

Antibiotic perturbations to the gut microbiome

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Abstract

Antibiotic-mediated perturbation of the gut microbiome is associated with numerous infectious and autoimmune diseases of the gastrointestinal tract. Yet, as the gut microbiome is a complex ecological network of microorganisms, the effects of antibiotics can be highly variable. With the advent of multi-omic approaches for systems-level profiling of microbial communities, we are beginning to identify microbiome-intrinsic and microbiome-extrinsic factors that affect microbiome dynamics during antibiotic exposure and subsequent recovery. In this Review, we discuss factors that influence restructuring of the gut microbiome on antibiotic exposure. We present an overview of the currently complex picture of treatment-induced changes to the microbial community and highlight essential considerations for future investigations of antibiotic-specific outcomes. Finally, we provide a synopsis of available strategies to minimize antibiotic-induced damage or to restore the pretreatment architectures of the gut microbial community.

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Introduction

The human gut microbiome is a microbial organ with substantial influence over health and disease¹. From birth, the gut microbiome works in cohesion with the gut epithelium and surrounding organs to digest and transform nutrients, provide colonization resistance against pathogens and regulate the immune system^{2–4}. Consequently, disruptions in the taxonomic and metabolic pathway architecture of the gut microbial community (referred to as dysbiosis) have been associated with an increased risk of numerous human pathologies, including inflammatory bowel disease⁵, obesity⁶, colorectal cancer^{7,8} and numerous gastrointestinal infections^{9–11}. Among the most notable aetiologies of gut microbiome dysbiosis is antibiotic exposure. In this Review, we focus on the relationship between antibiotics and the human gut microbiome, the complexities of understanding how antibiotics acutely and persistently perturb this microbial community, and strategies for remediating these perturbations.

Since the discovery of penicillin by Alexander Fleming¹², antibiotics have revolutionized modern medicine, saving countless lives from otherwise lethal bacterial infections¹³. For instance, in the USA, the antibiotic revolution changed the leading causes of death from infectious diseases (for example, pneumonia, tuberculosis or diphtheria) to non-communicable diseases (for example, cardiovascular diseases or cancer), adding decades to the average life expectancy^{13,14}. Thus, antibiotics have critically enabled our modern ways of living, particularly in high-income countries. However, although antibiotics were developed to target human and animal pathogens of interest, their molecular targets (for example, the cell wall, the ribosome and RNA polymerase) are highly conserved across the bacterial kingdom. Accordingly, antibiotic exposure in the gut can indiscriminately target both pathogenic and benign bacteria, thereby disrupting ecological niches that are responsible for myriad metabolic transformations. This change in taxonomic composition of the gut microbiome is often a risk factor for the onset of other diseases. Most notably, treatment-induced disturbance of the gut community enables the growth of potentially infectious pathobionts (opportunistic microorganisms that proliferate on microbiome perturbation) with the capacity to cause disease^{15,16}. Some of the most dangerous microbial threats (as named by the CDC in 2019 (ref. 17)), including drug-resistant *Enterobacteriaceae*, *Clostridioides difficile*, vancomycin-resistant *Enterococcus* and *Salmonella* spp., are responsible for a substantial amount of gastrointestinal disease¹⁸. In addition to enrichment of overtly pathogenic organisms following antibiotic exposure, antibiotics are increasingly considered key contributors to more complex immunological manifestations such as graft-versus-host disease, inflammatory bowel disease and even allergies in the host^{5,19–21}. Understanding how different antibiotics disturb the human gut microbiome is thus critical for mitigating diseases related to microbiome dysbiosis.

Another unintended consequence of the steady increase in antibiotic utilization worldwide²² has been the concomitant rise of antimicrobial resistance (AMR), compromising the treatment of resistant bacterial infections^{23,24}. In 2019, an estimated 4.95 million deaths were associated with bacterial AMR worldwide²⁵. Enrichment for AMR often begins within the gut microbiome of the patient, with antibiotics increasing the abundance of resident bacteria that carry antibiotic resistance genes (ARGs) and altering ARG content within the gut microbiome (termed the ‘resistome’)^{26,27}. However, the spread of ARGs is not exclusively attributed to their primary bacterial hosts: ARGs are commonly encoded within mobile genetic elements (MGEs)²⁸, which enable their acquisition by new hosts, including pathobionts²⁹.

Notably, the presence of ARG-encoding pathobionts in the gut is a risk factor for recurrent resistant infections post-treatment³⁰. Knowledge regarding the impact of specific antibiotics on gut resistome structure and dynamics, with a particular focus on mobilizable ARGs, could guide future treatment choices, leading to increased therapeutic efficacy long-term and minimizing the dissemination of AMR in the gut microbiome.

In this Review, we incorporate primary literature describing human cohort studies, animal studies and in vitro bacteriology to provide an overview of the factors governing the antibiotic exposure-induced restructuring of the gut microbial community. In each case, we highlight the general patterns of change in bacterial populations and encoded resistance traits observed during treatment, while emphasizing the multiple levels of complexity in understanding antibiotic action in a community of diverse, interconnected microorganisms within the human host. Finally, we review promising therapeutic avenues aimed at minimizing antibiotic-induced damage to the microbiome or restoring the community structure post-treatment.

Effects on the gut microbiome

A growing body of research has investigated the structure of the gut microbiome and its dynamics after different types of antimicrobial exposures (Supplementary Table 1). Antibiotic-specific effects on the gut microbiome are increasingly recognized, and both human and animal studies have revealed reproducible taxonomic and metabolic patterns of community perturbation, particularly in the context of broad-spectrum therapies. In this section, we first present general trends observed on antibiotic administration. Subsequently, to illustrate antibiotic-specific effects on the gut microbiome, we rely on the results of a few human clinical trials comparing the effects of distinct antibiotics on the microbiome in a controlled manner (Supplementary Table 1). Last, we highlight the growth and metabolic characteristics of bacterial life cycles in the gastrointestinal tract that alter bacterial resiliency during antibiotic treatment.

Generalized and antibiotic-specific principles of microbiome community perturbation

In general, exposure to antibiotics (across drug classes) often causes important changes in microbiome community structure, species composition and metabolic capacity (Fig. 1). Foundational studies in healthy adult humans have commonly demonstrated the acute decline in alpha diversity³¹ (in the first couple of days of treatment) and the incomplete recovery of the microbiome up to 6 months after treatment (often measured by beta diversity between baseline and post-treatment)^{32–37}. These community-level changes are accompanied by significant decreases in the relative abundance of important members of the phyla Firmicutes, Bacteroidetes and Actinobacteria, most notably *Faecalibacterium prausnitzii*, *Eubacterium* spp., *Roseburia* spp., *Bifidobacterium* spp., *Dorea* spp., *Anaerostipes* spp. and *Ruminococcus* spp.^{32,34,38,39}. These lost species provide critical metabolic functions to the gut microbiome, as antibiotic administration in both humans and mice is commonly associated with decreased bile acid transformation capacity (increased primary bile acids) and carbohydrate fermentation (decreased short-chain fatty acids (SCFAs))^{40–45}. Additionally, metabolomic investigations of antibiotic treatment and the gut microbiome have noted increased levels of simple carbohydrates and amino acids, which is probably due to the loss of species diversity in the community^{45,46}. Loss of this metabolic colonization resistance through antibiotic treatment precedes opportunistic infections

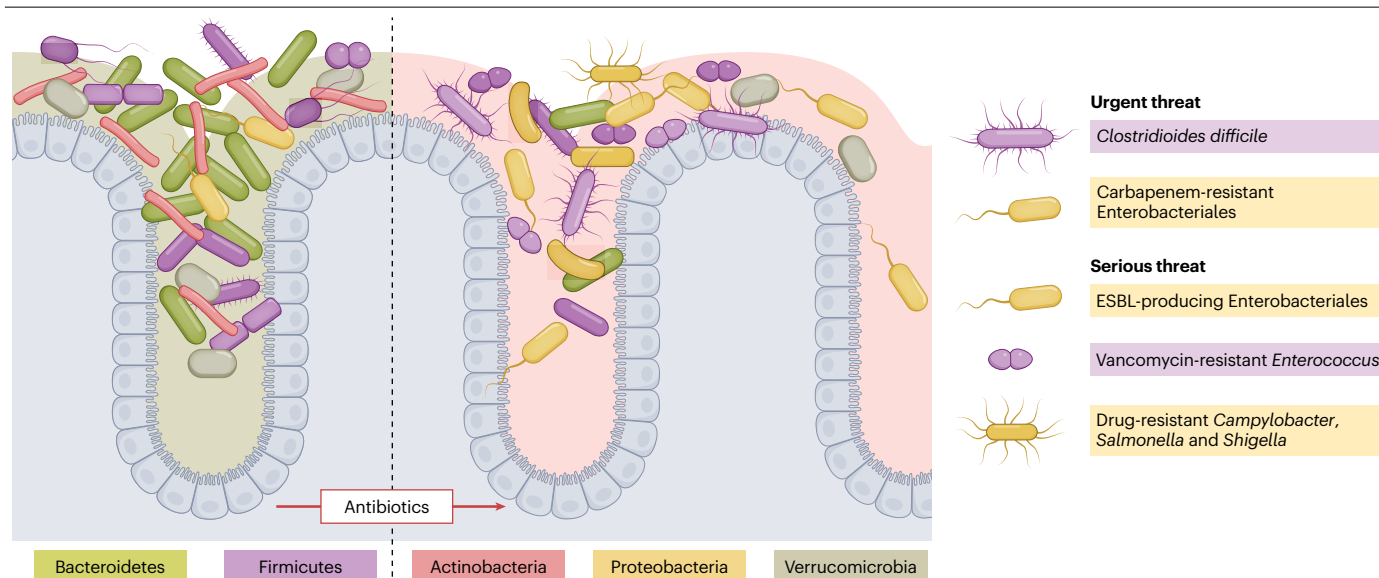


Fig. 1 | Antibiotic-mediated destruction of the gut microbiome opens an opportunistic niche. Antibiotics often eradicate Bacteroidetes, Firmicutes and Actinobacteria taxa responsible for important gut microbiome functions, although the species lost are community-specific and antibiotic-specific. This loss of diversity leaves an open niche for colonization or proliferation by opportunistic bacteria that are associated with gastrointestinal disease,

namely those from phylum Proteobacteria. These opportunistic bacteria often have mobilizable drug resistance or innate properties such as spore formation that enable them to survive antibiotic exposure. A number of organisms that can colonize an antibiotic-perturbed gut microbiome are recognized by the CDC as major threats to public health. ESBL, extended-spectrum β -lactamase.

(or blooms if pathobionts were existent at a lower abundance) by *C. difficile*, *Enterococcus* spp., *Candida albicans* and other gut pathogens^{46–50}. Correspondingly, antibiotic treatment in adult humans is often associated with an increase in the relative abundance of facultative anaerobes that were initially less abundant members of the community, such as Enterobacteriaceae, *Enterococcus* spp., *Clostridium* spp. and *Streptococcus* spp.^{34,37,51,52}. Although clinical studies of bacterial infections often emphasize the species and/or strain of opportunistic pathogens or pathobionts^{53,54}, literature describing commensal fluctuations during antibiotic exposure is currently limited to the taxonomic resolution of genus or species.

Assessment of gut microbiome composition is confounded by numerous host, microbial and environmental factors (both technical and biological), rendering comparisons of the reported effects of different antibiotics on the gut across studies currently intractable. Our understanding of the differential impact of antibiotics is largely informed by investigations in which multiple treatment agents are analysed in the same study population^{39,46,55,56}. For instance, vancomycin has been shown to cause a significant decrease in alpha diversity (more than twofold at the operational taxonomic unit level), an increase in primary bile acids (approximately threefold in faecal levels) and a decrease in the stool butyrate and acetate levels (more than fivefold and approximately twofold, respectively) relative to amoxicillin^{39,55}; paradoxically, of the two drugs, vancomycin has a narrower in vitro spectrum of activity. Furthermore, vancomycin significantly decreased the relative abundance of *Coprococcus eutactus*, *F. prausnitzii* and *Anaerostipes caccae* (SCFA-producing Firmicutes), while increasing the relative abundance of Enterobacteriaceae and *Enterococcus* spp.⁵⁵. Three different mouse models have reproduced the superior capacity of vancomycin to decrease alpha diversity or increase pathogen susceptibility

compared to more than five other antibiotic agents or combinations (including ampicillin, azithromycin, ciprofloxacin and ciprofloxacin-metronidazole)^{46,56,57}. Relative to cotrimoxazole and nitrofurantoin, ciprofloxacin has been shown to cause a greater reduction in species richness (less than twofold) and beta diversity^{58,59}, in addition to its effect in depleting *Bifidobacterium* and *Ruminococcus* spp. Azithromycin has been widely explored (in clinical trials of >1,000 children across sub-Saharan Africa) on its own and in comparison to other antibiotics^{60–66}. In a comparative study, azithromycin leads to a greater decrease in Simpson's alpha diversity (less than twofold) 5 days post-treatment than amoxicillin or cotrimoxazole⁶⁷. More recently, in healthy adult volunteers, microbiome analysis during four different antibiotic regimens identified that azithromycin-containing regimens were associated with delayed community recovery following treatment relative to the regimens without azithromycin³³. Understanding perturbation differences between regimens will be critical to enhancing antibiotic stewardship, and future studies incorporating more control over antibiotic administration parameters (for example, route of exposure, duration, dose or bioavailability) will be imperative for influencing antibiotic stewardship.

To systematically benchmark magnitudes of community destruction in the microbiome during different antibiotic perturbations, an increased taxonomic resolution of the commensal microbiome and a refined appreciation for antibiotic pharmacodynamics are required. To date, most studies of the microbiome have relied on stool short-read shotgun metagenomics or 16S rRNA amplicon sequencing as the main techniques for determining community structure, which generally does not provide strain-level resolution of the microbiome (a requirement for understanding gut microbiome AMR composition, as discussed below)^{68,69}. Metagenome-assembled genomes, particularly

those incorporating long-read metagenomic sequencing, can provide increased strain-level resolution^{70–72}. Additionally, depending on the chemical composition, formulation and administration route, antibiotics have different propensities to bioaccumulate in the gastrointestinal tract⁷³; this undoubtedly has a role in determining the magnitude of microbiome perturbation. For example, the superior ability of oral vancomycin administration to perturb the microbiome may be due to its poor absorption in mammals and perhaps higher concentration in the lumen⁷⁴. Bioavailability of antibiotics and their administration route have been rarely examined as influential variables in comparative studies of microbiome perturbation^{75,76}. Although published human studies provide some grounds for making antibiotic-specific claims about different magnitudes of microbiome perturbation capacity, human and

animal models require more rigorous control over dosing and route of administration of antibiotics to accurately interpret differences in effect size across antibiotics⁷⁷.

