How to Fight Back Against Antibiotic Resistance

Mapping the exchange of genes between pathogens and nonpathogens offers new ways to understand and manage the spread of drug-resistant strains.

Gautam Dantas and Morten O. A. Sommer

Not so long ago, it seemed like the fight against infectious diseases was nearly won. The discovery of penicillin in 1929 gave clinicians their first weapon to combat common ailments like pneumonia, gonorrhea, and rheumatic fever. In the decades that followed, medical researchers discovered more than 150 other types of antibiotics. These widely hailed "wonder drugs" were so successful that U.S. Surgeon General William Stewart announced in 1967, "The time has come to close the book on infectious diseases."

Stewart and most of his contemporaries greatly underestimated the ability of bacterial pathogens to adapt to these life-saving medicines. Almost as soon as clinical use of penicillin began in 1946, the first drug-resistant pathogens appeared. During the golden age of antibiotic development (the 1940s to the 1960s), the spread of antibiotic resistance was balanced by the continued discovery and deployment of new classes of antibiotics. But starting in the 1970s, a dwindling interest and ability of the pharmaceutical industry to develop new antibiotics resulted in a 40-year period when virtually no new broad-spectrum classes of antibiotics were brought to the market. Instead, companies focused on modi-

fying the chemical scaffolds of already approved classes of antibiotics.

During this innovation gap, bacterial evolution did not cease. Consequently, drugs that were previously effective in treating a broad spectrum of infectious bacteria are now useful for fewer and fewer infections. Certain bacteria, including strains of *Escherichia coli* and *Klebsiella pneumonia,* are now resistant to all major antibiotics—even carbapenems, which have long been the drug of last resort to treat afflictions such as lung infections. With dwindling treatment options, the mortality rate from those infections in the United States is approaching 50 percent. In effect, for some diseases we are now living in a post-antibiotic age.

According to a September 2013 report from the U.S. Centers for Disease Control and Prevention (CDC), treatment of antibiotic-resistant infections adds $35 billion in health care costs and 8 million hospital days in the United States per year. A recent drug-resistant *Salmonella* outbreak due to contaminated chicken meat was linked to nearly 300 illnesses across 18 states, sickening infants and nonagenarians alike. At least 23,000 Americans die each year from infections, many caused by the superbug methicillin-resistant *Staphylococcus aureus* (MRSA), because doctors have run out of drugs with which to treat them.

