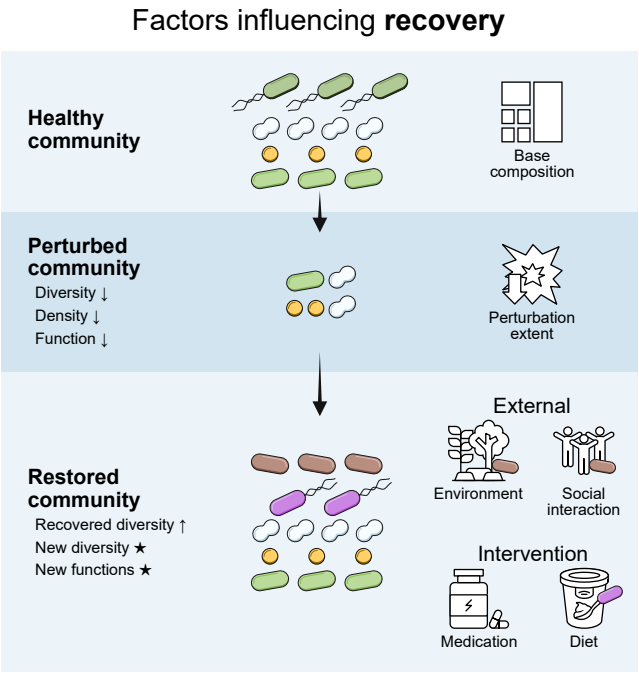

Figure 3. The recovery of microbial communities after perturbation is dependent on the initial composition of the healthy community and the severity of the disruption

Other external factors, such as the host environment and social interactions, as well as additional drug consumption, dietary changes, and the use of prebiotics or probiotics, can further affect the recovery process.

a rich extracellular metabolome. As a consequence of the increased metabolite efflux, members of such communities have an increased resistance to drugs.[41]

RECOVERY OR ESTABLISHMENT OF A NEW COMMUNITY STRUCTURE

The recovery of the gut microbiome after exposure to human-targeted drugs, in contrast to antibiotics, is not well understood. Yet, considering the varied antimicrobial effects of human-targeted drugs, ranging from mild to severe, antibiotics can serve as an extreme example of drug-induced perturbation (Figure 3). Microbiome disruption following drug exposure is transient, and the process of restoring community composition to a pre-perturbation state is complex, multifactorial, and sometimes imperfect due to varying levels of resilience. This was demonstrated by Chng and colleagues, who conducted a meta-analysis using data from five independent cohorts to examine the response of the human gut microbiome to antibiotics.[42] While more than half of individuals across all cohorts recovered pre-treatment diversity levels, the remaining participants exhibited lower diversity 3 months post-treatment. Taxa positively associated with recovery after antibiotic treatment included members of the genera *Bacteroides*, *Alistipes*, *Bifidobacterium*, and *Faecalibacterium*. The genomes of these taxa are enriched in genes involved in the degradation of dietary polysaccharides and mucin, potentially offering a metabolic advantage that promotes community restoration. The number and identity of recovery-associated taxa varied among cohorts, underscoring the context-dependent nature of the gut microbiome's resilience to drug perturbations. Anthony and colleagues reported similar findings when tracking the recovery of the microbiome in 20 healthy volunteers undergoing treatment with multiple antibiotics.[43] Despite the recovery of diversity in most volunteers, the overall microbiome composition remained changed 6 months post-treatment, partly due to the loss of microbial species; similar observations were reported by Palleja and colleagues.[44]

The recovery of the microbial community is influenced by the baseline composition and the extent of perturbation, as they dictate the interspecies interactions driving community readjustment. Chen and colleagues found a positive association between functional and taxonomic diversity and community restoration, suggesting that a more diverse starting point supports a more robust recovery process.[45] They confirmed that the presence and abundance of specific species, such as *Akkermansia muciniphila* and *Bacteroides uniformis*, predict community recovery. In mouse experiments, they showed that inoculating both species accelerates microbiome diversity restoration and promotes stable microbial networks. The authors attributed this to the substrate utilization of these species, using mucins and complex dietary carbohydrates to support their growth and cross-feed other microorganisms lacking such capabilities. Interspecies interactions promote a positive feedback loop involving short-chain fatty acid (SCFA) production, epithelial maturation, and increased mucin production, resulting in quicker recovery of the microbiome after treatment. Similarly, community stability in pulsed antibiotic treatment can be maintained by modulation of the growth rate of community members, clonal expansion of resistant haplotypes, and prophage induction, as shown by Münch and colleagues.[46]

External factors also impact community restoration. Ng and colleagues evaluated the influence of the host's social interactions on microbiome recovery post-antibiotic exposure. Single-caged mice exhibited slower recovery and increased stochasticity in taxa changes compared with mice co-housed with untreated counterparts.[47] While the contribution of interpersonal interactions on microbiome restoration in humans is not well understood,[48] it is known that an individual's social environment can serve as a source of microbes, even in adulthood.[49]

Extensive research has been conducted on interventions to restore the microbiome post-drug use, focusing on promoting existing microbial growth or introducing new microorganisms. These interventions can be broadly grouped into prebiotics (non-digestible compounds supporting microbial growth), probiotics (live microorganisms providing health benefits), or fecal transplants (transfer of fecal material with a diverse microbial community).[50,51] Although these approaches are promising for restoring microbial balance, their efficacy and benefits in the context of non-antibiotic drugs have yet to be proven in clinical practice.

CONCLUSIONS AND PERSPECTIVES

The integration of microbiome-informed approaches into drug development and clinical practice is expected to enhance therapeutic efficacy while minimizing adverse effects on the host and the microbial community. Likewise, a thorough understanding of the survival strategies employed by microbial cells and

human-associated microbial communities to cope with compounds is critical in the rational design of small molecules for host-directed therapy as well as for targeted microbiome modulation. We look forward to future studies that elucidate the mechanistic basis of drug-microbiome-host interactions, explore innovative therapeutic interventions, and translate scientific findings into clinical applications.

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DECLARATION OF INTERESTS

L.M. is listed as a co-inventor on the following patents (or patent applications): EP3838269A (published 23.06.2021) Compounds & Pharmaceutical Compositions for Prevention-Treatment of Dysbiosis, Antidotes for Microbiome Prevention; WO/2019/158559 (published 22.08.2019) Repurposing compounds for the treatment of infections and for modulating the composition of the gut microbiome; and WO/2019/154823 (published in 15.08.2019) *In vitro* model of the human gut microbiome and uses thereof in the analysis of the impact of xenobiotics.

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