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Effects of Antibiotics on Human Microbiota and Subsequent Disease

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Abstract

Although antibiotics have significantly improved human health and life expectancy, their disruption of the existing microbiota has been linked to significant side effects such as antibiotic-associated diarrhea, pseudomembranous colitis, and increased susceptibility to subsequent disease. By using antibiotics to break colonization resistance against *Clostridium*, *Salmonella*, and *Citrobacter* species, researchers are now exploring mechanisms for microbiota-mediated modulation against pathogenic infection, revealing potential roles for different phyla and family members as well as microbiota-liberated sugars, hormones, and short-chain fatty acids in regulating pathogenicity. Furthermore, connections are now being made between microbiota dysbiosis and a variety of different diseases such as rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, atopy, and obesity. Future advances in the rapidly developing field of microbial bioinformatics will enable researchers to further characterize the mechanisms of microbiota modulation of disease and potentially identify novel therapeutics against disease.

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UNEXPECTED CONSEQUENCES FROM ANTIBIOTIC USE

Without doubt, the discovery of antibiotics has greatly affected the world we live in, with significant health benefits. Before mass production of penicillin in the 1940s, sulfa drugs were widely used. Together with penicillin, antimicrobials ushered in a new era of modern medicine, ending a world where patients died from bacterial infections that led to bacterial endocarditis, bacterial meningitis, and pneumococcal pneumonia, saving millions of lives, and increasing the average life span by approximately 15 years (47). With such dramatic effects on human health, secondary effects almost seem inevitable. Several side effects from antibiotic use have been observed, including malabsorption characterized by a celiac-like syndrome, impaired absorption of medications, altered metabolism and absorption of vitamins, overgrowth of or colonization by resistant organisms, and altered susceptibility to infections (68). Perhaps one of the most common side effects observed immediately after administration of antibiotics was antibiotic-associated diarrhea (AAD), which causes patients to have frequent watery bowel movements. Some patients develop more severe pathologies, including inflammation of the colon (colitis), or a more severe form of colitis called pseudomembranous colitis. Many of these side effects have since been directly linked with antibiotic alterations of the microbial flora in the host, otherwise termed the microbiota. Until recently, characterizing how the microbiota is altered by antibiotics has been difficult, but recent advances in methodology have enabled researchers to define antibiotic-mediated population shifts and even to correlate individual microbiota constituents with susceptibility to disease. Here we describe how alterations to the host microbiota by antibiotics can result in increased susceptibility to subsequent diseases, including diarrhea, pathogenic infection, and other dysbiosis-linked, immune-mediated syndromes.

USE OF ANTIBIOTICS TO BREAK COLONIZATION RESISTANCE

The ability of antibiotics and antimicrobials to dramatically alter the microbiota in the gastrointestinal tract (GIT) was observed as early as 1950, when high doses of terramycin were demonstrated to eliminate the intestinal microbiota in patients who were preparing for intestinal surgery (30). Within a few years of the release of antibiotics, there was already mounting evidence that alterations to the host microbiota by antibiotics could lead to a GIT microbiota that is more favorable to colonization by opportunistic pathogens (72, 86). The concept that indigenous bacteria in

the GIT inhibit colonization by potentially pathogenic microorganisms is termed colonization resistance. In animal models for microbial-mediated disease, antibiotics are frequently used to break colonization resistance and elicit a more severe disease. For example, antibiotics are required in an animal model for pathogenesis of *Clostridium difficile*, a spore-forming, gram-positive, commensal anaerobe that causes a gastrointestinal infection in humans with effects ranging from severe diarrhea to death (32). Another animal model that requires antibiotics is *Salmonella enterica* serovar Typhimurium gastrointestinal infection in mice; streptomycin treatment makes the mice much more susceptible (59). Together, these mouse models underscore how important an intact host microbiota that is undisturbed by antibiotics is in protection against microbial disease. Recently, with advances in the methodology used to study the microbiota, it is now possible to study the mechanisms behind microbiota susceptibility to pathogenic infection and other immune-modulated diseases.

WHAT IS THE MICROBIOTA?

Basic Definition of the Microbiota

The microbiota is the collection of bacteria, archaea, fungi, protozoa, and viruses that inhabit various parts of a multicellular host. In the human host an incredibly large number of microbial cells colonize the oral and nasal cavities, the skin surface, and the gastrointestinal and urogenital tracts. It is estimated that microbial cells outnumber human cells 10 to 1, with the colon being the most heavily colonized site. Although we now know that the microbiota plays a role in a multitude of functions such as immunity, metabolism, and resistance to pathogens (90, 112), the definition of a healthy microbiota remains elusive. Throughout the twentieth century many studies tried to correlate microbiota composition with various pathological conditions but were always limited by the lack of adequate tools to identify and analyze the microbiota. In the following section, we briefly explain the rapidly evolving methods for analyzing the microbiota.