Species and community determinants of susceptibility during antibiotic exposure

From the perspective of an individual species of the gut microbiome, several intrinsic growth properties may enable a subset of bacterial species to persist on antibiotic treatment (Fig. 2). Members of the *Clostridium* and *Peptostreptococcus* genera (along with several other species in the phylum Firmicutes) have the capacity to form endospores, which are non-replicating forms of the cell that are intrinsically resistant to antibiotics, heat and oxygen^{78–80}. Spore-forming pathobionts such as

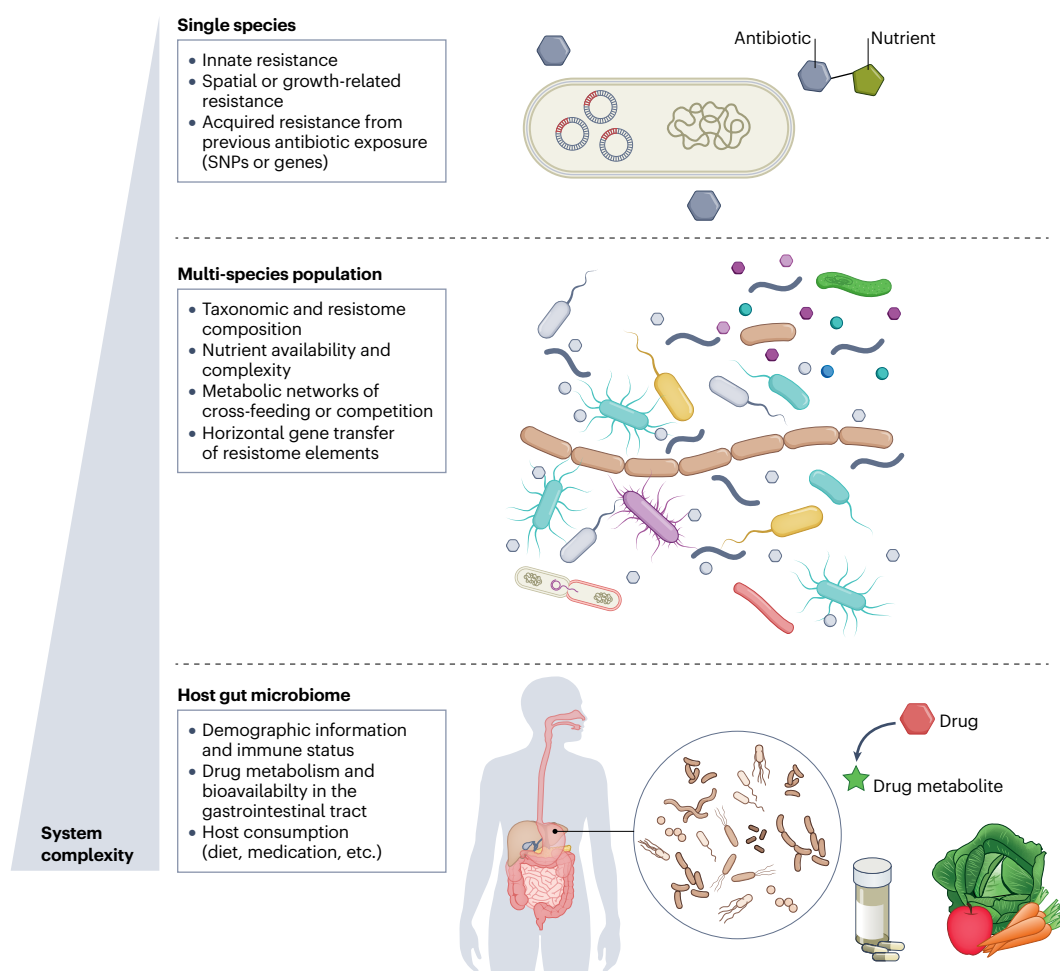


Fig. 2 | Integrated understanding of how antibiotics remodel the microbiome.

Mechanistic investigation into taxonomic, metabolic and resistome restructuring during antibiotic exposure requires multiple levels of study: single organisms, multi-species dynamics and in vivo microbiome studies in animal and human hosts. The susceptibility of a single organism is governed by physiological properties such as spore-forming capacity, the presence of drug resistance genetic elements, or defined differences in nutrients and minerals. Examination of bacterial interactions has revealed that the surrounding environment can influence differential susceptibility through provocation of horizontal gene transfer, metabolic conditioning or cell–cell signalling. In increasingly complex communities, the metabolic network of cross-feeding interactions

imparts unknown consequences on microbial susceptibility (at the species or community level). The exchange of antimicrobial resistance genes or other mobile genetic elements could also drive the outcome of community perturbation. Finally, at the whole-organism level, factors external to the microbial community may determine the magnitude of an antibiotic perturbation. Host genetics and immune status are likely to influence drug metabolism and absorption, in addition to drug-specific properties of absorption. Additionally, dynamic influences such as host consumption of dietary elements and medications are likely to change community trajectories during antibiotic perturbation. Other factors not discussed here are highlighted in Table 1.

C. difficile and *Clostridium perfringens* sporulate in response to population density and nutrient availability⁸¹. These spores survive antibiotic exposure in the gastrointestinal tract, and the presence of free nutrients and primary bile acids (as discussed above) provides molecular signals for spore germination and subsequent pathogenesis^{82,83}. Apart from spore-mediated persistence, many gut microorganisms can tolerate antibiotics through various resiliency mechanisms, including persister cell formation, perhaps in a mucin-embedded biofilm or deeper tissue niche where they can resist antibiotics^{84–87}. Antibiotic susceptibility of a gut commensal may thus be determined by its surrounding nutrient milieu (Box 1), a concept that remains difficult to investigate in vivo^{88–92}. Although it is often speculated that Bacilli and Enterobacteriaceae are more likely to bloom on antibiotic treatment of the gut microbiome because they possess more encoded resistance, these suppositions are based on resistance databases with likely overrepresentation of pathogens (discussed below) and do not account for the growth and metabolism of single organisms and communities.

In clinical practice, in vitro susceptibilities for respiratory and enteric pathogens serve as the gold standard for treatment of such infection and predict pathogen killing reasonably well. Yet, the in vitro spectrum of activity of an antibiotic does not always predict in vivo species trajectories in a multi-species microbiome during antibiotic exposure^{93–96}. For example, despite the in vitro Gram-positive-specific action of vancomycin, its oral administration (in human and animals) results in distinct loss of several Gram-negative gut microbiome commensals including *Bacteroides*, *Prevotella* and *Alistipes* spp.⁹⁷. These observations underline the complexity of microbial metabolic networks in the microbiome, built by a diversity of mutualistic and competitive relationships across phyla. These community networks almost certainly alter antibiotic killing in the microbiome⁹⁸. In vitro studies of interactions between two gut commensals during antibiotic treatment indicate that symbiotic relationships can have multiple outcomes in effecting differences in antibiotic tolerance in one⁹⁹ or both members of the community^{100,101}. Although these studies provide a high-resolution view of model gut microbiome relationships, extrapolating these findings to much larger networks of relationships within the human gut microbiome remains a critical hurdle in the field. This phenomenon is further complicated given that different investigations of microbiome perturbation by the same antibiotic often do not agree (Supplementary Table 1).

Host-related contributions

Synergy between antibiotics and metabolic or dietary elements can further shape microbiome dynamics and alter susceptibility to antibiotics within the community, leading to clinically relevant differences in disease¹⁰² (Fig. 2). Investigations into ‘Western-style’ diets (high in fat and simple sugars) have revealed that these diets correlate with an increased risk for obesity and heart disease, among other poor health outcomes^{103,104}. Antibiotic treatment of mice on a high-fat diet provokes pathological inflammation at the gut mucosa and increased levels of Enterobacterales, a taxonomic order that contains common pathobionts of the microbiome¹⁰⁵. These mice displayed immunological symptoms of pre-inflammatory bowel disease, which is probably due to the combined metabolic influence of the high-fat diet on the gut epithelial cells and the change in microbiome structure. From several other mouse studies using both dietary and chemical interventions to simulate the effects of a Western-style diet, it is clear that the combination of both diet and antibiotics changes microbial and host metabolism¹⁰⁶. Specifically, in one model, such synergistic

interventions freed up simple sugars and ethanolamines through an altered fatty acid metabolism, ultimately increasing susceptibility to enteric infection by *Salmonella enterica*¹⁰⁷. Alternatively, modulation of polysaccharide or microbiota-accessible carbohydrate levels in diet is highly influential over antibiotic treatment outcomes. Without fibre, mice with a conventional microbiome or a humanized microbiome have a delayed recovery in alpha diversity after ciprofloxacin treatment⁹⁶. In humans, volunteers fed omnivore and vegan diets during combination antibiotic treatment displayed an expedited microbiome recovery (as measured by alpha and beta diversity) relative to those on a fibre-free liquid diet. Specifically, fibre-containing diets encouraged a rapid restoration of stool butyrate levels and a return of Firmicutes with distinct amino acid metabolism¹⁰⁸.

In addition to understanding macronutrient dietary influences on antibiotic perturbations, consumption of antibiotics is likely to be accompanied by consumption of other medications, differential micronutrient intake and differential xenobiotic exposure. The interactions between these non-antibiotic factors and the microbiome have been reviewed elsewhere¹⁰⁹. Metagenomic analyses of large cohorts of humans with gastrointestinal inflammatory conditions reveal strong taxonomic and metabolic pathway associations with a proton-pump inhibitor, metformin (a type 2 diabetes medication) and laxative exposure¹¹⁰. These extrinsic host influences are not typically accounted for in antibiotic–microbiome investigations, despite the clear capacity of many of these xenobiotic compounds to affect growth promotion or inhibition in a species-specific manner^{111,112}. Correspondingly, some pharmaceuticals have the capacity to antagonize or synergize with antibiotics. For instance, dicumarol, an anticoagulant, was demonstrated in vitro and in vivo to antagonize the erythromycin-mediated killing of *Bacteroides vulgatus*, while allowing the killing of the opportunistic pathogen *Enterococcus faecalis* in a 12-member defined community¹¹³. Future human studies on coadministered medications and antibiotics will be critical, especially in immunocompromised hosts, to understanding the risks and benefits of coadministration related to microbiome consequences.

Effects on the gut resistome

Antibiotics have a pronounced but highly variable effect on the gut resistome (the suite of ARGs encoded by the microbiome)³³. Here, we summarize key insights from the literature regarding the effects of antibiotics on the resistome, while highlighting factors that correlate with variability in treatment outcomes. Although resistome composition is intimately connected to the taxonomic structure of the gut microbiome (defined by a distinct population of bacterial strains encoding resistance-conferring elements)⁵², our increasing understanding of horizontal gene transfer (HGT) has revealed that this connection is not absolute and that changes in the resistome are not fully explained by concurrent taxonomic shifts. In this context we focus on HGT within the microbiome, discussing the potential roles of MGEs in the spread of ARGs post-antibiotic challenge and emphasizing the challenges accompanying the investigations into the impact of antibiotics on HGT.

Antibiotics commonly enrich the gut resistome

Changes to the gut resistome through antibiotic exposure could provoke pathological blooms of drug-resistant pathobionts in the microbiome. Because of these phenomena, the composition and dynamics of the gut resistome have been the foci of numerous studies (Table 1), which have revealed a few general patterns of antibiotic-induced changes in the resistome. Notably, antibiotic treatment most commonly results

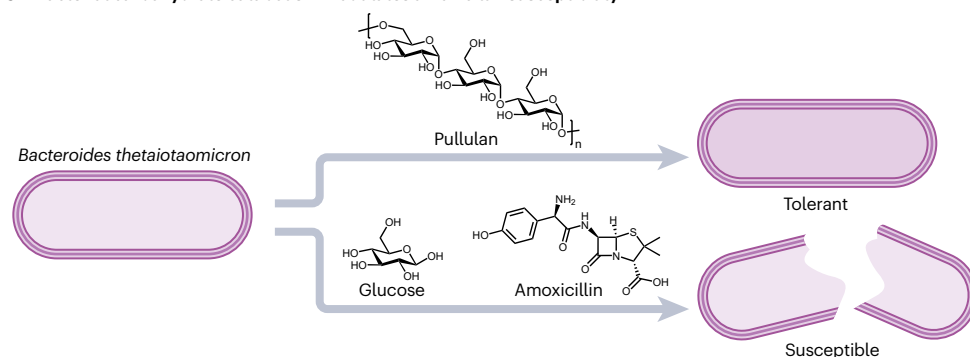
Box 1

Examples of metabolite–microorganism–antibiotic dynamics relevant to the gut microbiome

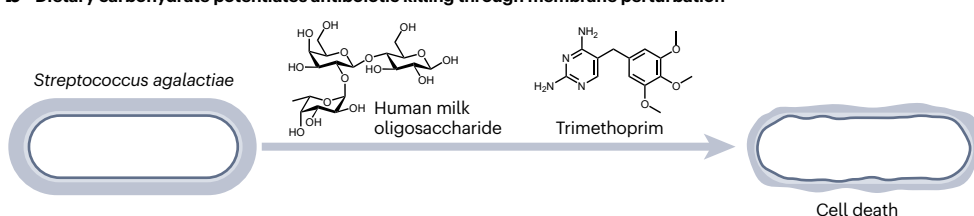
Host-derived metabolites and bacterial signalling molecules have the capacity to change the intrinsic antibiotic susceptibility of bacterial species. In phylum Bacteroidetes, *Bacteroides thetaiotaomicron* is more tolerant of amoxicillin in the presence of polysaccharides

such as pullulan, whereas glucose consumption is sensitizing⁸⁸ (see the figure, part **a**). Human milk oligosaccharides cause changes to membrane permeability, sensitizing *Streptococcus agalactiae* to trimethoprim⁸⁹ (see the figure, part **b**). As this species is not a

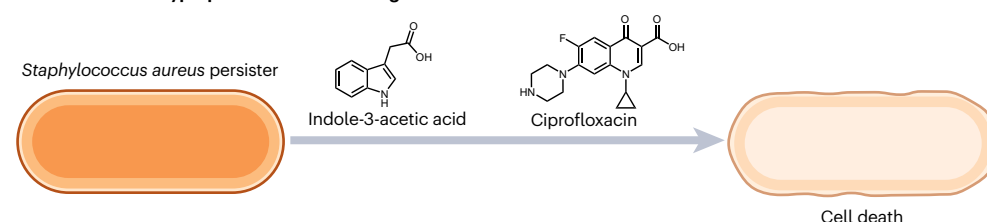
a Bacterial carbohydrate catabolism modulates amoxicillin susceptibility



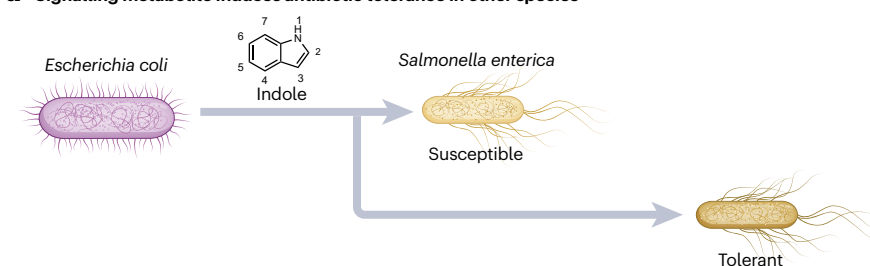
b Dietary carbohydrate potentiates antibiotic killing through membrane perturbation



c Metabolizable tryptophan derivative of the gut microbiome sensitizes MRSA to antibiotic



d Signalling metabolite induces antibiotic tolerance in other species



MRSA, methicillin-resistant *S. aureus*.