Government agencies are belatedly considering incentives to support renewed antibiotic drug development, but these initiatives have not yet had a direct impact on the drug development pipeline. As a result the number of antibiotics approved by the Food and Drug Administration (FDA) hit
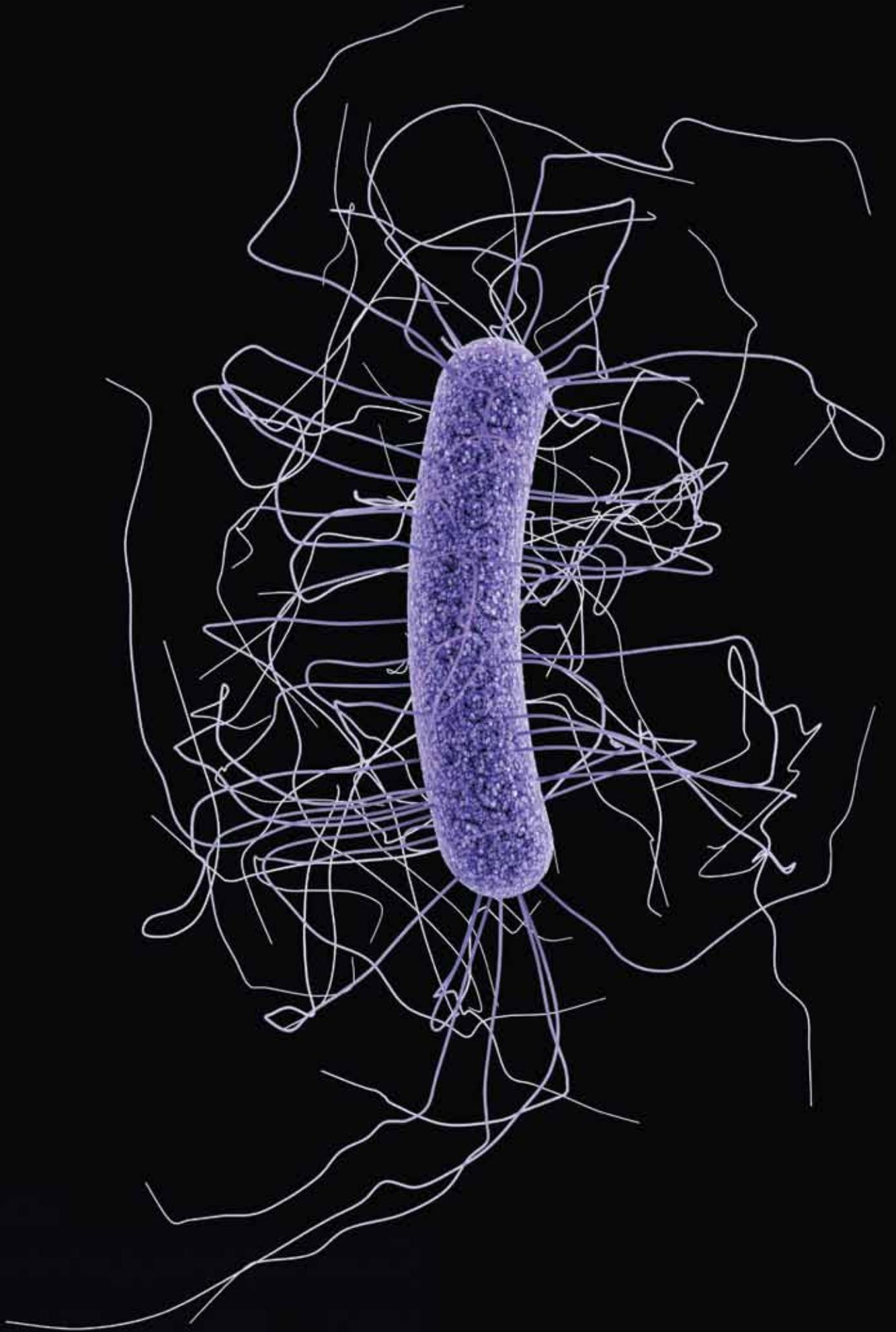
a record low of one new antibiotic in the five-year period from 2008 to 2012, down from 16 new drugs in the years from 1983 to 1987 *(see the figure on page 44).* CDC Director Tom Frieden recently warned, "If we don't act now, our medicine cabinet will be empty and we won't have the antibiotics we need to save lives." In reality, the development of new antibiotics is only part of the solution, as pathogens will inevitably develop resistance to even the most promising new compounds.
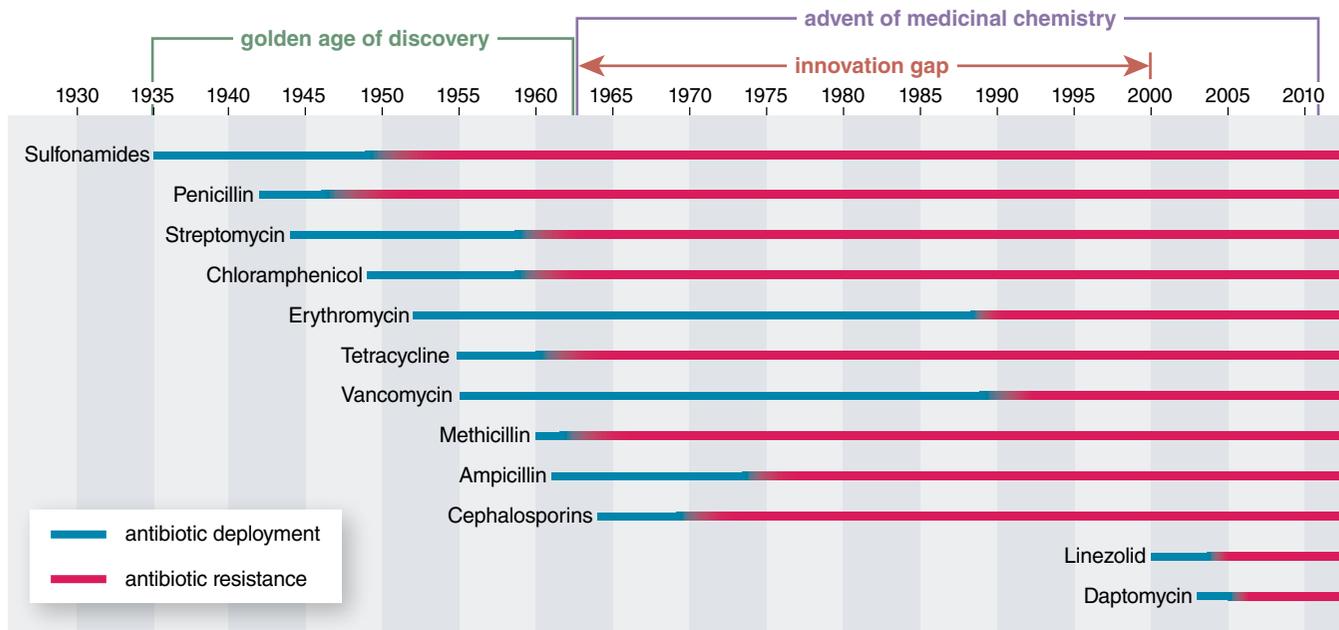
To save the era of antibiotics, scientists must figure out what it is about bacterial pathogens that makes resistance inevitable. By studying the suite of genes—collectively known as the "resistome"—that can turn a susceptible pathogen into a superbug, researchers may be able to uncover the Achilles heel of these multiple drug–resistant strains. Although most studies on drug resistance have focused on disease-causing pathogens, recent efforts by the two of us and by a number of our colleagues have shifted attention to the resistomes of nonpathogenic bacteria. Importantly, over the past decade advances in DNA sequencing have enabled us to explore the genomes of both pathogenic and nonpathogenic bacteria across a variety of different natural habitats.

This research has led to a growing understanding of how antibiotic re-

Gautam Dantas is an assistant professor of pathology/immunology and biomedical engineering and member of the Center for Genome Sciences and Systems Biology at Washington University in St. Louis. Morten O. A. Sommer is a professor of systems biology and member of the Novo Nordisk Foundation Center for Biosustainability at the Technical University of Denmark. E-mail for Dantas: dantas@wustl.edu

Clostridium difficile can cause life-threatening diarrhea. In 2000, a strain emerged with resistance to multiple antibiotics, including ciproflaxin and levofloxacin. The bacterium contributed to a 400 percent increase in *C. difficile*–associated deaths in the United States between 2000 to 2007.