Methods to Study the Microbiota

Early attempts to characterize the composition of the microbiota relied on culturing, but about 80% of the gut microbiota and 70% of the oral cavity microbiota are currently unculturable (19, 28, 64, 134). Progressive development of modern methods to characterize these communities has led to the discovery of novel taxa (11, 16, 33, 50) and has changed our view of host-pathogen interactions from one that involved only the pathogenic bacteria and the host cells to a more ecological view that involves a complex ecosystem associated with bacterial disease. Today, the most relevant methods used in microbiota research include targeted approaches, such as 16S rRNA gene next-generation sequencing (NGS) and large-scale meta-omics approaches, such as shotgun sequencing and metatranscriptomics.

16S rRNA gene next-generation sequencing—microbiota community surveys. Although other bacteria-specific targets have been used, the current gold standard for microbial community analysis is the amplification of the 16S rRNA gene, which harbors several hypervariable (V) regions that confer specificity to a large number of bacterial species. Currently there are mainly two sequencing platforms that are used in microbiology laboratories: the Roche 454 pyrosequencer (454 Life Sciences, Branford, Connecticut) and the Illumina platform (Illumina, San Diego, California). Longer reads can be sequenced using the 454 pyrosequencer, which translates into more taxonomic information. However, the Illumina platform, which provides sequencing of DNA fragments of up to 150 base pairs in length, sequences 10–100 more samples at a much higher sequencing depth than the 454 pyrosequencer. A large cohort study showed that reads

from Illumina clustered very close to the reads obtained from full-length Sanger sequencing, whereas reads from 454 pyrosequencing clustered separately (6). Postsequencing analysis from all these platforms involves a pipeline of bioinformatic steps that filter and classify large amounts of data, with high-quality sequences clustered into operational taxonomic units (OTUs), also referred to as molecular bacterial species.

Functional meta-omics approaches. Although 16S rRNA gene analysis of the microbial communities gives us a survey of the bacteria present in a particular environment, it fails to provide any functional information. For human clinical studies, this means that one cannot infer a possible mechanism that explains the associations between differences in microbial communities and particular disease states. An approach to obtain functional biochemical information includes metagenomics and metatranscriptomics. These large-scale studies aim to provide a gene-based (metagenomics) and gene-expression (metatranscriptomics) inventory within the microbial community. Metagenomics involves the sequencing of sheared genomic DNA without any previous amplification, and it is also known as shotgun sequencing, whereas metatranscriptomics is the sequencing of cDNA converted from extracted mRNA. To avoid missing the genetic information from very low abundance species, subsequent sequencing must be done at a great depth. This was evidenced in the recent Human Microbiome Project (HMP) study, in which each sample yielded $\sim 10^7$ reads (51). The short sequences obtained are assembled into longer contiguous reads and compared to databases of known genes, such as the NIH GenBank (**www.ncbi.nlm.nih.gov/genbank**). There are also databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG, **www.genome.jp/kegg**) (60), that organize the genes into biochemical pathways. In many cases, sequences do not match genes with known or even theoretical functions, but the proportion of these unknown sequences has decreased steadily every year, and it is expected to continue to decrease in the years to come.

Different Areas Have Different Core Compositions

The HMP recently utilized advances in our ability to sequence and analyze the microbiota. Samples were collected from multiple body sites of 250 volunteers, screened for absence of disease, and studied with the ultimate goal of "improvement of human health through monitoring or manipulation of the human microbiome" (88) (**Figure 1**). Perhaps the most staggering discovery of the HMP is the high level of diversity found between individuals at all of the tested body sites (52). No single phylum was present at any site in all individuals, and every sample could be viewed as unique in terms of phylum and genus composition.

Studies of the gastrointestinal microbiota have focused on defining the composition of fecal samples, primarily because of the difficult and invasive nature of sample collection from other GIT sites. However, given that the various anatomical parts of the GIT are characterized by very different physiological conditions, such as varying pH and nutrient availability (35, 107), it is reasonable to assume that the microbial composition also varies. Indeed, both animal and human studies where samples from different GIT sites could be obtained demonstrate that the composition of the fecal microbiota is highly divergent from the microbiota of other regions of the GIT, being most similar to that of the colon (14, 33, 44, 83) (**Figure 1**). The lower GIT (cecum and colon) and the duodenum show greater overall diversity than the jejunum and the ileum. Furthermore, the stomach and small intestine contain higher counts of aerobic and facultative anaerobic organisms than do the lower parts of the GIT (83). Despite the high level of variability in taxonomic composition, when samples collected from various body sites are analyzed by a metagenomic approach, the