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member of the gut microbiome, it is possible that streptococci and other organisms in the gut experience differences in membrane permeabilization due to fluctuations in dietary polysaccharides. Indole-3-acetic acid, which is a derivative of microbial-derived tryptophan metabolism, can sensitize *Staphylococcus aureus* to ciprofloxacin⁹⁰ (see the figure, part **c**). In phylum Proteobacteria,

indole (another microbially produced tryptophan derivative) is produced by Enterobacteriaceae and can induce tolerance to quinolones in nearby Enterobacteriaceae^{91,92} (see the figure, part **d**). These examples highlight representative and simple nutrient–microorganism relationships but remain difficult to generalize to the complex community within the gut microbiome.

in acutely increased ARG burden (as measured by changes in relative abundance) in the gut microbiome^{33,51,114–118}. Despite increasing the total resistome burden, antibiotics can concurrently lead to a substantial loss of specific ARGs¹¹⁹ and reduced resistome diversity¹¹⁶, which is probably due to taxonomic loss. Antibiotics expectedly enrich for ARGs that confer resistance to the treatment agent^{33,34,51,62–66,114,115,117,119–123} through selection of bacterial strains encoding these genes^{121,124,125}. However, concurrent enrichment of ARGs unrelated to the administered drug is common^{33,34,51,114,115,119–121,123}. This latter enrichment of ARGs after antibiotics is often species-specific, and the set of ARGs enriched on treatment can be attributed to specific multi-drug resistant (MDR) taxa^{52,126}. For example, the enrichment of most ARGs in the guts of preterm infants treated with meropenem and ticarcillin–clavulanate was found to be highly correlated with the enrichment of *Staphylococcus epidermidis* or *Klebsiella pneumoniae*⁵². However, it should be noted that ARG carriage is a strain-specific trait, and it is likely that individual MDR traits within these species are being selected for¹²⁴. Another factor driving the enrichment of genes unrelated to the treatment is the localization of diverse ARGs within MDR genetic clusters, which ensures the co-enrichment of groups of genes on selection for at least one of the encoded ARGs^{52,127}. However, such co-selection of ARGs is often short-lived: with prolonged treatment, ARGs conferring resistance to the administered antibiotic remain enriched even after years, but the abundance of enriched ARGs unrelated to treatment can diminish with time⁶². This suggests that over time, microorganisms undergo more specialized adaptation, isolating and maintaining the ARGs conferring growth advantage to the host while putatively shedding the genes unrelated to the treatment (likely due to the fitness costs associated with carriage of some ARGs)¹²⁸.

Importantly, the effects of antibiotics on the gut resistome vary substantially depending on the nature of the administered drug^{33,52,59,114,123}. For instance, in one study involving healthy adults, oral administration of azithromycin (a macrolide) or cefpodoxime (a cephalosporin) resulted in an increased relative abundance of ARGs, whereas no changes in the relative ARG abundance were observed on oral administration of levofloxacin (a fluoroquinolone)³³. Similar post-treatment effects of macrolides^{62–66,123}, cephalosporins^{119,120} and fluoroquinolones⁵⁹ on the relative abundance of ARGs have been reported elsewhere for diverse population segments (for example, children and elderly, healthy and diseased). Nonetheless, the effects of various antibiotic classes and individual antibiotics on the human gut resistome remain largely unexplored; reports focusing on single-treatment agents remain few and far between^{33,59,62,119–121,123}, with investigations of the resistome changes commonly involving combinations of antibiotics^{32,34,122} or with retrospective cohorts with variable treatment regimens, doses and durations^{114,116,118,129} (Table 1). Moreover, factors beyond the treatment agent can affect resistome dynamics during treatment, further obscuring our understanding of the antibiotic-induced resistome changes. Notably, administration

route is a potentially important determinant of the extent of resistome restructuring by antibiotics. In mice treated with tetracycline or ampicillin, relative to intravenous injection, oral administration of the same doses of drugs resulted in faster and higher increases in relative abundance of ARGs¹²⁵. To achieve a more in-depth understanding of antibiotic-specific effects on the gut resistome, there is a need for future research involving single-treatment agents tested within the same study populations with controls over dose and administration routes.

To use our nascent understanding of the antibiotic-mediated changes to the resistome in any predictive manner, the field would benefit from establishment and broader implementation of standardized approaches for resistome characterization. Recent investigations of the gut resistome have primarily relied on whole-metagenome shotgun sequencing with subsequent alignment of sequencing reads or annotated open reading frames from assembled contigs against one or a few of the available databases of known ARGs^{32–34,59,62,114,117–119,123,129,130}. ARG databases vary considerably in size (that is, the number of represented resistance determinants) and composition¹³¹, and, consequently, resistome classification is not consistent across databases¹³².

Table 1 | Biological variables that influence gut microbiome dynamics in response to antibiotics

Variable	Example	Refs.
Antibiotic type or class	Azithromycin (macrolide) versus levofloxacin (fluoroquinolone)	33,39,52,123, 126,210–214
Spectrum of activity	Broad versus narrow spectrum	55
Drug pharmacokinetics	Biliary elimination rate	215
Administration route	Oral versus intravenous	125,216
Antibiotic dose	Low-dose versus high-dose treatment	59,211,217
Duration of treatment	Short-term versus prolonged treatment	62–66,218
Concurrent medication or medications	Virostatic agents	59
Baseline microbiome composition	Abundance of <i>Bacteroides</i>	59,68,219
Diet	Low-fibre diet; breast milk	52,96,108,220
Host age	Postmenstrual age	52,126
Health status	Clinical Risk Index for Babies (CRIB) II score	52
Host organ system activity	Kidney and liver function	59
Sex	Male versus female	56,221,222
Host genetics	C57BL/6J mice versus 129S6 mice	223
Host type	Drug clearance rate in humans versus mice	224

There are instances of the same gene annotated by different names across databases¹³³, which further impedes accurate comparative analysis of corresponding resistome studies. Furthermore, such databases are typically curated from the existing biomedical literature^{134,135}, which suggests a strong bias in these collections towards ARGs from human pathogens and underrepresentation of ARGs from commensals and environmental organisms. These shortcomings could be addressed through functional metagenomic screens, which identify novel ARGs in a sequence-agnostic, function-centric manner¹³⁶. Future efforts to establish the effects of antibiotics on the gut resistome would benefit from enrichment of antimicrobial resistance databases with ARGs from a broader range of organisms and habitats, and from ongoing consolidation, cross-checking and benchmarking of these databases.

Horizontal gene transfer facilitates post-antibiotic resistome enrichment

The antibiotic-induced expansion of the gut resistome may also be driven by ARG-encoding MGEs^{114,137,138}. In the short term, the increase in resistome burden after antibiotic treatment is primarily driven by the enrichment of chromosomally encoded ARGs (that is, the expansion of resistant bacterial lineages). However, the abundance of microorganisms with chromosomal ARGs declines sharply shortly thereafter (~1 month)⁶⁸. Conversely, MGE-encoded ARGs persist for longer periods after therapy cessation, which is probably due to the parasitic nature of MGEs (that is, their persistence among bacterial populations despite the absence of antibiotic-mediated growth advantages or presence of fitness defects conferred on the bacterial host)^{32,68}. Antibiotics can increase the abundance of MGE-encoded ARGs in the gut⁵⁹ and lead to more frequent and broader (involving more diverse taxa) dissemination of ARGs and MGEs in patients¹³⁸. Overall, higher rates of horizontal transfer of ARGs are found in populations with higher reported clinical use and environmental antibiotic exposure¹³⁹. Such increased HGT rates are of concern owing to their potential implication in the spread of ARGs to both resident commensals and pathobionts within the gut microbiome¹⁴⁰, thus increasing the future risk of opportunistic infections.

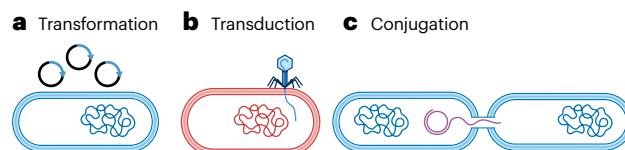
Antibiotics can reportedly facilitate all three mechanisms of HGT: transformation^{141–143}, transduction¹⁴⁴ and conjugation^{145,146}, the last being the best-studied form of horizontal transfer of ARGs^{138,147–152} (Box 2). ARGs are commonly encoded on conjugative plasmids, which can be transferred between taxonomically distinct residents of the gut microbiome¹³⁷. Low doses of antibiotics have been reported to facilitate plasmid conjugation within bacterial communities^{145,146}. Increased rates of conjugation can result from the antibiotic-mediated induction of the bacterial SOS response and increased membrane permeability^{153–157}, which may stem from the DNA damage inflicted by certain antibiotics. However, the effects of antibiotics on the rates of HGT (particularly conjugation) are contested, with other studies demonstrating highest plasmid transfer rates in the absence of antibiotics^{158–161}. These conflicting reports are likely to arise from the difficulty of disentangling the effects of antibiotics on HGT rates from the antibiotic-mediated selection of transconjugants (Fig. 3), which suggests that the reported post-antibiotic enrichments of ARG-encoding MGEs often result from the growth advantage conferred on transconjugants, not higher HGT rates per se¹⁶².

Investigation of HGT rates within the gut microbiome is still in its nascency; our understanding of how the frequency of horizontal transfer is affected by antibiotics is very limited, let alone our grasp on

Box 2

Mechanisms of horizontal gene transfer in the gut microbiome

Transformation involves bacterial uptake of extracellular DNA (see the figure, part **a**). During transduction, bacteriophages serve as vectors for the horizontal transfer of genetic material, including antimicrobial resistance genes, between bacterial hosts (see the figure, part **b**). Conjugation describes the direct transfer of genetic material from a bacterial donor to a bacterial recipient, a process that necessitates physical contact between the two cells (see the figure, part **c**).



the variability of this impact across drug classes. Further investigation into the effects of antibiotics on HGT are needed, with careful experimental design that enables the precise determination of the frequency of HGT while controlling for the effects of antibiotics on other aspects of bacterial biology (such as the growth rate). Furthermore, although shotgun sequencing has enabled a comprehensive characterization of the taxonomic and resistance profiles of microbial communities (albeit with limitations noted earlier), technological limitations of short-read sequencing make it ill-suited for investigations of HGT (Box 3). For one, short-read metagenome assemblies are highly fragmented and cannot be used for identification of genetic origins (that is, whether from chromosome or plasmid) and taxonomic origins of ARGs¹⁵¹. Hybrid assemblies (based on both short and long reads) can address many of these limitations, enabling the contextualized characterization of ARGs²⁸. Additionally, because of the total DNA extraction step that typically precedes sequencing library preparation, shotgun metagenomic sequencing does not enable the efficient linking of MGEs with bacterial hosts, obscuring community HGT dynamics. These limitations can be addressed through Hi-C, a technique in which crosslinking before cell lysis preserves interacting DNA molecules and the information regarding the MGE–bacterial host associations¹³⁸. Hence, in addition to the aforementioned controls, future investigations into HGT dynamics would benefit from application of long-read sequencing and Hi-C platforms, offering further insight into the frequency and direction of ARG mobilization within the context of an antibiotic-perturbed microbiome.

Towards predictive models

The growing body of research directed at elucidating antibiotic-induced microbiome perturbations could ultimately grant us the capacity to model the post-treatment microbiome dynamics and to forecast off-target therapeutic effects, guiding the selection of treatments that minimize collateral microbiome damage. However, the immense complexity and interindividual variation of the gut microbiome is

well established, with its composition affected by innumerable factors. Consequently, and as we describe in previous sections, the post-antibiotic community dynamics depends on (and is confounded by) numerous treatment, microbial, host and environmental factors (Table 1), making it currently non-trivial to generate accurate generalized predictive models of microbiome or host outcomes related to antibiotic exposure. Nonetheless, some early efforts in generating predictive models of microbiome perturbation in specific cases and populations have been made by accounting for and, when possible, controlling for the variables of interest (Table 1). For instance, in a study involving a cohort of preterm infants from a single hospital, and testing over 70 metadata variables, a model based on only six predictors (postmenstrual age, breast milk consumption, CRIB (Clinical Risk Index for Babies) II score, and administration of meropenem, cefotaxime and ticarcillin–clavulanate) explained 33% of the variance in the species richness in this cohort⁵². Another investigation involving infants within the same hospital system similarly found that a predictive model based on day of life (age) and antibiotic treatment information alone could account for 57% of the variability in Shannon diversity¹²⁶. Thus, despite the complexity of the compositional dynamics of the microbiome, control over deterministic factors through careful selection of the study cohort can enable the development of accurate predictive models in specific settings. Conversely, careful selection of variables to control often decreases the generalizability of the study (for example, successful prediction of microbiome outcomes may be confined to only preterm infants in the region or hospital under investigation). To generate models with higher predictive accuracy and breadth, it is important that future investigations into the compositional consequences of microbiome perturbation integrate extensive metadata on treatment, microbial and host variables, some of which are outlined in Table 1. Moreover, it is imperative to subject the resulting models

to cross-cohort validation to determine the generalizability of the underlying predictors.