Science Source

golden age of discovery

advent of medicinal chemistry

innovation gap

| 1930 | 1935 | 1940 | 1945 | 1950 | 1955 | 1960 | 1965 | 1970 | 1975 | 1980 | 1985 | 1990 | 1995 | 2000 | 2005 | 2010 |

Sulfonamides
Penicillin
Streptomycin
Chloramphenicol
Erythromycin
Tetracycline
Vancomycin
Methicillin
Ampicillin
Cephalosporins
Linezolid
Daptomycin

— antibiotic deployment
— antibiotic resistance

Clinical deployment of new antibiotics (blue bars) has quickly been followed by the evolution of bacteria able to resist their effects (red). During the golden age of discovery, 150 types of antibiotics were developed. Since then, the spread of resistance has greatly outpaced the rate of drug development. The Infectious Disease Society of America estimates that 70 percent of hospital-acquired infections in the United States are now resistant to one or more antibiotics.

sistance evolves in specific bacteria, as well as how it gets passed between different bacteria and between different environments. We are still a long way from Stewart's old proclamation of victory, but the recent advances are helping frame new strategies to complement the 90-year-old paradigm of just tr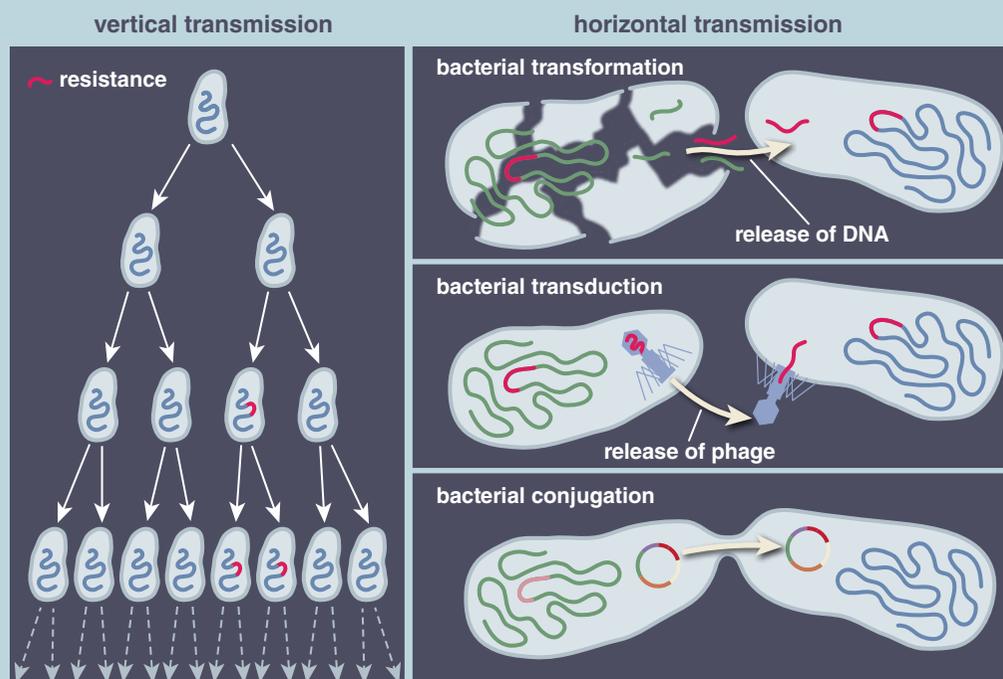ying to defeat resistant bacteria by discovering newer drugs. Understanding factors that influence resistome evolution and dissemination may both extend the life of current drugs and point toward new disease-fighting strategies.

Origins of Resistance

There are two ways that pathogenic bacteria can develop antibiotic resistance: vertically, through the accumulation of genetic changes during the natural process of copying its genome, and horizontally, by swapping resistant genes from one microbe to another *(see box below)*.

Vertical transmission is the fundamental evolutionary process by which a cell can accumulate errors in its genome during replication, such that the resulting progeny differ genetically from their bacterial ancestors. The genome replication or copying error rate is rather low, so typically one in a thousand growing bacteria will