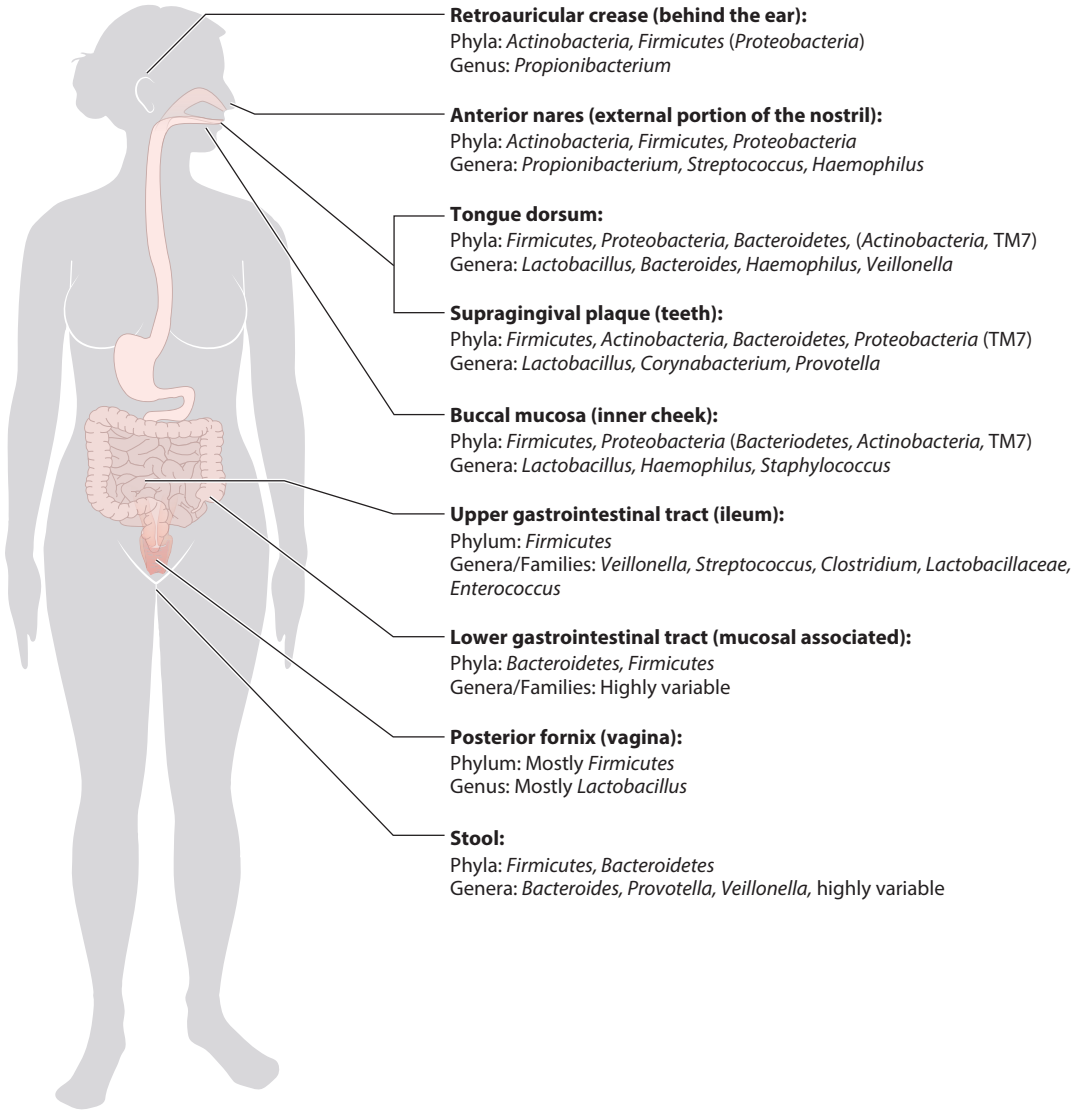

Figure 1

Composition of the microbiota at various sites of the human body. A schematic representation of the composition of bacterial flora in different parts of the human anatomy, with the less abundant community members in parentheses (14, 33, 52). Whereas some regions, such as the mouth, have high levels of diversity, others, like the vagina, have a simple composition. In the gastrointestinal tract differences have been found between the bacterial composition of the lower and upper gastrointestinal tract. On the phylum level, *Firmicutes* are present in all the tested compartments, whereas *Bacteroidetes* are highly prevalent in the stool but are relatively few in number at other body sites. *Proteobacteria* were found mainly in the oral and nasal cavities, and *Actinobacteria* seem to be a feature of the nasal cavity.

variability is greatly reduced if genes that encode proteins for metabolic pathways are selected for sequencing. The majority of the metabolic pathways considered were similarly distributed among body sites and were almost identical between individuals, leading to the conclusion that different microbiota compositions can perform the same function (52).

The high level of diversity in the human microbiome begs the question: Is there even such a thing as a healthy microbiota? The answer to this may be rather complex. Although microbial diversity is indeed very great, some consensus in bacterial phylum composition can be found even between human and murine microbiota (70). It is also very likely that the overall composition is not as important as the balance between certain microbial groups or organisms that leads to the conserved metagenome observed by the HMP consortium. Dysbiosis is defined as an altered state of microbial composition that breaks host homeostasis (117). Whereas we do not know definitively what defines a healthy microbiota, we do know numerous studies have linked microbial dysbiosis in humans with various pathological conditions, leaving us certain that the microbiota is crucial for health and that some form of proper composition likely exists.