Ameliorating perturbations

The gastrointestinal tract requires a high, diverse microbial load to carry out requisite microbiome functions¹⁶³. Thus, efforts towards the maintenance or restoration of richness and diversity in the gut microbiome on antibiotic exposure are of extreme medical importance^{113,164,165}. Here, we present an overview of approaches (established and in development) aimed at either minimizing antibiotic-induced damage to the gut community during treatment or restoring microbiome diversity post-exposure (Fig. 4). Furthermore, alternative strategies with improved precision and decreased off-target effects relative to antibiotics are urgently needed and could be used to mitigate treatment-induced gut dysbiosis. Engineered probiotic microorganisms have shown great promise in treatment and prevention of pathogenic colonization with increasing precision, and may offer an avenue for the development of the aforementioned alternative anti-infective strategies¹⁶⁶. Given this potential, we discuss the use of engineered probiotic therapies with demonstrated anti-pathogen activity^{167,168}. Based on the shortcomings of many of these approaches, or the likelihood of community evolution to outpace a given therapy, we expect that the most successful therapies in the future are likely to come from a combination of approaches in mitigating gut dysbiosis and preventing gastrointestinal infections.

Minimizing antibiotic-induced perturbations to the gut

Given that antibiotics vary in their spectrum of activity against the diverse members of the commensal gut microbiome, the choice of treatments with a narrower spectrum could result in less perturbation to the microbiome and faster community recovery¹⁶⁹. For instance, relative

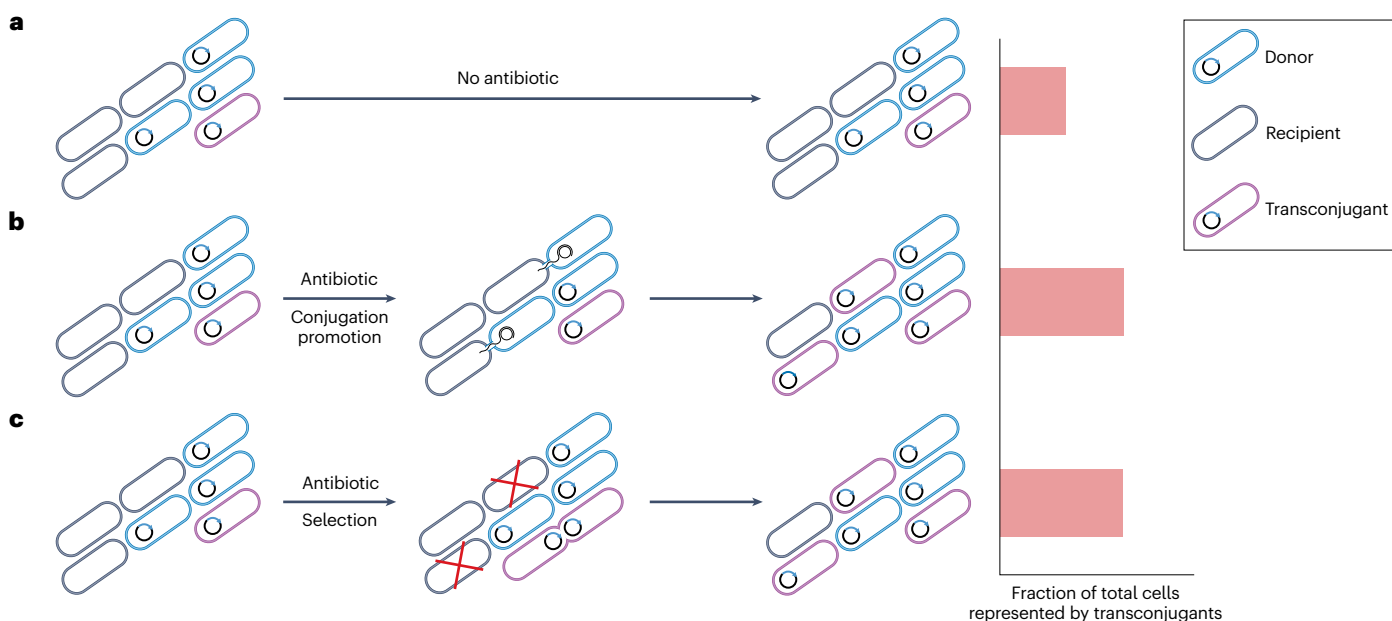


Fig. 3 | Assessing the effect of antibiotics on horizontal gene transfer rates. **a**, A community in equilibrium is depicted. In the absence of an antibiotic selection pressure, the basal horizontal gene transfer rates are maintained. The chart on the right depicts transconjugants as a fraction of the total cells in the community. **b,c**, During antibiotic treatment, either conjugation rates are increased (part **b**)

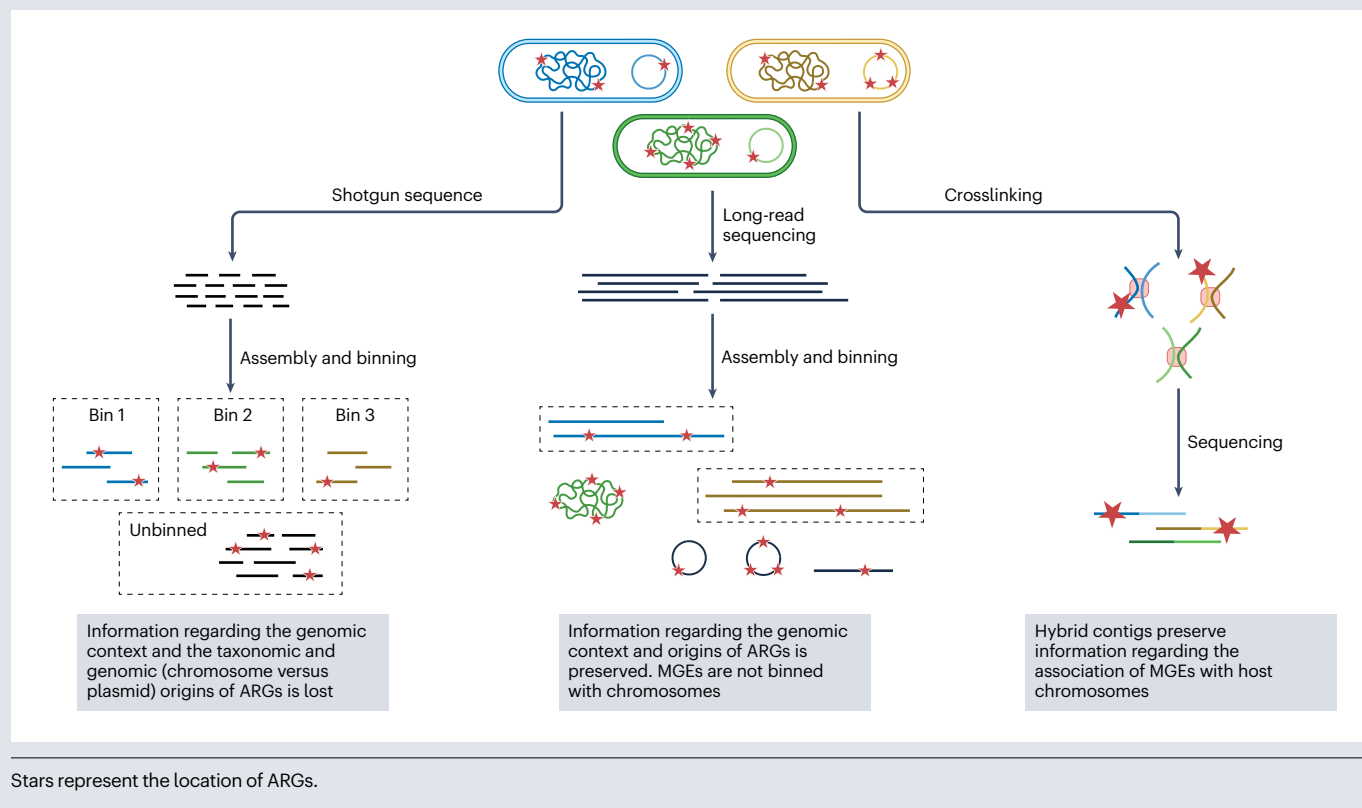
or selection promotes the growth of plasmid-containing populations (part **c**); these two outcomes could also take place concurrently. Both cases result in a higher fraction of transconjugants relative to the community in the absence of antibiotics. This leads to difficulties in distinguishing the effects of antibiotics on conjugation rates from those of antibiotic-mediated selection.

Box 3

Approaches for profiling the mobile fraction of the community resistome

To perform shotgun sequencing, the total metagenomic DNA of the community is first extracted and then sequenced to produce short (~150–300 bp) sequencing reads¹⁴⁹. The reads can subsequently be assembled into larger contigs, which are then grouped into bins based on the predicted common microbial origin¹⁴⁹ (see the figure). Despite computation advancements in assembly, binning and classification, many of the assembled contigs, particularly those corresponding to mobile genetic elements (MGEs), remain unbinned, obscuring their taxonomic origins¹⁵⁰. Furthermore, the relatively short length of the assembled contigs makes it impractical to determine the genomic origins (chromosome versus plasmid) of these contigs¹⁵¹. Consequently, although shotgun metagenomics enables a comprehensive characterization of antibiotic resistance genes (ARGs), the information regarding the taxonomic and genomic origins of ARGs is lost. Recent developments in long-read sequencing can address many of these limitations. Longer reads often enable the

assembly of complete (that is, circular) plasmidic contigs and yield much more informative (if not complete) chromosomal contigs¹⁵². This increases the efficiency of identification of the genomic origins of ARGs and pairing of chromosomal ARGs to taxa of origin. The longer contigs also reveal the genetic context of ARGs and their potential localization within MGEs¹⁵¹. However, it should be noted that the sequencing error rates of long-read sequencing platforms are often substantially higher than those of short-read methods; hence, a hybrid approach, taking advantage of longer contigs of long-read sequencing and higher accuracy of short-read sequencing, presents an optimal approach¹⁵¹. Last, given that the total metagenomic DNA is similarly extracted before sequencing, the information regarding the MGE–chromosome associations is lost. To this end, crosslinking of interacting DNAs before cellular lysis preserves the chromosome–MGE pairs. Hi-C, in which this strategy is used, yields hybrid contigs that can be used to identify the MGE hosts¹³⁸.



to vancomycin treatment (broad spectrum), fidaxomicin treatment (narrow spectrum) resulted in a smaller shift from the pretreatment taxonomic structure of the mouse gut, promoted a faster recovery of the taxonomic structure and preserved the *C. difficile* colonization resistance capacity of the gut to a higher extent¹⁶⁹. However, it should

be noted that the in vitro spectra of activities do not directly translate in vivo, and, as mentioned previously, higher levels of gut microbiome perturbation may result from agents with narrower in vitro spectra. The specificity of antibiotics can also be modulated through coadministration of other antibiotic and non-antibiotic medications: combination

treatments can have differential effects (neutral, synergistic or antagonistic) on the activity of the given antibiotic against microorganisms, and this modulation is variable across microbial taxa¹⁷⁰. Correspondingly, it may be possible to achieve species-specific treatment by using drug combinations that have synergistic effects against pathogens and antagonistic effects against commensal microorganisms^{113,170}.

During systemic administration of antibiotics (for example, in response to suspected bloodstream infections), reducing the levels of antimicrobials in the gut can also minimize off-target treatment effects and damage to the gut microbiome. As proof of principle, gut commensal *Bacteroides* spp. can release cephalosporinases that protect susceptible members of the community from lethal doses of cefotaxime¹⁷¹. Similarly, oral administration of a β -lactamase concurrently with systemic β -lactam antibiotic treatment maintains the taxonomic structure of the gut community^{164,172}. Although coadministration of the β -lactamase reduced the antibiotic concentrations in the gut, the serum levels of antimicrobials remained unaltered¹⁶⁴. Production and purification of antibiotic-inactivating agents at scale is non-trivial; expression and delivery of these agents by engineered probiotics provides a viable alternative. The probiotic *Lactococcus lactis* was engineered to express and secrete the TEM1 β -lactamase; oral gavage of the engineered microorganism with concomitant intraperitoneal injections of ampicillin reduced the gut levels of the drug (without affecting the serum concentrations of the antibiotic), lessened collateral damage to the gut microbiome and maintained *C. difficile* colonization resistance in mice¹⁷³. Notably, TEM1 was designed to be expressed by two gene segments as two enzymatically inactive components that reconstitute the functional enzyme

on secretion: this split nature of the gene minimizes the risk of its spread through HGT¹⁷³.

Antibiotic adsorbing agents have also been used in the context of the gut microbiome, as they have the advantage of inactivating a broad range of compounds. The most notable example is DAV132, an adsorbent based on activated charcoal, which reduces the faecal concentrations of moxifloxacin and fluoroquinolones without altering the serum levels of the antibiotics^{165,174}. DAV132 can further adsorb antibiotics belonging to five other drug classes *ex vivo*¹⁶⁵. Coadministration of DAV132 with antibiotics protects gut bacterial richness and diversity, impedes the spread of pathobionts and resistant organisms, and maintains the colonization resistance capacity of the gut against *C. difficile*^{165,174,175}. DAV132 can be further modified to achieve site-specific inactivation of antibiotics as well: coating of the adsorbent in a pH-dependent polymer ensured its release and activity in the colon, without compromising the antibiotic activity in the small intestine¹⁷⁶.