vertical transmission

~ resistance

horizontal transmission

bacterial transformation

release of DNA

bacterial transduction

release of phage

bacterial conjugation

Antibiotic resistance can be acquired in two basic ways. In vertical transmission, a bacterium accumulates errors or mutations in its genome during replication; some of those changes *(red)* give the ability to resist antibiotics and are passed on to subsequent generations. In horizontal transmission, resistant genes are swapped from one microbe to another. This can occur via three mechanisms: *transformation,* when bacteria scavenge resistance genes from dead bacterial cells and integrate them into their own genomes; *transduction,* when resistance genes are transferred by bacteriophages (viruses that infect bacteria); or *conjugation,* when genes are transferred between bacterial cells through tubes called *pilli.*
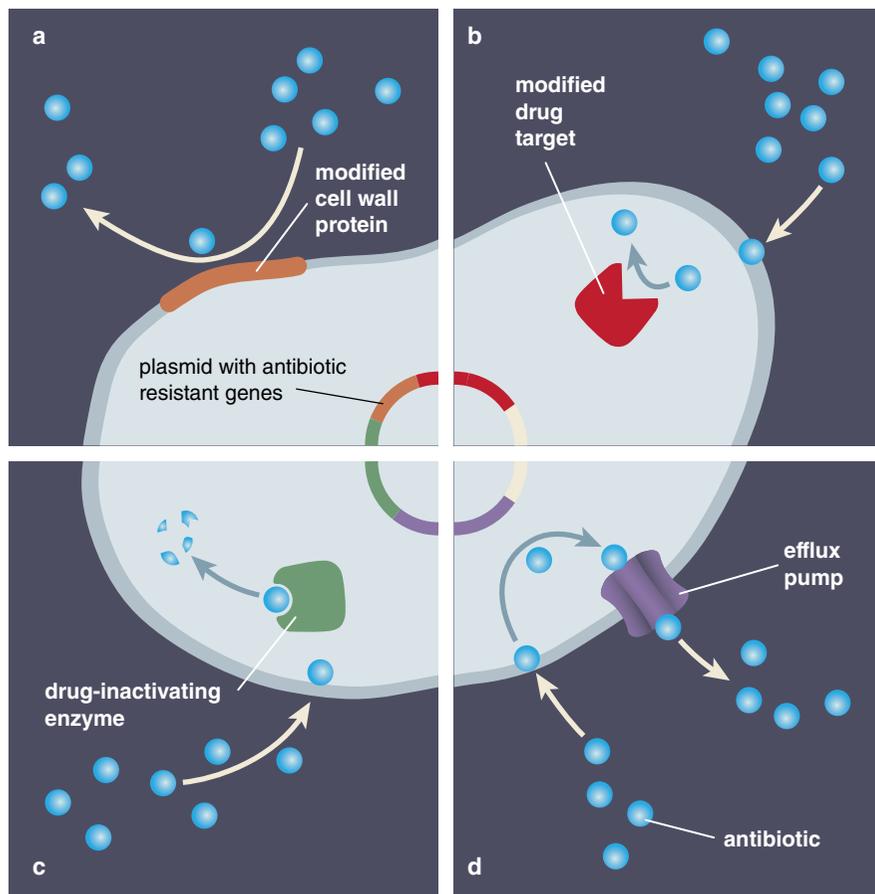
Four mechanisms of resistance: impermeable barrier (*a*) **blocks antibiotics** (*blue spheres*) **because the bacterial cell membrane is now impermeable to the drug. Target modification** (*b*) **alters the proteins inhibited by the antibiotic, so the drug cannot bind properly. Antibiotic modification** (*c*) **produces an enzyme that inactivates the antibiotic. Efflux** (*d*) **employs genes coding for enzymes that actively pump the antibiotic out of the cell.**

introduce an error (called a *mutation*) into the genome. Not all of these mutations are advantageous, but about one in a billion will generate mutants that can grow faster or tolerate higher concentrations of antibiotics than their predecessors. When such bacterial mutants are exposed to antibiotics, those possessing antibiotic resistance genes will increase in prevalence to the point of taking over the entire population. Multiple cycles of such mutation and selection are often necessary to evolve high-level antibiotic resistance.

Genes that have evolved to confer antibiotic resistance to one type of bacteria can be transferred to another by a mechanism known as *horizontal gene transfer.* While some pathogens acquire resistance via vertical transmission, recent studies have suggested that horizontal transmission may be the dominant force behind growing antibiotic resistance. During horizontal gene transfer, antibiotic resistance genes catch a ride on mobile genetic elements that carry the genetic material between different cells. Mobile genetic elements can be linear or circular pieces of DNA, called *plasmids,* which are replicated by the cell along with its genome.

These DNA fragments make their way into a new cell through three mechanisms: transformation, transduction, and conjugation. In transformation, bacterial cells scavenge DNA remnants from dead bacterial cells and integrate them into their own genome. In transduction, genetic material is transferred by bacteriophages (viruses that infect bacteria). Bacteriophages can insert their DNA into the genome of a host cell, where they are maintained for many generations before they pack up their DNA and leave to infect another cell. Along the way, the bacteriophage may coincidentally integrate a section of the bacterial host cell genome into the bacteriophage genome, enabling genetic material from one cell to hitchhike a ride to another cell on the bacteriophage.

The final way antibiotic resistance genes can be passed from one microbe to another is through conjugation, also known as bacterial sex. The discovery of this process, now thought to be the main mechanism of horizontal gene transfer, earned Joshua Lederberg the 1958 Nobel Prize in Physiology or Medicine. During conjugation, plasmids hijack the cellular machinery to create structures called *pilli* that protrude from the donor cell to penetrate the membrane of the recipient cells, enabling the transfer of the conjugative plasmid and all the functions it encodes. Many hospital-associated pathogens, including the carbapenem-resistant bacteria mentioned previously, harbor large conjugative plasmids that contain tens of resistance genes, offering the host cells immunity to virtually all antibiotics.

Recent large-scale efforts sequencing the genomes of many bacterial pathogens, by such groups as the Wellcome Trust Sanger Institute in the

Understanding factors which influence resistome evolution and dissemination may both extend the life of current drugs and point toward new disease-fighting strategies.