IMPACT OF ANTIBIOTICS ON THE HUMAN MICROBIOTA

Antibiotics Alter Microbiota Composition

The effects that clinically relevant antibiotics have on different phyla, classes, and families in the community setting of the human GIT are at the early stages of discovery. **Table 1** outlines the findings from a few studies describing populations affected by different antibiotic cocktails; however, the methods used in these studies are considered preliminary. With the development of more comprehensive and cheaper methods to characterize composition shifts, future research will undoubtedly unlock how specific antibiotics alter not only the composition of the human microbiota, but also the composition of the metabolites each altered community produces. In the past, culture-based studies (reviewed extensively in Reference 89) revealed crude changes in the composition of oral, gastrointestinal, vaginal, and periurethral microbial communities in patients treated with different antimicrobial agents. Microbiota analysis by culturing, however, is only able to detect global changes to large groups of organisms (such as some anaerobic bacteria), and as such these studies may not fully reveal the complex effect antibiotic treatment has on the body's normal flora. For example, ciprofloxacin, a broad-spectrum antibiotic widely prescribed for the treatment of different bacterial infections, reduced the number of enterobacteria in fecal samples in culture-based studies (89) but seemed to have little effect on the overall composition of the GIT microbiota in a study utilizing denaturing gradient gel electrophoresis (DGGE) (31) (**Table 1**). However, when the fecal microbiota of patients treated with ciprofloxacin was analyzed by 16S rRNA sequencing over a four-week period, major changes in microbial composition were observed (27, 26). Up to 70% of the bacterial composition of feces was shifted by a single course of treatment with the antibiotic, and whereas the composition for the most part reverted to the pretreated state four weeks after treatment, some OTUs were never regained by the GIT microbiota. Repeated treatment with ciprofloxacin generated even bigger short-term and long-term changes to the composition of the GIT microbiota (27).

Indeed, a key issue with antibiotic administration is whether the microbial community regains its original composition after treatment. A few studies have looked at the long-term effects of antibiotic use in humans, but the findings differ between high-resolution and low-resolution analysis techniques (**Table 1**). On the one hand, in a DGGE-based study of six patients treated with a short course of various antibiotics, only a single individual had not regained his original GIT microbiota 60 days posttreatment (25). On the other hand, a study that used 16S rRNA sequencing to identify changes of *Bacteroidetes* in the feces of patients who received a short course of clindamycin found extreme long-term changes in the species composition of treated individuals (56).

In all these studies, none of the patients developed common antibiotic side effects such as diarrhea, implying the shifted postantibiotic flora had normal basic function. This is consistent

Table 1 Antibiotic treatment alters the gut microbiota[a]

Reference	Antibiotic treatment	N	Method of analysis	General findings
31	**Ciprofloxacin**, 500 mg, 2 times a day, 7 days; **clindamycin**, 500 mg, 3 times a day, 7 days One after the other, not at the same time	1	DGGE coupled with specific band sequencing	Treatment with ciprofloxacin resulted in minor changes (73% similarity to original state); clindamycin led to extreme change (18% similarity to original state, 11% similarity to postciprofloxacin state)
132	**Amoxicillin/ clavulanate**	2	DGGE coupled with specific band sequencing	19–34% similarity to original state
	Cefazolin	1	DGGE coupled with specific band sequencing	81% similarity to original state
	Amoxicillin (875 mg) and **clavulanic acid** (125 mg), 2 times a day, 10 days	1	16S rRNA clone library screen	Changes to all major bacterial groups (*Bacteroides* spp., *Clostridium* cluster IV, *Clostridium* cluster XIVa, *Bifidobacterium* spp.). Overgrowth of *Enterobacteriaceae* by day 4; general reversal of the community by day 24, except for *Bifidobacterium* spp., which did not return
25	**Amoxicillin**, 500 mg, 3 times a day, 5 days	6	DGGE coupled with specific band sequencing	Microbiota modulated within 3–5 days posttreatment, mostly regenerated (87% of bands) within 60 days
92	Mostly **amoxicillin**, oral administration in the first month of life	28	Real-time PCR	Decreased bifidobacteria and *B. fragilis*–group species compared to untreated children
56	**Clindamycin**, 150 mg 4 times a day, 7 days	4	Clonal typing by rep-PCR (*Bacteroides* specific) T-RFLP	Reduction in *Bacteroides* spp.; the strains did not fully regenerate; resistant clones appeared after antibiotic treatment
26	**Ciprofloxacin**, 500 mg, 2 times a day, 5 days	3	16S rRNA tag pyrosequencing	30% of the taxa in the gut were affected by the treatment; the microbiota mostly regenerated 4 weeks posttreatment
27	**Ciprofloxacin**, two 5-day treatments 6 months apart	3	16S rRNA tag pyrosequencing	25–50% of taxa wiped out by treatment, mostly regenerated over time