Prebiotic-mediated expansion of beneficial taxa

Given the metabolic capacity of the microbiome to digest complex dietary fibres, prebiotic compounds (which are compounds that stimulate the growth of beneficial organisms in the microbiome) have been increasingly used to both combat enteropathogens and restore commensal gut microbiome structure during or post-perturbation. Antibiotics disrupt the production of SCFAs in the gut. Polysaccharide-rich diets can be used to restore the production of these molecules; this restoration is largely associated with colonization resistance against pathogens^{49,177,178}. Defined polysaccharides have been examined in the context of mitigating *C. difficile* infection. In one study, purified mucin

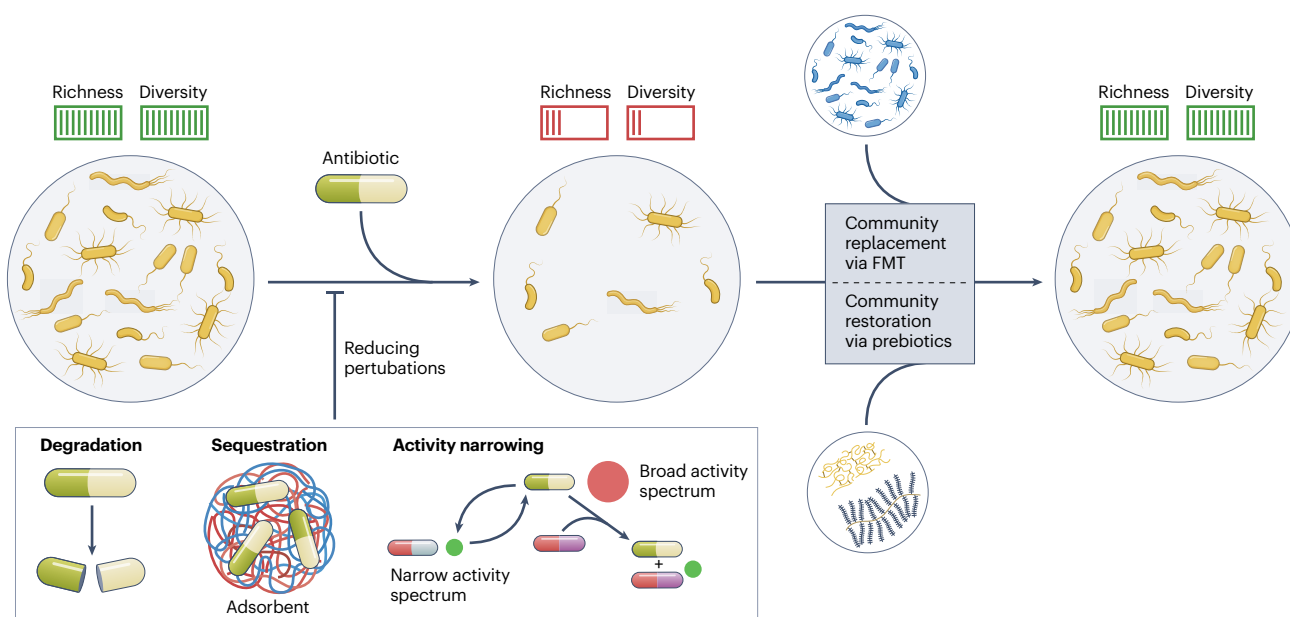


Fig. 4 | Approaches aimed at maintaining or restoring gut microbiome structure on antibiotic treatment. Antibiotics reduce the microbial richness and diversity of the gut microbiome. The gut community post-antibiotics can be restored via community replacement (faecal microbiota transplant (FMT)) or prebiotic administration, which may facilitate the growth of commensal members of the microbiome and/or prevent the colonization of pathogenic bacteria. Alternatively, antibiotic-mediated destruction of the microbiome can be prevented by bioengineering approaches targeting the antibiotic.

Specifically, the gut concentrations of the drugs can be diminished through enzymatic degradation or physical sequestration of antibiotics, preventing a broader reduction in the richness and diversity of the gut community. Last, the narrowing of the spectrum of activity of the treatment regimen might also prevent broader damage to the gut microbial community. This could be achieved by choosing antibiotics with inherently narrower activity spectra or through combinations of antibiotics with other drugs, which may similarly result in a narrower spectrum of antimicrobial activity.

glycans were used to increase community diversity post-antibiotic treatment; this activity decreases the proliferation of *C. difficile* on infection¹⁷⁹. In another study, dietary xanthan gum demonstrated a capacity to increase fibre-degrading taxa, although without significantly altering alpha diversity, and prevented *C. difficile* colonization, probably owing to the maintenance of the commensal microbiota during antibiotic treatment¹⁸⁰. In addition to restructuring the microbiome, it is also possible that several soluble non-starch polysaccharides could serve as a physical barrier against pathogen adherence to the epithelial barrier^{181,182}. Most recently, a symbiotic combination, *Bifidobacterium infantis* and milk oligosaccharides, was used to stably reverse dysbiotic human microbiomes¹⁸³.

Restoring antibiotic-perturbed gut communities via faecal microbiota transplants

The microbiome structure can be restored via whole-community replacement, or faecal microbiota transplants (FMTs), from healthy donors. Originally designed for its use in the treatment of recurrent *C. difficile* infection (rCDI), the use of FMTs has expanded to other infectious diseases, including the treatment of enteric infections, MDR bacterial colonization and viral infections (as reviewed elsewhere)¹⁸⁴. Following antibiotic treatment, administration of an autologous FMT resulted in faster restoration of the microbiome than a 12-member probiotic cocktail, which notably delayed the recovery in taxonomic diversity in the gut microbiome¹⁸⁵. In the context of rCDI, several microbial communities (derived from human donor stool or from defined bacterial culture) have shown promise in providing decreased risk of recurrent episodes^{186–189}. The most recent success story is SER-109 (produced by Seres Therapeutics), a compilation of purified Firmicutes spores that shows success against rCDI in phase III clinical trials¹⁸⁸. Importantly, SER-109 improves the production of secondary bile acids, which presumably prevent *C. difficile* germination^{190,191}. Another defined community, VE303 (produced by Vedanta Biosciences), is composed of fewer members than SER-109 and demonstrates robust restoration of butyrate production and secondary bile acid production in dysbiotic human microbiomes engrafted into mice; this community is well tolerated when administered with vancomycin to healthy volunteers¹⁸⁹. In all, FMTs and defined communities have been generally demonstrated to be safe and hold great promise in post-antibiotic restoration of the gut community. Nonetheless, there are noteworthy therapeutic risks, including the unintended introduction of pathogens and ARGs into the gut of the recipient^{185,192}. In the case of the microbiome restoration product RBX2660, although there is a clear benefit against rCDI, high-resolution profiling of microbiome dynamics indicated that it also engrafted donor antibiotic-resistant organisms and ARGs into the new host¹⁹². These unintended consequences may prove dangerous to patients; although rarely, previous FMT administration has resulted in severe opportunistic infections from a donor pathobiont^{193,194}. As therapies such as SER-109 and VE303 are further evaluated, it will be important to identify recipient microbiome-specific effects on community trajectory post-administration.

Probiotics as alternatives to antibiotics

Probiotics, which are live microorganisms with potential health benefits¹⁹⁵, can provide alternatives to antibiotics in treatment and prevention of microbial infections. However, probiotics may perform less well than whole-community replacement strategies (discussed above) in microbiome restoration and could result in resistome expansion in an antibiotic-pretreated gut¹⁹⁶. Nonetheless, probiotic organisms have been extensively used therapeutically against

various pathogens, commonly achieving their native anti-infective activity through competitive exclusion, sequestration and release of antimicrobial compounds (constitutive or induced), as reviewed elsewhere¹⁶⁶. Recent expansions of the toolsets aimed at genetic modification of bacterial and yeast probiotics^{197–199} have enabled the engineering of probiotic organisms with more potent and/or targeted anti-pathogenic activities. For instance, probiotic *Lactobacillus casei* strains were engineered to express and display adhesion proteins that enabled them to robustly colonize the intestine; the engineered probiotic prevented the intestinal colonization by a foodborne pathogen, *Listeria monocytogenes*, and protected mice from lethal infection¹⁶⁷. Similarly, the anti-pathogen activity of the probiotic *Escherichia coli* Nissle 1917 was enhanced by expressing nanobodies as bacterial curli fibre fusions, which enables the engineered strains to sequester pathogens (for example, enteropathogenic *E. coli* and *Shigella flexneri*) or pathogen toxins (for example, Shiga toxin and *C. difficile* toxin TcdA)²⁰⁰.

Probiotics can also be engineered to express and release pathogen-specific antimicrobial agents²⁰¹. To further reduce the off-target effects of the treatment, the release of antimicrobials can be engineered to be induced on sensing of external stimuli produced by the target pathogen^{202,203}. For example, *E. coli* Nissle 1917 was engineered to lyse itself on sensing the *Pseudomonas aeruginosa* quorum-sensing molecules, releasing cytosolic lysis and anti-biofilm molecules²⁰⁴. The engineered probiotic reduced gut *P. aeruginosa* titres in both mouse and *Caenorhabditis elegans* infection models²⁰⁴. The release of antimicrobials can alternatively be engineered to be induced by molecules characteristic of gut damage (dysbiosis and inflammation)²⁰⁵. Notably, probiotic *E. coli* was engineered to sense extracellular sialic acid, a proxy for gut dysbiosis, and respond by producing a bile salt hydrolase, deconjugating primary bile salts in the gut¹⁶⁸. The engineered probiotic reduced the germination of *C. difficile* endospores, resulting in 100% survival and improved clinical symptoms in murine models of *C. difficile* infection¹⁶⁸.

Conclusion and future directions

Because the gut microbiome is increasingly linked to human health, research on how antibiotics reshape the microbiome and endanger the health of the host is of considerable interest for public health. Furthermore, a greater understanding of the mechanisms by which antibiotics perturb the human microbiome is critical to maximizing the therapeutic benefits of these agents while reducing both the damage imposed on the commensal gut microbiome and the broader spread of antimicrobial resistant pathogens^{23,24}. As we have outlined here, the gut microbiome response to antibiotics is highly complex and variable³³, driven by a multitude of factors^{33,59,206,207}. To quantitatively understand and predict the most important biological variables that influence antibiotic-mediated destruction of this complex ecology, techniques for host-informed sample collection¹⁶⁸, sequencing methodologies and statistical analyses need to be standardized within the field. Recent collaborative advocacy for standardized reporting guidelines for microbiome studies will enable the field to move towards this goal²⁰⁸.

In the effort to achieve globally robust predictions of antibiotic-microbiome outcomes, there lies a difficulty in balancing generalizability with reproducibility. Specifically, it is imperative for future models of antibiotic-mediated damage to the gut community to control for and test the predictive capacities of broader sets of host and microbial variables to yield more predictive models of the community dynamics^{52,59,126}. Among the notable variables that are traditionally overlooked in microbiome studies is geographical origin of the studied population.

Investigations of the gut microbiome have predominantly focused on industrialized populations in the USA and Europe, representing a minority of the world population. Thus, our current models of microbiome dynamics are critically limited in their scope and application²⁰⁹.

The proliferation of high-throughput, low-cost sequencing technologies in the academic, medical and private sectors provides the field with an unprecedented opportunity to further develop predictive statistical methods that can be used as part of a public health framework to prevent antibiotic resistance emergence and future infections. Complementing such strategies, a wealth of scholarship highlights the translational potential of emerging microbiome restorative strategies, such as narrow-spectrum engineered probiotics^{167,168,173,200,204,205} or defined FMTs^{185–188,190–192}. To realize the frequently touted goal of ‘personalized medicine’ in the treatment of infectious diseases, much basic and translational work is still needed to accurately understand and predict the host, microbial and environmental factors that control the response of the microbiome to specific antimicrobials, the restoration of pre-perturbation microbiome composition and function, and the response to novel microbiome-directed therapeutics.