United Kingdom and the Broad Institute of MIT and Harvard in the United States, have added another complication to this story. These studies have shown that the genes conferring resistance toward antibiotics in pathogens can be acquired via horizontal gene transfer from another gene reservoir entirely, such as city soil, waste water, or processed meat. Accordingly, there

is a substantial interest in broadly characterizing these gene reservoirs to improve our overall understanding of how swapping genes from one reservoir to another contributes to the evolution of antibiotic resistance in bacterial pathogens.

How Pathogens Fight Back

Just as there are a number of different ways for bacteria to acquire an antibiotic resistance gene, the genes themselves represent a number of different strategies to encode resistance. The genes that confer antibiotic resistance can be loosely separated into four groups, each with their own unique mechanism for combating antibiotic exposure (*see figure on page 45*).

Bacteria in the impermeable-barrier group are naturally resistant to certain antibiotics, either because they lack the target of the antibiotic or because their membranes are impermeable to the drug. In the second group, target modification, bacteria acquire mutations in genes that modify the target of the antibiotic, dampening its effectiveness. Each antibiotic is designed to target a well-defined essential bacterial process. For example, fluoroquinolones are a widely used class of antibiotics, for the treatment of skin, lung, or urinary tract infections. These antibiotics target DNA, disrupting the proper functioning of proteins involved in unwinding DNA's helix during replication. Mutations that confer resistance toward fluoroquinolone antibiotics often change the conformation of these proteins, reducing the binding of the drug to its target and thus increasing the concentration necessary to block the process.

In antibiotic modification, a resistance gene can encode an enzyme that helps break down or modify the antibiotic before it can kill the bacteria. This tactic is often used against beta-lactams, the most widely prescribed and diverse chemical class of antibiotics, which include the well-known drug penicillin. Penicillin inhibits enzymes that remodel the bacterial cell wall and are essential for the cell during growth. Resistance toward penicillin is frequently conferred by beta-lactamases, enzymes that cleave the penicillin molecule to render it ineffective in inhibiting the cell wall modification enzymes.

Finally, efflux occurs when a resistance gene codes for proteins that actively pump the antibiotic out of the cell, keeping its internal concentration low enough to prevent inhibition. This resistance mechanism is deployed for all antibiotics that have targets within the cell; in many cases such efflux pumps are able to pump out several different antibiotics, resulting in multidrug resistance. An example of this has been observed for tetracycline, an agent used to treat a wide variety of infections. Resistance to the drug can stem from tetracycline efflux genes, which code for proteins that sit in the bacterial membrane and export the antibiotic out of the cell.

Further complicating matters, resistance toward any one drug typically results from more than one mechanism. For instance, tetracycline resistance has been observed to occur through target modification, antibiotic modification, and efflux mechanisms.

Though the term "antibiotic resistome" didn't emerge until five years ago, the concept encapsulates decades of research on the transmission and

Exploring Resistance Outside the Petri Dish

In the first several decades after the discovery of antibiotics, researchers studied the emerging problem of antibiotic resistance by growing target microorganisms in the lab. The reliance on such methods traces back to the founder of modern bacteriology, Robert Koch, whose work in cultured bacteria made "pure culture" the gold standard in clinical microbiology laboratories. We know now that studying individual organisms grown in pure culture ignores the increasing number of diseases caused not by one pathogenic bacterium but by several acting in concert. In addition, most environmental microbes cannot be readily cultivated in the lab. Recent technical breakthroughs have led to three established culture-independent strategies for exploring antibiotic resistance much more fully, in both pathogenic and non-pathogenic bacteria.

The polymerase chain reaction (PCR), selectively amplifies specific antibiotic resistance genes from complex microbial communities so they can be easily identified. PCR is well suited for studying the prevalence of already known genes but cannot be used to discover new ones.

Metagenomic sequencing identifies all of the DNA from a particular environment, irrespective of its origin. The sequences are then assembled and scanned for new genes that are similar to already known resistance genes

Metagenomic functional selections combine the cultivation-based methods of old with new, culture-independent techniques. A host organism that is normally susceptible to antibiotics is genetically engineered to possess various chunks of DNA taken from a microbial community of interest. The modified hosts are then exposed to antibiotics. The only survivors will be those that acquired a resistance gene. The microbes can then be characterized to reveal the sequence that confers resistance.

In addition, we have developed a novel approach called **PARFuMS** (for Parallel Annotation and Reassembly of Functional Metagenomic Selections) that integrates culture-independent functional metagenomic selections, next-generation DNA sequencing, and optimized computational sequence assembly and annotation algorithms to profile resistomes.

Scientists once relied heavily on methods that required culturing bacteria in the laboratory (usually in petri dishes, as shown at left) to study antibiotic resistance in various microbial populations. Today these culture-dependent methods are being eclipsed by other approaches that enable researchers to characterize the large percentage—as much as 99 percent—of bacteria that cannot be grown in pure culture.