[a]Abbreviations: DGGE, denaturing gradient gel electrophoresis; PCR, polymerase chain reaction; T-RFLP, terminal restriction fragment length polymorphism.

with the finding that various gut microbial compositions can perform the same basic functions (52). In some cases, however, the disruption in the microbiota can lead to clinical effects such as AAD and pseudomembranous colitis, an inflammation of the colon that is characterized by diarrhea, abdominal pain, fever, and sometimes even death (118). In a case study of a 39-year-old, healthy man treated with amoxicillin, AAD was also associated with the dramatic disappearance of all members of clostridial cluster XIVa from the GIT microbiota (132). The AAD observed in this study is presumed to be the result of the loss of butyrate-producing bacteria, which resulted in a dysfunctional microbial flora.

Antibiotics Increase Susceptibility to Pathogens

Researchers have begun to answer fundamental questions regarding the role of the host microbiota in colonization resistance by studying the effect of antibiotics on infection by different bacterial pathogens.

Clostridium difficile. First isolated from the gut microbiota of infants by Hall & O'Toole in 1935 (45), *C. difficile* was named such because of the difficulty of isolating and culturing the bacterium. Although it was isolated from healthy infants, *C. difficile* was shown to be a toxin-secreting pathogen that can impair the GIT epithelium and cause colitis and death in animals, and in humans it is often the causative agent for the more acute form of AAD, pseudomembranous colitis (62). Despite early discovery, *C. difficile* received little attention until it began to emerge as a major cause of illness in hospitalized patients in the 1970s (94). Although *C. difficile*–associated diarrhea (CDAD) is rare, in epidemic conditions in the hospital setting, up to 20 in 100 hospital cases can progress to CDAD (9, 81). A recent increase in acute cases of CDAD can be at least partly attributed to the emergence of a new hypervirulent strain that produces increased amounts of toxins, causing greater pathology and as such more frequent diagnosis (128).

Many aspects of CDAD remain uncertain. Whereas it is clear that *C. difficile* is naturally present in the GI microbiota of at least some healthy individuals, the exact percentage of carriers differs between the surveyed populations, with some studies reporting less than 1% of the population are carriers (9) and others showing that as many as 20% of individuals carry the bacterium (82). It is also not entirely clear whether individuals who develop CDAD are carriers of *C. difficile* that experiences overgrowth due to GIT dysbiosis or whether they newly acquire the bacterium at a time when their GIT is susceptible. A comprehensive analysis of studies involving hospitalized patients tested for the presence of *C. difficile* revealed that those who had detectable levels of the bacterium were less likely to develop CDAD during hospitalization; this effect was enhanced in cases where *C. difficile* overgrowth was the result of antibiotic treatment (109). These results indicate that CDAD may be the result not of indigenous overgrowth but rather of pathogen acquisition from the environment. Furthermore, they suggest that some form of adaptive immunity may be involved in resistance to *C. difficile* colonization.

In a murine model of CDAD, some of the infected mice that were treated with vancomycin to eliminate the bacteria became more resistant to repeated infection (20). This finding supports the data from human patient studies showing that initial colonization with *C. difficile* may in fact protect against CDAD by the development of adaptive immunity to the bacterium. One concept that emerges rather clearly from both human studies and animal models is that GIT dysbiosis is the biggest factor in *C. difficile* colonization. Antimicrobial therapy is probably the biggest risk factor in CDAD development in humans (62), and in murine models effective infection with *C. difficile* is only possible in germ-free animals or after conventionally raised animals are treated with a complex antibiotic cocktail (20, 22).

***Salmonella* Typhimurium.** Antibiotics also increase the susceptibility of human patients to salmonellosis, where patients can develop diarrhea, fever, and abdominal cramps after infection. In most patients, the illness lasts four to seven days before recovery, but a subset of patients develop a severe illness requiring hospitalization. Patients that are more susceptible to this severe infection are often immunocompromised, such as elderly people and young people, but they also include patients who are recovering from antibiotic treatment. For example, antibiotic-treated infants were more likely than non-antibiotic-treated infants to be infected by *Salmonella indiana* in a hospital outbreak in the late 1960s (1). In other early examples from the 1960s and 1970s, chronic carriers of *S.* Typhimurium developed systemic disease after tetracycline, ampicillin, oxacillin, and gentamicin treatment (24, 99, 105). Early studies also demonstrated that after a patient develops acute salmonellosis, use of sulfonamide, tetracycline, ampicillin, and chloramphenicol can extend the carrier status of the patient (15, 80). Some of the most compelling early evidence supporting the observation that antibiotics increase the susceptibility to infection was mouse work in 1964 that demonstrated that after a single dose of streptomycin, mice were at least 100,000-fold more susceptible to salmonellae (13).