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References

- Gomaa, E. Z. Human gut microbiota/microbiome in health and diseases: a review. *Antonie van Leeuwenhoek* **113**, 2019–2040 (2020).
- Derrien, M., Alvarez, A. S. & de Vos, W. M. The gut microbiota in the first decade of life. *Trends Microbiol.* **27**, 997–1010 (2019).
- Gilbert, J. A. et al. Current understanding of the human microbiome. *Nat. Med.* **24**, 392–400 (2018).
- Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. & Gordon, J. I. Host–bacterial mutualism in the human intestine. *Science* **307**, 1915–1920 (2005).
- Nguyen, L. H. et al. Antibiotic use and the development of inflammatory bowel disease: a national case-control study in Sweden. *Lancet Gastroenterol. Hepatol.* **5**, 986–995 (2020).
- Cox, L. M. & Blaser, M. J. Antibiotics in early life and obesity. *Nat. Rev. Endocrinol.* **11**, 182–190 (2015).
- Farhana, L., Banerjee, H. N., Verma, M. & Majumdar, A. P. N. Role of microbiome in carcinogenesis process and epigenetic regulation of colorectal cancer. *Methods Mol. Biol.* **1856**, 35–55 (2018).
- Akimoto, N. et al. Rising incidence of early-onset colorectal cancer — a call to action. *Nat. Rev. Clin. Oncol.* **18**, 230–243 (2021).
- Johnson, S. et al. Epidemics of diarrhea caused by a clindamycin-resistant strain of *Clostridium difficile* in four hospitals. *N. Engl. J. Med.* **341**, 1645–1651 (1999).
- Kim, J. H. et al. Maternal antibiotic exposure during pregnancy is a risk factor for community-acquired urinary tract infection caused by extended-spectrum beta-lactamase-producing bacteria in infants. *Pediatr. Nephrol.* **37**, 163–170 (2022).
- Lynch, J. J. & Martinez, F. J. Clinical relevance of macrolide-resistant *Streptococcus pneumoniae* for community-acquired pneumonia. *Clin. Infect. Dis.* **34**, S27–S46 (2002).
- Fleming, A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae*. 1929. *Bull. World Health Organ.* **79**, 780–790 (2001).
- Adediji, W. A. The treasure called antibiotics. *Ann. Ib. Postgrad. Med.* **14**, 56–57 (2016).
- Centers for Disease Control and Prevention. Life Expectancy. *Centers for Disease Control and Prevention* <https://www.cdc.gov/nchs/fastats/life-expectancy.htm> (2021).
- Ng, K. M. et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature* **502**, 96–99 (2013).
- Fishbein, S. R. S. et al. Randomized controlled trial of oral vancomycin treatment in *Clostridioides difficile*-colonized patients. *mSphere* <https://doi.org/10.1128/mSphere.00936-20> (2021).
- Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States. *Centers for Disease Control and Prevention* <https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf> (2019).
- Anthony, W. E., Burnham, C. D., Dantas, G. & Kwon, J. H. The gut microbiome as a reservoir for antimicrobial resistance. *J. Infect. Dis.* **223**, S209–S213 (2021).
- Hayase, E. et al. Mucus-degrading *Bacteroides* link carbapenems to aggravated graft-versus-host disease. *Cell* **185**, 3705–3719.e14 (2022).
- This study highlights an underexplored area in identifying antibiotic-induced microbiome perturbation as a contributor to intestinal graft-versus-host disease.**
- Shono, Y. et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. *Sci. Transl. Med.* **8**, 339ra371 (2016).
- Wypych, T. P. & Marsland, B. J. Antibiotics as instigators of microbial dysbiosis: implications for asthma and allergy. *Trends Immunol.* **39**, 697–711 (2018).
- Klein, E. Y. et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl Acad. Sci. USA* **115**, E3463–E3470 (2018).
- Gould, I. M. A review of the role of antibiotic policies in the control of antibiotic resistance. *J. Antimicrob. Chemother.* **43**, 459–465 (1999).
- Olesen, S. W. et al. The distribution of antibiotic use and its association with antibiotic resistance. *eLife* <https://doi.org/10.7554/eLife.39435> (2018).
- Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **399**, 629–655 (2022).
- Sommer, M. O. A., Dantas, G. & Church, G. M. Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science* **325**, 1128–1131 (2009).
- Gasparrini, A. J. et al. Antibiotic perturbation of the preterm infant gut microbiome and resistome. *Gut Microbes* **7**, 443–449 (2016).
- Mahmud, B. et al. Epidemiology of plasmid lineages mediating the spread of extended-spectrum beta-lactamases among clinical *Escherichia coli*. *mSystems* <https://doi.org/10.1128/mSystems.00519-22> (2022).
- Forster, S. C. et al. Strain-level characterization of broad host range mobile genetic elements transferring antibiotic resistance from the human microbiome. *Nat. Commun.* **13**, 1445 (2022).
- Stracy, M. et al. Minimizing treatment-induced emergence of antibiotic resistance in bacterial infections. *Science* **375**, 889–894 (2022).
- Using a machine-learning approach, this study leverages a massive bacterial genomics database tied to infection data to identify genomic predictors by which treatment-induced emergence of antibiotic resistance can be avoided.**
- Dethlefsen, L., Huse, S., Sogin, M. L. & Relman, D. A. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol.* **6**, e280 (2008).
- Palleja, A. et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat. Microbiol.* **3**, 1255–1265 (2018).
- Anthony, W. E. et al. Acute and persistent effects of commonly used antibiotics on the gut microbiome and resistome in healthy adults. *Cell Rep.* **39**, 110649 (2022).
- Dubinsky, V. et al. Predominantly antibiotic-resistant intestinal microbiome persists in patients with pouchitis who respond to antibiotic therapy. *Gastroenterology* **158**, 610–624.e13 (2020).
- Van Engelen, T. S. R. et al. Gut microbiome modulation by antibiotics in adult asthma: a human proof-of-concept intervention trial. *Clin. Gastroenterol. Hepatol.* **20**, 1404–1407.e4 (2022).
- Doan, T. et al. Mass azithromycin distribution and community microbiome: a cluster-randomized trial. *Open Forum Infect. Dis.* **5**, ofy182 (2018).
- Reyman, M. et al. Effects of early-life antibiotics on the developing infant gut microbiome and resistome: a randomized trial. *Nat. Commun.* **13**, 893 (2022).
- Raymond, F. et al. The initial state of the human gut microbiome determines its reshaping by antibiotics. *ISME J.* **10**, 707–720 (2016).
- Reijnders, D. et al. Short-term microbiota manipulation and forearm substrate metabolism in obese men: a randomized, double-blind, placebo-controlled trial. *Obes. Facts* **11**, 318–326 (2018).
- Zarrinpar, A. et al. Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. *Nat. Commun.* **9**, 2872 (2018).
- Kelly, C. P. et al. *Saccharomyces boulardii* CNCM I-745 modulates the fecal bile acids metabolism during antimicrobial therapy in healthy volunteers. *Front. Microbiol.* **10**, 336 (2019).
- Tsukuda, N. et al. Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. *ISME J.* **15**, 2574–2590 (2021).
- Young, V. B. & Schmidt, T. M. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. *J. Clin. Microbiol.* **42**, 1203–1206 (2004).
- Lloyd-Price, J. et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* **569**, 655–662 (2019).
- Theriot, C. M. et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to *Clostridium difficile* infection. *Nat. Commun.* **5**, 3114 (2014).
- Isaac, S. et al. Microbiome-mediated fructose depletion restricts murine gut colonization by vancomycin-resistant *Enterococcus*. *Nat. Commun.* **13**, 7718 (2022).
- Giel, J. L., Sorg, J. A., Sonenshein, A. L. & Zhu, J. Metabolism of bile salts in mice influences spore germination in *Clostridium difficile*. *PLoS ONE* **5**, e8740 (2010).
- This study underscores the importance of microbial transformation of bile salts in conferring microbiome-mediated colonization resistance to a spore-forming pathogen.**
- Reed, A. D., Nethery, M. A., Stewart, A., Barrangou, R. & Theriot, C. M. Strain-dependent inhibition of *Clostridioides difficile* by commensal clostridia carrying the bile acid-inducible (bai) operon. *J. Bacteriol.* <https://doi.org/10.1128/JB.00039-20> (2020).
- Guinan, J., Wang, S., Hazbun, T. R., Yadav, H. & Thangamani, S. Antibiotic-induced decreases in the levels of microbial-derived short-chain fatty acids correlate with increased gastrointestinal colonization of *Candida albicans*. *Sci. Rep.* **9**, 8872 (2019).
- Buffie, C. G. & Pamer, E. G. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.* **13**, 790–801 (2013).

51. MacPherson, C. W. et al. Gut bacterial microbiota and its resistome rapidly recover to basal state levels after short-term amoxicillin-clavulanic acid treatment in healthy adults. *Sci. Rep.* **8**, 11192 (2018).
52. Gibson, M. K. et al. Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. *Nat. Microbiol.* **1**, 16024 (2016).
This study shows that in infant guts, the ARGs enriched after antibiotic treatment highly correlate with the abundance of single species. This study also utilizes functional screening to identify novel ARGs in stool metagenomes.
53. Loo, V. G. et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N. Engl. J. Med.* **353**, 2442–2449 (2005).
54. Weill, F. X. et al. Genomic insights into the 2016–2017 cholera epidemic in Yemen. *Nature* **565**, 230–233 (2019).
55. Reijnders, D. et al. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: A randomized double-blind placebo-controlled trial. *Cell Metab.* **24**, 63–74 (2016).
56. Gao, H. et al. Antibiotic exposure has sex-dependent effects on the gut microbiota and metabolism of short-chain fatty acids and amino acids in mice. *mSystems* <https://doi.org/10.1128/mSystems.00048-19> (2019).
57. Sim, C. K. et al. A mouse model of occult intestinal colonization demonstrating antibiotic-induced outgrowth of carbapenem-resistant Enterobacteriaceae. *Microbiome* **10**, 43 (2022).
58. Stewardson, A. J. et al. Collateral damage from oral ciprofloxacin versus nitrofurantoin in outpatients with urinary tract infections: a culture-free analysis of gut microbiota. *Clin. Microbiol. Infect.* **21**, 344.e1–344.e11 (2015).
59. Willmann, M. et al. Distinct impact of antibiotics on the gut microbiome and resistome: a longitudinal multicenter cohort study. *BMC Biol.* **17**, 76 (2019).
60. Hintewirh, A. et al. Rapid reduction of *Campylobacter* species in the gut microbiome of preschool children after oral azithromycin: a randomized controlled trial. *Am. J. Trop. Med. Hyg.* **103**, 1266–1269 (2020).
61. Chaima, D. et al. Biannual administrations of azithromycin and the gastrointestinal microbiome of Malawian children: a nested cohort study within a randomized controlled trial. *Front. Public Health* **10**, 756318 (2022).
62. Arzika, A. M. et al. Gut resistome of preschool children after prolonged mass azithromycin distribution: a cluster-randomized trial. *Clin. Infect. Dis.* **73**, 1292–1295 (2021).
63. Doan, T. et al. Macrolide and nonmacrolide resistance with mass azithromycin distribution. *N. Engl. J. Med.* **383**, 1941–1950 (2020).
64. Doan, T. et al. Macrolide resistance in MORDOR I — a cluster-randomized trial in Niger. *N. Engl. J. Med.* **380**, 2271–2273 (2019).
65. Doan, T. et al. Gut microbiome alteration in MORDOR I: a community-randomized trial of mass azithromycin distribution. *Nat. Med.* **25**, 1370–1376 (2019).
66. Pickering, H. et al. Impact of azithromycin mass drug administration on the antibiotic-resistant gut microbiome in children: a randomized, controlled trial. *Gut Pathog.* **14**, 5 (2022).
67. Oldenburg, C. E. et al. Effect of commonly used pediatric antibiotics on gut microbial diversity in preschool children in Burkina Faso: a randomized clinical trial. *Open Forum Infect. Dis.* **5**, ofy289 (2018).
68. Yassour, M. et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci. Transl. Med.* **8**, 343ra381 (2016).
69. Zlitni, S. et al. Strain-resolved microbiome sequencing reveals mobile elements that drive bacterial competition on a clinical timescale. *Genome Med.* **12**, 50 (2020).
70. Goltsman, D. S. A. et al. Metagenomic analysis with strain-level resolution reveals fine-scale variation in the human pregnancy microbiome. *Genome Res.* **28**, 1467–1480 (2018).
71. Olm, M. R. et al. Necrotizing enterocolitis is preceded by increased gut bacterial replication, *Klebsiella*, and fimbriae-encoding bacteria. *Sci. Adv.* **5**, eaax5727 (2019).
72. Brito, I. L. et al. Transmission of human-associated microbiota along family and social networks. *Nat. Microbiol.* **4**, 964–971 (2019).
73. Vinarov, Z. et al. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review. *Eur. J. Pharm. Sci.* **162**, 105812 (2021).
74. Rao, S., Kupfer, Y., Pagala, M., Chapnick, E. & Tessler, S. Systemic absorption of oral vancomycin in patients with *Clostridium difficile* infection. *Scand. J. Infect. Dis.* **43**, 386–388 (2011).
75. Connelly, S., Subramanian, P., Hasan, N. A., Colwell, R. R. & Kaleko, M. Distinct consequences of amoxicillin and ertapenem exposure in the porcine gut microbiome. *Anaerobe* **53**, 82–93 (2018).
76. Singh, J., Burr, B., Stringham, D. & Arrieta, A. Commonly used antibacterial and antifungal agents for hospitalized paediatric patients: implications for therapy with an emphasis on clinical pharmacokinetics. *Paediatr. Drugs* **3**, 733–761 (2001).
77. Leopold, S. R. et al. Murine model for measuring effects of humanized-dosing of antibiotics on the gut microbiome. *Front. Microbiol.* **13**, 813849 (2022).
78. Browne, H. P. et al. Host adaptation in gut Firmicutes is associated with sporulation loss and altered transmission cycle. *Genome Biol.* **22**, 204 (2021).
79. Fawley, W. N. et al. Efficacy of hospital cleaning agents and germicides against epidemic *Clostridium difficile* strains. *Infect. Control. Hosp. Epidemiol.* **28**, 920–925 (2007).
80. Baines, S. D., O'Connor, R., Saxton, K., Freeman, J. & Wilcox, M. H. Activity of vancomycin against epidemic *Clostridium difficile* strains in a human gut model. *J. Antimicrob. Chemother.* **63**, 520–525 (2009).
81. Antunes, A. et al. Global transcriptional control by glucose and carbon regulator CcpA in *Clostridium difficile*. *Nucleic Acids Res.* **40**, 10701–10718 (2012).
82. Normington, C. et al. Biofilms harbour *Clostridioides difficile*, serving as a reservoir for recurrent infection. *NPJ Biofilms Microbiomes* **7**, 16 (2021).
83. Theriot, C. M., Bowman, A. A. & Young, V. B. 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* <https://doi.org/10.1128/mSphere.00045-15> (2016).
84. Duncan, K., Carey-Ewend, K. & Vaishnav, S. Spatial analysis of gut microbiome reveals a distinct ecological niche associated with the mucus layer. *Gut Microbes* **13**, 1874815 (2021).
This analysis utilizes a number of imaging techniques to characterize the microbial composition of the mucus layer in a mouse.
85. Elhenawy, W. et al. High-throughput fitness screening and transcriptomics identify a role for a type IV secretion system in the pathogenesis of Crohn's disease-associated *Escherichia coli*. *Nat. Commun.* **12**, 2032 (2021).
86. Bakkeren, E. et al. Pathogen invasion-dependent tissue reservoirs and plasmid-encoded antibiotic degradation boost plasmid spread in the gut. *eLife* <https://doi.org/10.7554/eLife.69744> (2021).
87. Bakkeren, E. et al. *Salmonella* persisters promote the spread of antibiotic resistance plasmids in the gut. *Nature* **573**, 276–280 (2019).
88. Cabral, D. J. et al. Microbial metabolism modulates antibiotic susceptibility within the murine gut microbiome. *Cell Metab.* **30**, 800–823.e7 (2019).
89. Chambers, S. A. et al. A solution to antifolate resistance in group B *Streptococcus*: untargated metabolomics identifies human milk oligosaccharide-induced perturbations that result in potentiation of trimethoprim. *mBio* <https://doi.org/10.1128/mBio.00076-20> (2020).
90. Liu, Y. et al. Gut microbiome alterations in high-fat-diet-fed mice are associated with antibiotic tolerance. *Nat. Microbiol.* **6**, 874–884 (2021).
91. Vega, N. M., Allison, K. R., Samuels, A. N., Klempner, M. S. & Collins, J. J. *Salmonella typhimurium* intercepts *Escherichia coli* signaling to enhance antibiotic tolerance. *Proc. Natl Acad. Sci. USA* **110**, 14420–14425 (2013).
92. Zarkan, A. et al. Inhibition of indole production increases the activity of quinolone antibiotics against *E. coli* persisters. *Sci. Rep.* **10**, 11742 (2020).
93. Varga, J. J. et al. Antibiotics drive expansion of rare pathogens in a chronic infection microbiome model. *mSphere* **7**, e0031822 (2022).
94. Amor, D. R. & Gore, J. Fast growth can counteract antibiotic susceptibility in shaping microbial community resilience to antibiotics. *Proc. Natl Acad. Sci. USA* **119**, e2116954119 (2022).
95. Bottery, M. J. et al. Inter-species interactions alter antibiotic efficacy in bacterial communities. *ISME J.* **16**, 812–821 (2022).
This study utilizes a simple defined community framework to identify quantitative features of a small bacterial community that predict antibiotic susceptibility in the community.
96. Ng, K. M. et al. Recovery of the gut microbiota after antibiotics depends on host diet, community context, and environmental reservoirs. *Cell Host Microbe* **26**, 650–665.e4 (2019).
97. Isaac, S. et al. Short- and long-term effects of oral vancomycin on the human intestinal microbiota. *J. Antimicrob. Chemother.* **72**, 128–136 (2017).
98. Coyte, K. Z. & Rakoff-Nahoum, S. Understanding competition and cooperation within the mammalian gut microbiome. *Curr. Biol.* **29**, R538–R544 (2019).
99. Aranda-Diaz, A. et al. Bacterial interspecies interactions modulate pH-mediated antibiotic tolerance. *eLife* <https://doi.org/10.7554/eLife.51493> (2020).
100. Adamowicz, E. M., Flynn, J., Hunter, R. C. & Harcombe, W. R. Cross-feeding modulates antibiotic tolerance in bacterial communities. *ISME J.* **12**, 2723–2735 (2018).
101. Adamowicz, E. M., Muza, M., Chacon, J. M. & Harcombe, W. R. Cross-feeding modulates the rate and mechanism of antibiotic resistance evolution in a model microbial community of *Escherichia coli* and *Salmonella enterica*. *PLoS Pathog.* **16**, e1008700 (2020).
102. Hazleton, K. Z. et al. Dietary fat promotes antibiotic-induced *Clostridioides difficile* mortality in mice. *NPJ Biofilms Microbiomes* **8**, 15 (2022).
103. Guasch-Ferre, M. et al. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. *Am. J. Clin. Nutr.* **102**, 1563–1573 (2015).
104. Leone, V., Chang, E. B. & Devkota, S. Diet, microbes, and host genetics: the perfect storm in inflammatory bowel diseases. *J. Gastroenterol.* **48**, 315–321 (2013).
105. Lee, J. Y. et al. High-fat diet and antibiotics cooperatively impair mitochondrial bioenergetics to trigger dysbiosis that exacerbates pre-inflammatory bowel disease. *Cell Host Microbe* **28**, 273–284.e6 (2020).
106. Cabral, D. J., Wurster, J. I., Korry, B. J., Penumutthu, S. & Belenky, P. Consumption of a Western-style diet modulates the response of the murine gut microbiome to ciprofloxacin. *mSystems* <https://doi.org/10.1128/mSystems.00317-20> (2020).
107. Wurster, J. I. et al. Streptozotocin-induced hyperglycemia alters the cecal metabolome and exacerbates antibiotic-induced dysbiosis. *Cell Rep.* **37**, 110113 (2021).
108. Tanes, C. et al. Role of dietary fiber in the recovery of the human gut microbiome and its metabolome. *Cell Host Microbe* **29**, 394–407.e5 (2021).
This human clinical trial provides promising evidence that modulation of dietary elements can aide the microbiome in recovering its metabolic capacity following broad-spectrum antibiotic treatment.