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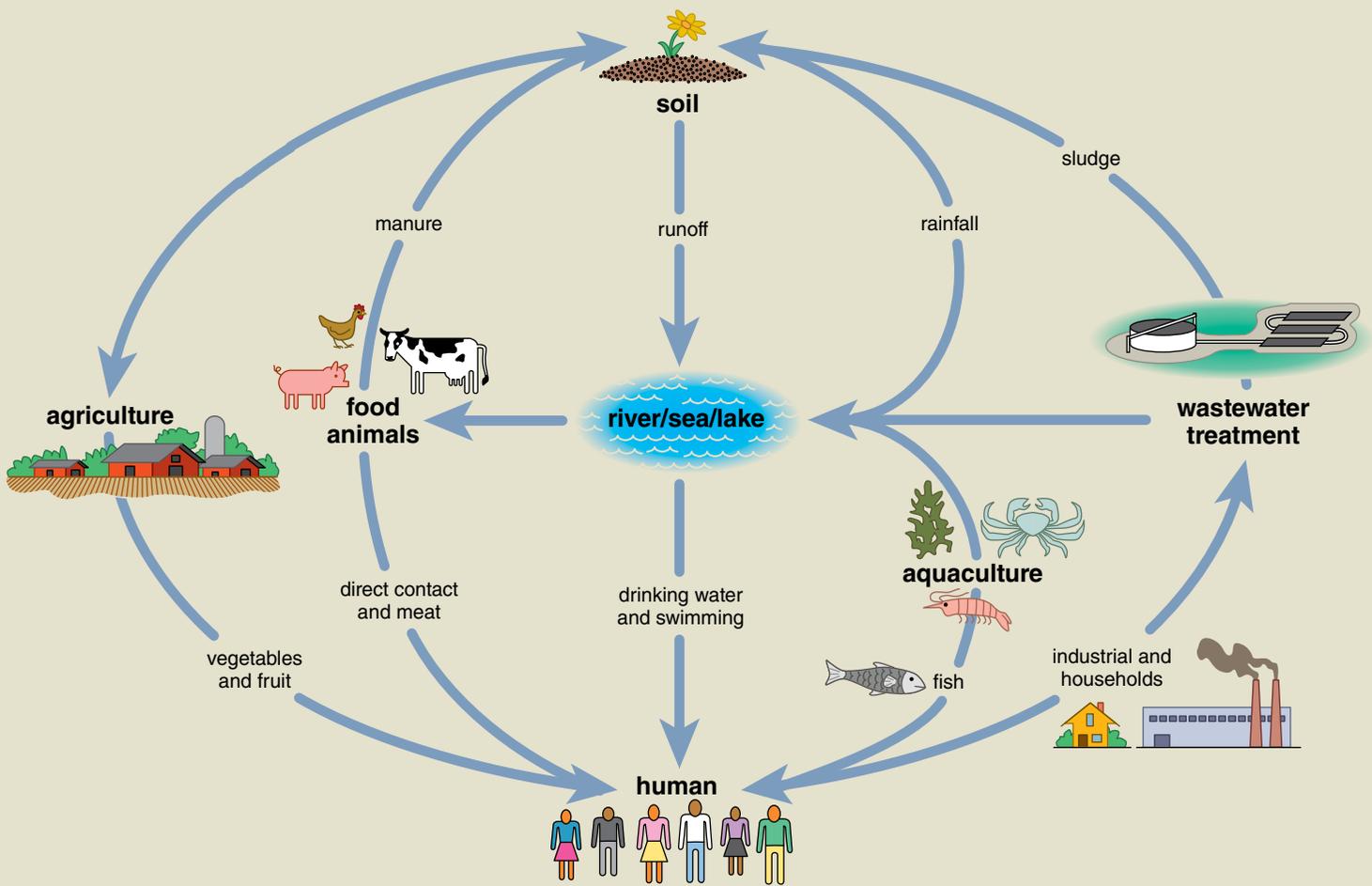
Interconnections between people, animals, and the environment make it easy for antibiotic-resistant bacteria to jump from one species to another. For instance, a resistant strain living in soil could travel through runoff and get passed on to humans via drinking water or recreational swimming. Multiple routes of exchange propel the evolution and spread of resistance.

evolution of antibiotic resistance genes between different microbes and different environments. The resistome as it is currently defined is the entire suite of genes, from a particular microbe or microbes, which confers antibiotic resistance. It includes all antibiotic resistance genes in a group of microbes at any scale, from a single organism to all of the microbes in an arbitrary environmental sample. Viewed this way, the resistome from one environment can be evaluated for its capacity to exchange resistance genes with another environment.

Mounting evidence indicates that essentially all microbial environments contain antibiotic resistomes; that myriad natural and human-driven activities influence their exchange within and between environments; and that a complex web of interactions connects various resistomes. Two of the most important environments for microbial

resistome evolution and exchange are the soil and the human body, but there are other resistomes as well *(see the box on page 49)*.

The Soil's Reservoir of Resistance

Antibiotic resistance is everywhere, even in your backyard. Soil microbes likely represent the evolutionary reservoir of most resistance, and the resistome of the soil is easily the largest and most diverse of any environment. The majority of antibiotics used in the clinic were originally discovered as or derived from natural products of soil-dwelling microbes, primarily of the *Streptomyces* genus of the Actinomycete phylum.

In 1973, Julian Davies, now at the University of British Columbia, and his colleagues first hinted at the idea of a resistome in the Producer Hypothesis on the origin of clinical resistance. He argued that the "producer" bac-

teria Actinomycetes, a major source of antimicrobials for the commercial drug industry, must contain elements protecting against the antibiotics they produced. These elements were by definition antibiotic resistance genes. Given that these production capacities likely evolved hundreds of millions of years ago, the corresponding resistance genes are likely just as old.