More recently, it was found that vancomycin and streptomycin induced changes to the GIT microbiota and increased susceptibility to *S.* Typhimurium colonization in a dose-dependent manner (108). Subsequent studies with multiple antibiotic treatments in 129S1/SvImJ mice, which are more resistant to *S.* Typhimurium colonization than other mouse strains, such as C57Bl/6, revealed that *S.* Typhimurium elicits different disease and pathology outcomes depending on the antibiotic used (38). Enterocolitis was only seen after streptomycin or vancomycin treatment, whereas metronidazole-treated 129S1/SvImJ mice had no significant pathology. By studying the composition of the microbiota in these mice, Ferreira et al. correlated *Bacteroidetes* and *Porphyromonadaceae* members to resistance against colitis (38). A separate study demonstrated that antibiotic-treated mice have an altered intestinal metabolome, with streptomycin treatment reducing steroid and eicosanoid hormones that are normally increased after *S.* Typhimurium infection (3).

It will be interesting to follow future research in this field, as it is possible that examining the metabolites these altered microbial communities generate will reveal a mechanism behind differential microbiota modulation of *S.* Typhimurium pathology. Indeed, a recent study demonstrated that after antibiotic treatment, mucosal carbohydrates such as sialic acids are increasingly available, which corresponds to an increased presence of bacteria, such as *Bacteroides thetaiotaomicron*, that are able to liberate these carbohydrates from the mucosa (87). *S.* Typhimurium and *C. difficile* mutants that lacked catabolic pathways for sialic acid had a competitive defect in colonization in streptomycin-treated mice, supporting the theory that microbiota-generated nutrients that are differentially produced after antibiotic treatment influence pathogen colonization of the host.

***Citrobacter rodentium*.** *C. rodentium* is a natural pathogen of mice that is used as a model for human attaching and effacing pathogens, including enteropathogenic *Escherichia coli* and enterohemorrhagic *E. coli*. Breaking colonization resistance by administering a high dose of the antibiotic streptomycin (20 mg per mouse) results in 10- to 50-fold greater *C. rodentium* colonization (10). Treatment with another antibiotic, metronidazole, leads to increased attachment of *C. rodentium* to the intestinal epithelium and an exacerbated severity of *C. rodentium*–induced colitis (131). The increased pathology corresponds to a reduction in the *Bacteroidales* order within the *Bacteroidetes* phylum, a reduced population of *Porphyromonadaceae*, and an increased number of lactobacilli. Additionally, there was reduced expression of a major component of the mucous layer, Muc2, and subsequent thinning of the inner mucin layer (131). Short-chain fatty acids, produced by intestinal microbiota members such the *Bacteroidetes* phylum, are known to stimulate epithelial cells to produce Muc2, suggesting a possible mechanism for decreased Muc2 levels in metronidazole-treated

mice (18, 29, 57, 63, 76, 113). Unlike the microbiota of infected wild-type mice, the microbiota of Muc2-deficient mice interacts with *C. rodentium* and host tissues, and the *C. rodentium* colonization burden is 10- to 100-fold greater than in wild-type mice, indicating that Muc2 may regulate pathogen and intestinal microbiota access to the gut surface. Together, these studies suggest that the composition of the microbiota may modulate the thickness of the inner mucous layer and that the host may in turn use mucin to flush *C. rodentium* away from the mucosal surface (10). Another recent study suggests that the ability of *C. rodentium* to outcompete the microbiota is controlled by temporal expression of the type 3 secretion system (T3SS), a channel encoded by gram-negative bacterial pathogens to transport effector proteins into host cells. This work suggested that whereas the pathogen may require the T3SS to attach and replicate on the epithelium in the presence of the host microbiota during the early stages of colonization, during later stages of infection, which correspond to the pathogen's migration from the epithelial surface to the intestinal lumen, expression of the LEE, a pathogenicity island that encodes the T3SS, may no longer be as critical for *C. rodentium* survival against the microbiota (59).

By exploiting an experimental treatment for ulcerative colitis and *C. difficile* overgrowth, studies with fecal microbial transplantation have shown that the microbiota plays a role in resistance to intestinal infection by *C. rodentium* by modulating the development of intestinal Th17 cells and the ensuing IL-17 and IL-22 innate immune response (41, 54, 130). At the family level, resistance was correlated with a decrease in *Porphyromonadaceae* (130) and an increase in *Lachnospiraceae*, *Bacteroidaceae*, and unclassified *Clostridiales* species (130). At the phylum level, resistance was correlated with an increase in *Bacteroidetes* (41, 130) and a decrease in *Firmicutes* (130).

Together, the above studies with *C. difficile*, *S.* Typhimurium, and *C. rodentium* link antibiotic treatment with susceptibility to infection and furthermore suggest that the composition of the microbiota, as evidenced by differential effects by different antibiotics, is critical for prevention or susceptibility to subsequent disease.