109. Lindell, A. E., Zimmermann-Kogadeeva, M. & Patil, K. R. Multimodal interactions of drugs, natural compounds and pollutants with the gut microbiota. *Nat. Rev. Microbiol.* **20**, 431–443 (2022).
110. Vich Vila, A. et al. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat. Commun.* **11**, 362 (2020).
111. Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R. & Goodman, A. L. Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature* **570**, 462–467 (2019).
112. Javdan, B. et al. Personalized mapping of drug metabolism by the human gut microbiome. *Cell* **181**, 1661–1679.e22 (2020).
113. Maier, L. et al. Unravelling the collateral damage of antibiotics on gut bacteria. *Nature* **599**, 120–124 (2021).
114. de Nies, L. et al. Evolution of the murine gut resistome following broad-spectrum antibiotic treatment. *Nat. Commun.* **13**, 2296 (2022).
115. Loof, T. et al. In-feed antibiotic effects on the swine intestinal microbiome. *Proc. Natl Acad. Sci. USA* **109**, 1691–1696 (2012).
116. Duan, Y. et al. Gut resistomes, microbiota and antibiotic residues in Chinese patients undergoing antibiotic administration and healthy individuals. *Sci. Total Environ.* **705**, 135674 (2020).
117. D'Souza, A. W. et al. Cotrimoxazole prophylaxis increases resistance gene prevalence and alpha-diversity but decreases beta-diversity in the gut microbiome of HIV-exposed, uninfected infants. *Clin. Infect. Dis.* <https://doi.org/10.1093/cid/ciz1186> (2019).
118. Thanert, R. et al. Antibiotic-driven intestinal dysbiosis in pediatric short bowel syndrome is associated with persistently altered microbiome functions and gut-derived bloodstream infections. *Gut Microbes* **13**, 1940792 (2021).
119. Kokai-Kun, J. F. et al. Ribaxamase, an orally administered β -lactamase, diminishes changes to acquired antimicrobial resistance of the gut resistome in patients treated with ceftriaxone. *Infect. Drug Resist.* **13**, 2521–2535 (2020).
120. Li, J. et al. Antibiotic treatment drives the diversification of the human gut resistome. *Genomics Proteom. Bioinforma.* **17**, 39–51 (2019).
121. Lofmark, S., Jernberg, C., Jansson, J. K. & Edlund, C. Clindamycin-induced enrichment and long-term persistence of resistant *Bacteroides* spp. and resistance genes. *J. Antimicrob. Chemother.* **58**, 1160–1167 (2006).
122. Jakobsson, H. E. et al. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS ONE* **5**, e9836 (2010).
123. Oldenburg, C. E. et al. Gut resistome after oral antibiotics in preschool children in Burkina Faso: a randomized, controlled trial. *Clin. Infect. Dis.* **70**, 525–527 (2020).
124. Jernberg, C., Lofmark, S., Edlund, C. & Jansson, J. K. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J.* **1**, 56–66 (2007).
125. Zhang, L., Huang, Y., Zhou, Y., Buckley, T. & Wang, H. H. Antibiotic administration routes significantly influence the levels of antibiotic resistance in gut microbiota. *Antimicrob. Agents Chemother.* **57**, 3659–3666 (2013).
126. Gasparini, A. J. et al. Persistent metagenomic signatures of early-life hospitalization and antibiotic treatment in the infant gut microbiota and resistome. *Nat. Microbiol.* **4**, 2285–2297 (2019).
127. Johnson, T. A. et al. Clusters of antibiotic resistance genes enriched together stay together in swine agriculture. *mBio* **7**, e02214–e02215 (2016).
128. Rajer, F. & Sandegren, L. The role of antibiotic resistance genes in the fitness cost of multiresistance plasmids. *mBio* <https://doi.org/10.1128/mBio.03552-21> (2022).
129. Esaiassen, E. et al. Effects of probiotic supplementation on the gut microbiota and antibiotic resistance development in preterm infants. *Front. Pediatr.* **6**, 347 (2018).
130. Rahman, S., Olm, M. R., Morowitz, M. J. & Banfield, J. F. Machine learning leveraging genomes from metagenomes identifies influential antibiotic resistance genes in the infant gut microbiome. *mSystems* <https://doi.org/10.1128/mSystems.00123-17> (2018).
131. Papp, M. & Solymosi, N. Review and comparison of antimicrobial resistance gene databases. *Antibiotics* <https://doi.org/10.3390/antibiotics11030339> (2022).
132. Lal Gupta, C., Kumar Tiwari, R. & Cytryn, E. Platforms for elucidating antibiotic resistance in single genomes and complex metagenomes. *Env. Int.* **138**, 105667 (2020).
133. Xavier, B. B. et al. Consolidating and exploring antibiotic resistance gene data resources. *J. Clin. Microbiol.* **54**, 851–859 (2016).
134. Alcock, B. P. et al. CARD 2020: antibiotic resistance surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res.* **48**, D517–D525 (2020).
135. Doster, E. et al. MEGARes 2.0: a database for classification of antimicrobial drug, biocide and metal resistance determinants in metagenomic sequence data. *Nucleic Acids Res.* **48**, D561–D569 (2020).
136. Mahmud, B., Boolchandani, M., Patel, S. & Dantas, G. Functional metagenomics to study antibiotic resistance. *Methods Mol. Biol.* **2601**, 379–401 (2023).
137. Goren, M. G. et al. Transfer of carbapenem-resistant plasmid from *Klebsiella pneumoniae* ST258 to *Escherichia coli* in patient. *Emerg. Infect. Dis.* **16**, 1014–1017 (2010).
138. Kent, A. G., Vill, A. C., Shi, Q., Satlin, M. J. & Brito, I. L. Widespread transfer of mobile antibiotic resistance genes within individual gut microbiomes revealed through bacterial Hi-C. *Nat. Commun.* **1**, 4379 (2020).
- This study describes the implementation of Hi-C towards elucidating HGT networks in microbial communities.**
139. Groussin, M. et al. Elevated rates of horizontal gene transfer in the industrialized human microbiome. *Cell* **184**, 2053–2067.e18 (2021).
140. Stecher, B. et al. Gut inflammation can boost horizontal gene transfer between pathogenic and commensal Enterobacteriaceae. *Proc. Natl Acad. Sci. USA* **109**, 1269–1274 (2012).
141. Charpentier, X., Polard, P. & Claverys, J. P. Induction of competence for genetic transformation by antibiotics: convergent evolution of stress responses in distant bacterial species lacking SOS. *Curr. Opin. Microbiol.* **15**, 570–576 (2012).
142. Slager, J., Kjos, M., Attaiach, L. & Veening, J. W. Antibiotic-induced replication stress triggers bacterial competence by increasing gene dosage near the origin. *Cell* **157**, 395–406 (2014).
143. Prudhomme, M., Attaiach, L., Sanchez, G., Martin, B. & Claverys, J. P. Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. *Science* **313**, 89–92 (2006).
144. Modi, S. R., Lee, H. H., Spina, C. S. & Collins, J. J. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. *Nature* **499**, 219–222 (2013).
- In this study, the authors demonstrate that antibiotic treatment results in the expansion of the frequency of phage–bacteria interactions, resulting in broader dissemination of phage-encoded antimicrobial resistance genes.**
145. Jutkina, J., Marathe, N. P., Flach, C. F. & Larsson, D. G. J. Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations. *Sci. Total Environ.* **616–617**, 172–178 (2018).
146. Cairns, J. et al. Ecology determines how low antibiotic concentration impacts community composition and horizontal transfer of resistance genes. *Commun. Biol.* **1**, 35 (2018).
147. Barlow, M. What antimicrobial resistance has taught us about horizontal gene transfer. *Methods Mol. Biol.* **532**, 397–411 (2009).
148. Huddleston, J. R. Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. *Infect. Drug Resist.* **7**, 167–176 (2014).
149. Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J. & Segata, N. Shotgun metagenomics, from sampling to analysis. *Nat. Biotechnol.* **35**, 833–844 (2017).
150. Maguire, F. et al. Metagenome-assembled genome binning methods with short reads disproportionately fail for plasmids and genomic islands. *Microb. Genom.* <https://doi.org/10.1099/mgen.0.000436> (2020).
151. Brown, C. L. et al. Critical evaluation of short, long, and hybrid assembly for contextual analysis of antibiotic resistance genes in complex environmental metagenomes. *Sci. Rep.* **11**, 3753 (2021).
152. Moss, E. L., Maghini, D. G. & Bhatt, A. S. Complete, closed bacterial genomes from microbiomes using nanopore sequencing. *Nat. Biotechnol.* **38**, 701–707 (2020).
153. Lu, J. et al. Triclosan at environmentally relevant concentrations promotes horizontal transfer of multidrug resistance genes within and across bacterial genera. *Environ. Int.* **121**, 1217–1226 (2018).
154. Jutkina, J., Rutgersson, C., Flach, C. F. & Joakim Larsson, D. G. An assay for determining minimal concentrations of antibiotics that drive horizontal transfer of resistance. *Sci. Total Environ.* **548–549**, 131–138 (2016).
155. Beaber, J. W., Hochhut, B. & Waldor, M. K. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* **427**, 72–74 (2004).
156. Zhang, P. Y. et al. Combined treatment with the antibiotics kanamycin and streptomycin promotes the conjugation of *Escherichia coli*. *FEMS Microbiol. Lett.* **348**, 149–156 (2013).
157. Wang, Y. et al. Antiepileptic drug carbamazepine promotes horizontal transfer of plasmid-borne multi-antibiotic resistance genes within and across bacterial genera. *ISME J.* **13**, 509–522 (2019).
158. Handel, N., Otte, S., Jonker, M., Brul, S. & ter Kuile, B. H. Factors that affect transfer of the Inc11 β -lactam resistance plasmid pESBL-283 between *E. coli* strains. *PLoS ONE* **10**, e0123039 (2015).
159. Johnsen, A. R. & Kroer, N. Effects of stress and other environmental factors on horizontal plasmid transfer assessed by direct quantification of discrete transfer events. *FEMS Microbiol. Ecol.* **509**, 718–728 (2007).
160. Lopatkin, A. J. et al. Antibiotics as a selective driver for conjugation dynamics. *Nat. Microbiol.* **1**, 16044 (2016).
161. Feld, L. et al. Selective pressure affects transfer and establishment of a *Lactobacillus plantarum* resistance plasmid in the gastrointestinal environment. *J. Antimicrob. Chemother.* **61**, 845–852 (2008).
162. Lopatkin, A. J., Sysoeva, T. A. & You, L. Dissecting the effects of antibiotics on horizontal gene transfer: analysis suggests a critical role of selection dynamics. *Bioessays* **38**, 1283–1292 (2016).
- In this examination of the reports on the effects of antibiotics on conjugation, the authors make a compelling case for how antibiotic selection confounds the experimental results and their interpretation. The authors call for more careful experimental design that enables decoupling of the antibiotic effects on conjugation and bacterial growth rates.**
163. Deng, F., Li, Y. & Zhao, J. The gut microbiome of healthy long-living people. *Aging* **11**, 289–290 (2019).
164. Connolly, S. et al. SYN-004 (ribaxamase), an oral beta-lactamase, mitigates antibiotic-mediated dysbiosis in a porcine gut microbiome model. *J. Appl. Microbiol.* **123**, 66–79 (2017).
165. de Gunzburg, J. et al. Protection of the human gut microbiome from antibiotics. *J. Infect. Dis.* **217**, 628–636 (2018).
166. Wan, M. L. Y., Forsythe, S. J. & El-Nezami, H. Probiotics interaction with foodborne pathogens: a potential alternative to antibiotics and future challenges. *Crit. Rev. Food Sci. Nutr.* **59**, 3320–3333 (2019).
167. Drolia, R. et al. Receptor-targeted engineered probiotics mitigate lethal *Listeria* infection. *Nat. Commun.* **11**, 6344 (2020).