The antibiotic resistance genes observed in nonproducer organisms (including pathogens) may have been acquired directly from the producers or from their soil-dwelling neighbors who evolved them in response to the selection pressure of natural antibiotic production from other microbes in the soil. In fact, Davies's group showed the activity of the generally nonpathogenic soil bacteria *Streptomyces* coded for resistance enzymes that modify specific antibiotics known as aminoglycosides identical to those found in clinical pathogens.

In the 40 years since Davies's proposal, a large body of literature has provided general support for this hypothesis, as well as some key refine-
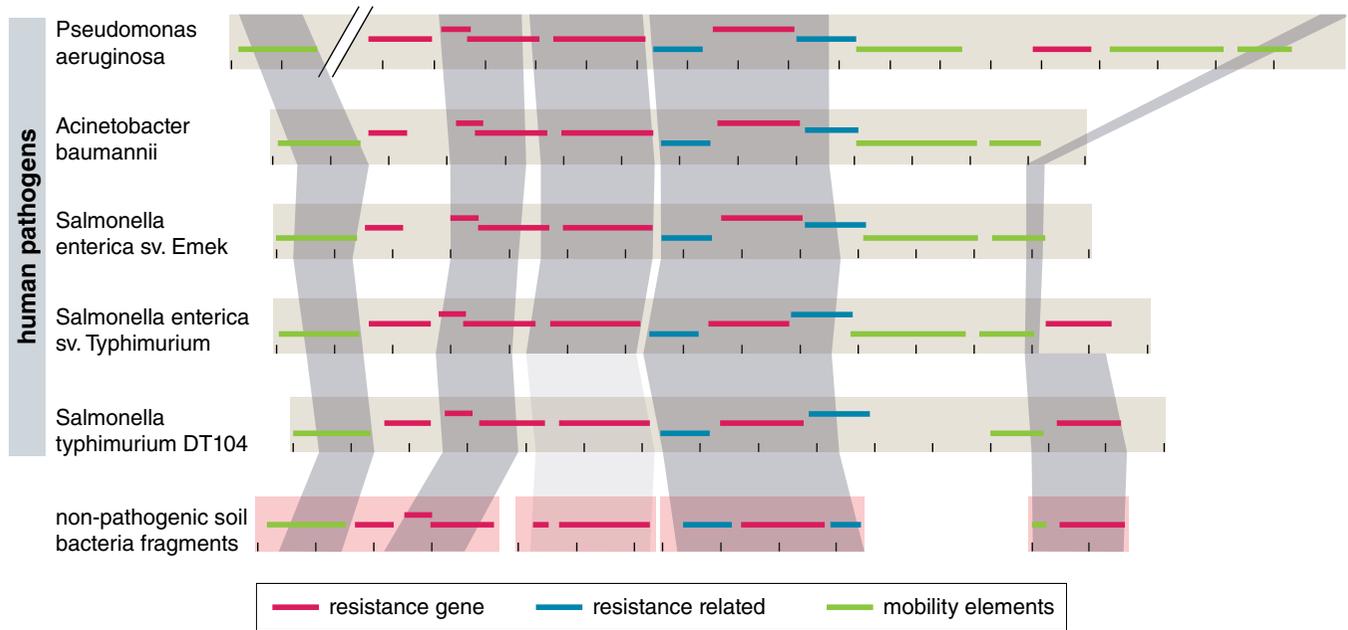
Antibiotic resistance genes from nine multidrug-resistant soil bacterial cultures (*bottom row*) **were genetically identical** (*shown by gray shading*) **to genes in several clinical pathogens** (*top five rows*), **providing evidence for recent exchange between the resistomes of nonpathogenic soil bacteria and human pathogens.** (Adapted from K. J. Forsberg et al, *Science* 337:1107.)

ments. A landmark paper from Gerry Wright and colleagues at McMaster University in 2006, which also formally introduced the resistome, showed that about 400 randomly isolated soil *Streptomyces* samples were highly mul-

world, namely, the Proteobacteria, Bacteroidetes, and Actinomycetes—that can actually feed and grow on antibiotics. These bacteria were on average resistant to 17 of 18 clinically relevant antibiotics profiled, likely displaying

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Just one gram of soil is estimated to contain about one billion bacterial cells, and no current method gets even remotely close to sampling this diversity.

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tidrug resistant against a large panel of clinically relevant antibiotics. On average, these bacteria were resistant to 7 to 8 drugs, and one superbug was resistant to 15 different compounds of the 21 tested, including drugs that were entirely synthetic and ones that were only recently approved for clinical use. Wright's observation was startling, since such high levels of multidrug resistance exceed those found in most pathogens.

In 2008, our own research further expanded the view of a substantial multidrug soil resistome by describing about 600 soil bacteria—from 3 of the 60 phyla or divisions of the bacterial

this incredibly high multidrug resistance because they were cultured under the selective pressure of extremely high antibiotic concentrations. Other censuses of cultured soil bacteria populations have since confirmed these high levels of multidrug resistance.