MICROBIOTA DYSBIOSIS LEADS TO OTHER DISEASE STATES

Gut dysbiosis, induced from antibiotics or other environmental and genetic factors, is strongly correlated with diseases such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), type 1 diabetes (T1D), atopy, and obesity (**Table 2**). A common feature of diseases linked to microbial dysbiosis is a significant reduction in bacterial diversity (67, 75). Additionally, the ratio of *Firmicutes* to *Bacteroides*, two of the most abundant phyla in the human intestinal microbiota, is altered in dysbiosis-linked disorders (111, 129).

Whereas genetic, environmental, and epigenetic factors are all known to play a role in the development of these diseases, there is mounting evidence that the intestinal microbiota may also influence the early stages of these diseases by modulating the tone of host immune development, as there is a constant interaction between certain members of the microbiota and the host's immune system (21, 96). For example, using animal models, researchers have recently revealed a possible causal relationship between the microbiota and allergic disease, where exposure to different clostridial strains induces regulatory T cells (Treg) in the gut of mice, which go on to induce Treg expansion and prevent colitis (7). However, the effect of intestinal dysbiosis may not be strictly localized to the site of the imbalance: Under dysbiotic conditions and in a genetically susceptible host, immune-mediated diseases could originate in the intestine and manifest later systemically (RA, T1D, atopy, and obesity) or in specific organs (IBD) (**Table 2**).

It is still unclear if and how certain bacteria may guide the immune response into the development of extraintestinal diseases such as asthma and atopy. Animal models for these diseases have provided us with important clues to how the microbiota may drive their development.

Table 2 Intestinal microbial dysbiosis in inflammatory bowel disease, rheumatoid arthritis, type 1 diabetes, obesity, atopy, and asthma[a]

Disease	Evidence of dysbiosis	Microorganisms identified
IBD, CD, and UC	■ Intestinal microbiota are disturbed in patients with CD (95) and UC (39, 67) ■ Disease occurs in the presence of certain bacterial species in genetically susceptible animal models (116)	■ Overall reduction in *Firmicutes*, and *Faecalibacterium prausnitzii* is associated with higher recurrence risk (110) ■ 46 clostridial strains from clusters IV and XIVa prevent colitis in mice (7) ■ *Enterobacteria* species (40, 73, 115) and *Enterococcus faecalis* (61) are increased in CD patients ■ Decreased *Bifidobacterium* strains in CD patients (71) ■ *Bacteroides fragilis* prevents colitis in mice (79)
Rheumatoid arthritis	■ Patients with RA have reduced intestinal bacterial diversity (106) ■ Altered proportion of certain members of the oral microbiota in patients with RA (77) ■ Mouse models raised in SPF conditions do not develop RA-like disease until exposed to microbial β-glucans (102).	■ Increase in of *Prevotella* spp. in patients with recently diagnosed RA, particularly *Prevotella copri* (106) ■ Reduced group XIV *Clostridia*, *Lachnospiraceae*, and *Bacteroides* species (106) ■ Reduced bifidobacteria and *Bacteroides fragilis* (125) ■ Increased *Porphyromonas gingivalis* (77) and *Bacteroides* spp. (125)
Type 1 diabetes	■ Reduced *Firmicutes*:*Bacteroidetes* ratio and reduced bacterial diversity in children that progress to T1D (42). ■ The diabetes-prone BBDP rat shows alterations in gut microbiota prior to the onset of disease (17) ■ Treatment with antibiotic Sulfatrim prevented diabetes in the virus-induced T1D rat model (LEW1.WR1 rat) (46)	■ Increased *Bacteroides ovatus* in patients with T1D (42) ■ Decreased OTUs within order *Clostridiales* (42) ■ BBDP rats have lower levels of *Bacteroides* (17), *Bifidobacterium*, and *Lactobacillus* species (98)
Obesity	■ Increased *Firmicutes*:*Bacteroidetes* ratio in obese individuals (124) and mouse models (2, 34, 69) ■ Transfer of microbiota of obese mice into germ-free mice resulted in weight gain (122) ■ Antibiotics, including tetracycline, glycopeptide, macrolides, and penicillin, induced weight gain in animals (85) ■ Weight gain in children 1–3 years old when antibiotics administered before 6 months of age (121) ■ Vancomycin, but not penicillin, induced weight gain in adult patients with endocarditis (120) ■ Azithromycin caused weight gain in children and adolescent patients (6–18 years) treated for cystic fibrosis (93, 103, 104) ■ Weight gain in adult patients treated with clarithromycin for *Helicobacter pylori* infection (66)	■ Reduced *Bacteroidetes* species in obese individuals (4, 123, 124, 135) ■ Increased *Methanobrevibacter smithii* (133) ■ Increased *Lactobacillus* species (4, 84) ■ Increased *Faecalibacterium prausnitzii* in obese children (8)

(*Continued*)

Table 2 (*Continued*)