168. Koh, E. et al. Engineering probiotics to inhibit *Clostridioides difficile* infection by dynamic regulation of intestinal metabolism. *Nat. Commun.* **13**, 3834 (2022).
This paper reports on the engineering of *E. coli* Nissle 1917 to selectively secrete a bile salt hydrolase in a dysbiotic environment, restoring the intestinal bile salt metabolism and impeding *C. difficile* germination and disease.
169. Ajami, N. J., Cope, J. L., Wong, M. C., Petrosino, J. F. & Chesnel, L. Impact of oral fidaxomicin administration on the intestinal microbiota and susceptibility to *Clostridium difficile* colonization in mice. *Antimicrob. Agents Chemother.* <https://doi.org/10.1128/AAC.02112-17> (2018).
170. Brochado, A. R. et al. Species-specific activity of antibacterial drug combinations. *Nature* **559**, 259–263 (2018).
In this paper, the authors demonstrate that the combined activity of an antibiotic combination is commonly species specific and strain specific, providing evidence for the possibility of developing narrow-spectrum therapies based on drug combinations.
171. Stentz, R. et al. Cephalosporins associated with outer membrane vesicles released by *Bacteroides* spp. protect gut pathogens and commensals against β -lactam antibiotics. *J. Antimicrob. Chemother.* **70**, 701–709 (2015).
172. Kokai-Kun, J. F. et al. Use of ribaxamase (SYN-004), a β -lactamase, to prevent *Clostridium difficile* infection in β -lactam-treated patients: a double-blind, phase 2b, randomised placebo-controlled trial. *Lancet Infect. Dis.* **19**, 487–496 (2019).
173. Cubillos-Ruiz, A. et al. An engineered live biotherapeutic for the prevention of antibiotic-induced dysbiosis. *Nat. Biomed. Eng.* <https://doi.org/10.1038/s41551-022-00871-9> (2022).
The authors of this paper generate an engineered *L. lactis* strain that degrades intestinal β -lactams through secretion of a heterodimeric β -lactamase. Notably, given its split nature and extracellular assembly, the β -lactamase protects the microbial community from β -lactams but does not confer resistance to the host *L. lactis*.
174. Vehreschild, M. et al. An open randomized multicentre phase 2 trial to assess the safety of DAV132 and its efficacy to protect gut microbiota diversity in hospitalized patients treated with fluoroquinolones. *J. Antimicrob. Chemother.* **77**, 1155–1165 (2022).
175. Burdet, C. et al. Protection of hamsters from mortality by reducing fecal moxifloxacin concentration with dav131a in a model of moxifloxacin-induced *Clostridium difficile* colitis. *Antimicrob. Agents Chemother.* <https://doi.org/10.1128/AAC.00543-17> (2017).
176. de Gunzburg, J. et al. Targeted adsorption of molecules in the colon with the novel adsorbent-based medicinal product, DAV132: a proof of concept study in healthy subjects. *J. Clin. Pharmacol.* **55**, 10–16 (2015).
177. Hryckowian, A. J. et al. Microbiota-accessible carbohydrates suppress *Clostridium difficile* infection in a murine model. *Nat. Microbiol.* **3**, 662–669 (2018).
178. McDonald, J. A. K. et al. Inhibiting growth of *Clostridioides difficile* by restoring valerate, produced by the intestinal microbiota. *Gastroenterology* **155**, 1495–1507.e15 (2018).
179. Pruss, K. M. et al. Mucin-derived O-glycans supplemented to diet mitigate diverse microbiota perturbations. *ISME J.* **15**, 577–591 (2021).
180. Schnitzlein, M. K., Vendrov, K. C., Edwards, S. J., Martens, E. C. & Young, V. B. Dietary xanthan gum alters antibiotic efficacy against the murine gut microbiota and attenuates *Clostridioides difficile* colonization. *mSphere* <https://doi.org/10.1128/mSphere.00708-19> (2020).
181. Simpson, H. L. et al. Soluble non-starch polysaccharides from plantain (*Musa × paradisiaca* L.) diminish epithelial impact of *Clostridioides difficile*. *Front. Pharmacol.* **12**, 766293 (2021).
182. Roberts, C. L. et al. Soluble plantain fibre blocks adhesion and M-cell translocation of intestinal pathogens. *J. Nutr. Biochem.* **24**, 97–103 (2013).
183. Button, J. E. et al. Dosing a synbiotic of human milk oligosaccharides and *B. infantis* leads to reversible engraftment in healthy adult microbiomes without antibiotics. *Cell Host Microbe* **30**, 712–725.e7 (2022).
184. Ghani, R., Mullish, B. H., Roberts, L. A., Davies, F. J. & Marchesi, J. R. The potential utility of fecal (or intestinal) microbiota transplantation in controlling infectious diseases. *Gut Microbes* **14**, 2038856 (2022).
185. Suez, J. et al. Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. *Cell* **174**, 1406–1423.e16 (2018).
186. Orenstein, R. et al. Durable reduction of *Clostridioides difficile* infection recurrence and microbiome restoration after treatment with RBX2660: results from an open-label phase 2 clinical trial. *BMC Infect. Dis.* **22**, 245 (2022).
187. Kao, D. et al. The effect of a microbial ecosystem therapeutic (MET-2) on recurrent *Clostridioides difficile* infection: a phase 1, open-label, single-group trial. *Lancet Gastroenterol. Hepatol.* **6**, 282–291 (2021).
188. Feuerstadt, P. et al. SER-109, an oral microbiome therapy for recurrent *Clostridioides difficile* infection. *N. Engl. J. Med.* **386**, 220–229 (2022).
189. Dsouza, M. et al. Colonization of the live biotherapeutic product VE303 and modulation of the microbiota and metabolites in healthy volunteers. *Cell Host Microbe* **30**, 583–598.e8 (2022).
This study is the first clinical demonstration of the therapeutic efficacy and safety of a rationally defined bacterial consortium developed to treat recurrent *C. difficile* infections.
190. Francis, M. B., Allen, C. A., Shrestha, R. & Sorg, J. A. Bile acid recognition by the *Clostridium difficile* germinant receptor, CspC, is important for establishing infection. *PLoS Pathog.* **9**, e1003356 (2013).
191. Winston, J. A. & Theriot, C. M. Impact of microbial derived secondary bile acids on colonization resistance against *Clostridium difficile* in the gastrointestinal tract. *Anaerobe* **41**, 44–50 (2016).
192. Kwak, S. et al. Impact of investigational microbiota therapeutic RBX2660 on the gut microbiome and resistome revealed by a placebo-controlled clinical trial. *Microbiome* **8**, 125 (2020).
193. DeFilipp, Z. et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N. Engl. J. Med.* **381**, 2043–2050 (2019).
194. Zellmer, C. et al. Shiga toxin-producing *Escherichia coli* transmission via fecal microbiota transplant. *Clin. Infect. Dis.* **72**, e876–e880 (2021).
195. Guarner, F. & Schaafsma, G. J. Probiotics. *Int. J. Food Microbiol.* **39**, 237–238 (1998).
196. Montassier, E. et al. Probiotics impact the antibiotic resistance gene reservoir along the human GI tract in a person-specific and antibiotic-dependent manner. *Nat. Microbiol.* **6**, 1043–1054 (2021).
197. Zuo, F., Chen, S. & Marcotte, H. Engineer probiotic bifidobacteria for food and biomedical applications — current status and future perspective. *Biotechnol. Adv.* **45**, 107654 (2020).
198. Goh, Y. J. & Barrangou, R. Harnessing CRISPR–Cas systems for precision engineering of designer probiotic lactobacilli. *Curr. Opin. Biotechnol.* **56**, 163–171 (2019).
199. Kwak, S., Mahmud, B. & Dantas, G. A tunable and expandable transactivation system in probiotic yeast *Saccharomyces boulardii*. *ACS Synth. Biol.* **11**, 508–514 (2022).
200. Gelfat, I. et al. Single domain antibodies against enteric pathogen virulence factors are active as curli fiber fusions on probiotic *E. coli* Nissle 1917. *PLoS Pathog.* **18**, e1010713 (2022).
201. Forkus, B., Ritter, S., Vlysidis, M., Geldart, K. & Kaznessis, Y. N. Antimicrobial probiotics reduce *Salmonella enterica* in turkey gastrointestinal tracts. *Sci. Rep.* **7**, 40695 (2017).
202. Tscherner, M., Giessen, T. W., Markey, L., Kumamoto, C. A. & Silver, P. A. A synthetic system that senses *Candida albicans* and inhibits virulence factors. *ACS Synth. Biol.* **8**, 434–444 (2019).
203. Jayaraman, P., Holowko, M. B., Yeoh, J. W., Lim, S. & Poh, C. L. Repurposing a two-component system-based biosensor for the killing of *Vibrio cholerae*. *ACS Synth. Biol.* **6**, 1403–1415 (2017).
204. Hwang, I. Y. et al. Engineered probiotic *Escherichia coli* can eliminate and prevent *Pseudomonas aeruginosa* gut infection in animal models. *Nat. Commun.* **8**, 15028 (2017).
205. Palmer, J. D. et al. Engineered probiotic for the inhibition of salmonella via tetrathionate-induced production of microcin H47. *ACS Infect. Dis.* **4**, 39–45 (2018).
206. Willmann, M. et al. Antibiotic selection pressure determination through sequence-based metagenomics. *Antimicrob. Agents Chemother.* **59**, 7335–7345 (2015).
207. Buelow, E. et al. Effects of selective digestive decontamination (SDD) on the gut resistome. *J. Antimicrob. Chemother.* **69**, 2215–2223 (2014).
208. Mirzayi, C. et al. Reporting guidelines for human microbiome research: the STORMS checklist. *Nat. Med.* **27**, 1885–1892 (2021).
209. Brewster, R. et al. Surveying gut microbiome research in Africans: toward improved diversity and representation. *Trends Microbiol.* **27**, 824–835 (2019).
210. Stokholm, J. et al. Antibiotic use during pregnancy alters the commensal vaginal microbiota. *Clin. Microbiol. Infect.* **20**, 629–635 (2014).
211. Dubos, R., Schaedler, R. W. & Stephens, M. The effect of antibacterial drugs on the fecal flora of mice. *J. Exp. Med.* **117**, 231–243 (1963).
212. Hertz, F. B. et al. Effects of antibiotics on the intestinal microbiota of mice. *Antibiotics* <https://doi.org/10.3390/antibiotics9040191> (2020).
213. Zhang, Y., Limaye, P. B., Renaud, H. J. & Klaassen, C. D. Effect of various antibiotics on modulation of intestinal microbiota and bile acid profile in mice. *Toxicol. Appl. Pharmacol.* **277**, 138–145 (2014).
214. Sun, L. et al. Antibiotic-induced disruption of gut microbiota alters local metabolomes and immune responses. *Front. Cell Infect. Microbiol.* **9**, 99 (2019).
215. Burdet, C. et al. Ceftriaxone and cefotaxime have similar effects on the intestinal microbiota in human volunteers treated by standard-dose regimens. *Antimicrob. Agents Chemother.* <https://doi.org/10.1128/AAC.02244-18> (2019).
216. Tirello, P. et al. Comparison of different modes of antibiotic delivery on gut microbiota depletion efficiency and body composition in mouse. *BMC Microbiol.* **20**, 340 (2020).
217. Stokholm, J., Sevelsted, A., Bonnelykke, K. & Bisgaard, H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. *Lancet Respir. Med.* **2**, 631–637 (2014).
218. Tao, C., Zhang, Q., Zeng, W., Liu, G. & Shao, H. The effect of antibiotic cocktails on host immune status is dynamic and does not always correspond to changes in gut microbiota. *Appl. Microbiol. Biotechnol.* **104**, 4995–5009 (2020).
219. Lavelle, A. et al. Baseline microbiota composition modulates antibiotic-mediated effects on the gut microbiota and host. *Microbiome* **7**, 111 (2019).
220. Akbar, S. et al. Changes in the life history traits of *Daphnia magna* are associated with the gut microbiota composition shaped by diet and antibiotics. *Sci. Total Environ.* **705**, 135827 (2020).
221. Harrison, C. A. et al. Sexual dimorphism in the response to broad-spectrum antibiotics during T cell-mediated colitis. *J. Crohns Colitis* **13**, 115–126 (2019).
222. Ruczizka, U. et al. Early parenteral administration of ceftiofur has gender-specific short- and long-term effects on the fecal microbiota and growth in pigs from the suckling to growing phase. *Animals* <https://doi.org/10.3390/ani10010017> (2019).
223. Fujisaka, S. et al. Antibiotic effects on gut microbiota and metabolism are host dependent. *J. Clin. Invest.* **126**, 4430–4443 (2016).
224. Jansen, K., Pou Casellas, C., Groenink, L., Wever, K. E. & Masereeuw, R. Humans are animals, but are animals human enough? A systematic review and meta-analysis on interspecies differences in renal drug clearance. *Drug Discov. Today* **25**, 706–717 (2020).

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Author contributions

S.R.S.F. and B.M. researched data for the article. S.R.S.F., B.M. and G.D. substantially contributed to discussion of content, wrote the article, and reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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