Disease	Evidence of dysbiosis	Microorganisms identified
Atopy and asthma	■ Differences in intestinal microbiota between children with atopy and healthy controls (12, 58, 126, 127) ■ Mice deficient in the Toll-like receptor 4 gene develop a worse disease (23) ■ Vancomycin, but not streptomycin, worsened asthma in mice (100) ■ A meta-analysis of 23 studies concluded that infants born via cesarean section have a 20% increased risk of developing asthma during childhood (119)	■ *Mycobacterium vaccae* (53) and *Helicobacter pylori* (5) significantly reduced airway disease in mice ■ Vancomycin-treated mice showed a decrease in *Bacteroides* groups and an increase in members of the *Lactobacillaceae* family (100) ■ *Clostridium* spp. induced higher IgE titers in a mouse asthma model (7) ■ Babies born via cesarean section have fewer members of *Bifidobacterium* and *Bacteroides* spp. in the gut (36, 43)

[a]Abbreviations: BBDP, biobreeding diabetes prone; CD, Crohn disease; IBD, irritable bowel disease; OTU, operational taxonomic unit; RA, rheumatoid arthritis; SPF, specific pathogen free; T1D, type 1 diabetes; UC, ulcerative colitis.

For example, attenuation of asthma in a germ-free mouse model upon microbial colonization is associated with decreased invariant natural killer T cells in the gut and the lung (91). Additionally, Treg decreases and IgE increases have been found to worsen disease in mouse models of asthma treated with vancomycin (101). In a similar study, animals treated with a strong cocktail of antibiotics showed increased IgE titers and elevated numbers of circulating basophils. The authors suggest that the intestinal microbiota, via MyD88 signaling in B cells, downregulates the induction of IgE and that in the absence or significant reduction of microbiota, IgE production drives basophil hematopoiesis in the bone marrow (49). Although at birth these animals exhibit a primitive, Th2-polarized immune response, they exhibit an increase in asthma severity (48, 49, 100), and it is not until microbial colonization that these effects are minimized (5, 53). Furthermore, treatment with vancomycin, and not streptomycin, worsened asthma severity in mice, suggesting that specific microbiota compositions differentially affect the development of these diseases (100). Animals treated with vancomycin showed a marked decline in members of the *Bacteroides* groups and an increase in members of the *Lactobacillaceae* family, implying that the lack of the former and/or the overrepresentation of the latter group may be involved in directing the immune response toward an exacerbated allergic phenotype. In another study, select *Clostridium* species were critical in the modulation of an allergic response, as higher IgE blood titers were observed in mice that were not colonized by these bacteria (7).

There seems to be a window of opportunity during which shifts in the microbiota are critical in immune modulation and disease development (100). In asthma animal models, antibiotic treatment to neonate but not adult mice results in an increase in asthma severity, suggesting that the vulnerability of the intestinal microbiota composition occurs early in its development and that later shifts may not result in allergic disease. Several studies have found that in children, antibiotic use results in an increased risk of asthma (65, 74, 97). In a study examining the fecal microbiota of 1,176 infants in the Netherlands, researchers found that those treated with antibiotics in the first month of life ($n = 55$) had decreased numbers of bifidobacteria, in comparison with untreated children (92) (**Table 1**). However, given that antibiotics are often prescribed for respiratory infections, the correlation between antibiotic treatment and asthma may be confounded by indication.

Obesity research also suggests antibiotics may lead to disease development. Antibiotics have historically been used to induce weight gain in livestock (85). In humans, administration of antibiotics during the first six months of life leads to a small, yet significant, weight increase in

children at one to three years of age (121), suggesting that there might be a window of opportunity in obesity as well. There are many other reports that associate antibiotic use and weight gain (**Table 2**). Of special relevance is a meta-analysis that concluded that macrolides lead to weight gain in children and adolescents treated for cystic fibrosis (114). Again, it is difficult to conclude from these studies that the effect on weight occurs via shifts in the intestinal microbiota, as it cannot be ruled out that the observed outcome is due to the individual's improvement after eliminating a bacterial infection or a direct effect of the drug on the host's metabolism.

FUTURE DIRECTIONS

Antibiotics have irreversibly altered the field of modern medicine, significantly improving the quality of life for countless individuals. However, with advancement in methodologies, we are just beginning to appreciate that these life-altering drugs did not arrive without imposing some collateral damage on the microbiota, which protects the host against pathogenic infection and potentially other dysbiosis-linked diseases such as IBD, obesity, and asthma. In the future, modern sequencing methods will enable us to more fully analyze the composition of the microbiota and understand more completely how antibiotic-altered communities and their products influence disease development. Furthermore, researchers may be able to exploit these new data to identify novel therapies against microbiota-mediated diseases, such as identifying metabolites that beneficial communities generate and applying them to patients in an effort to prevent colitis (55), identifying more effective probiotics (37), or even applying microbes that provide immunostimulatory signals to patients in an effort to prevent pathogen colonization (78). By using antibiotics to alter microbiota-mediated colonization resistance, it has been possible to identify mechanisms for pathogen interaction with the host GIT community, as well as to suggest ways forward to a better understanding of the effect of antibiotics on the microbiota.

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