The discovery of ubiquitous multidrug resistance in soil microbes suggests that the soil resistome is immense. Complementary investigations of the genes conferring this multidrug resistance corroborate this prediction. Numerous PCR-based assays (using the polymerase chain reaction to amplify DNA samples) have shown that although resistance genes are already present in vir-

tually any soil, these natural resistomes are likely being enriched by human activity. David Graham and colleagues at Newcastle University analyzed a panel of Dutch soils archived between 1940 and 2008 for both the presence and the abundance of a series of specific resistance gene types. They found a dramatic increase in levels of key beta-lactam, macrolide, and tetracycline resistance genes over the 70-year study period, closely matching the era of large-scale human antibiotic production.

The surveys of known resistance genes in the soil resistome are just the tip of the iceberg. Jo Handelsman, now at Yale University, and her colleagues pioneered the application of *culture-independent functional metagenomics*, a technique that provides the functional gene composition of environmental samples, to characterize soil resistomes from both human-affected and pristine environments. Using this approach, they identified a number of novel antibiotic resistance genes, some with never-before-seen mechanisms of resistance. Taken together, these studies indicated the soil resistome was diverse, ancient, and recently enriched by human activity. Direct support for the notion that the soil resistome long predated clinical use of antibiotics comes from recent work from Gerry Wright's group. In 2011, they reported on DNA sequencing of 30,000-year-old Beringian permafrost that uncovered evolutionary relatives of resistance genes against important modern antibiotics, including beta-lactams, tetracyclines, and vancomycin.

One might expect that this wealth of information on the breadth and depth of the soil resistome would confirm the key prediction of the Producer Hypothesis, that the same resistance genes identified in clinical pathogens would also be identified in soil bacteria, suggesting recent exchange between the clinical and soil resistomes. Surprisingly, such evidence was lacking until very recently. The overwhelming majority of soil resistome studies revealed only limited similarity to resistance genes found in pathogens.

To account for this unexpected result, we hypothesized that a key subset of soil bacteria—the notoriously multidrug-resistant soil Proteobacteria—may represent a conduit for recent exchange with pathogens. We reasoned that since the greatest current clinical burden for multidrug resistance comes from multidrug resistant proteobacterial pathogens, their closest cousins in the soil might show evidence for recent resistome exchanges.

We then set out to test this idea using culture-based selections to selectively enrich about 100 highly multidrug-resistant soil bacterial cultures, composed primarily of Proteobacteria. We profiled their resistomes using a novel approach that integrates culture-independent functional metagenomic selections, next-generation DNA sequencing, and optimized computational sequence assembly and annotation algorithms *(see sidebar on page 46)*. Through this method, named PAR-FuMS (for Parallel Annotation and Reassembly of Functional Metagenomic Selections), we uncovered nine different antibiotic resistance genes from diverse U.S. soils that were genetically identical to a number of globally distributed clinical pathogens, finally providing broad support for recent exchange between the resistomes of non-pathogenic soil bacteria and human pathogens *(see figure on page 48)*.

Despite recent key advances in our knowledge of soil resistomes, we are still in the infancy of exploring this incredibly diverse ecosystem. Just 1 gram of soil is estimated to contain about 1 billion bacterial cells, and no current method gets even remotely close to sampling this diversity. Approximately half of the 60 predicted phyla or divisions of the bacterial world cannot be cultured in a lab, and even the ones that can are still not completely characterized. Fortunately, advances in both culture-based and culture-independent experimental methods, as well as improved computational tools for their analysis, enable scientists to scan and sequence the DNA of individual microbes without having to culture them first so they can fill in these holes.

The Resistome Inside You

Although the soil resistome is the most important reservoir of resistance from an evolutionary perspective, the microbes living in and on us—known as the *human commensal microbiota*—incorporate the resistome that is most accessible to human pathogens. These microbes outnumber human cells by 10-fold, and their collective genes (the *microbiome*) outnumber the genes in the human genome by over 100-fold. Specialized and relatively stable ecosystems of microbes inhabit various parts of the body, with the densest and most diverse community housed in the human intestine. Nearly every aspect of the human condition, in health

On the Trail of the Other Resistomes

In addition to those in soil and in the human gut, microbes from agriculture and aquaculture are believed to contribute substantially to the exchange of antibiotic resistance genes. In the United States and Europe, antibiotics are used four times as often in the food industry as in human medicine. Frank Aarestrup from the Technical University of Denmark has shown that this high consumption has led to high levels of antibiotic resistance in gut bacteria from farm animals. Furthermore, computational genomics analysis from Eric Alm's group at MIT reveals frequent transfer of resistance genes between microbes from farm animals and those in the human microbiota. Antibiotic usage is also growing at an alarming rate in aquaculture as more and more seafood is harvested this way. Felipe Cabello of New York Medical College and his colleagues have shown a concomitant increase in antibiotic resistance of bacteria associated with farmed fish.

Agriculture- and aquaculture-associated resistomes most likely act as intermediates between the human microbiota and human pathogens living in more pristine environments such as soil, sea, and fresh water. The transfer of antibiotic resistance genes probably goes both ways. Antibiotic-resistant bacteria from farm animals are spread through manure onto the soil, where they can disseminate resistance to soil bacteria. Conversely, farm animals are in frequent direct contact with soil, allowing genes to be transferred from the soil microbiota back to the animals. Although pinpointing specific sources of resistance is difficult, it is clear that heavy use of antibiotics in food production is fueling the evolution and spread of antibiotic resistance genes.

Arterra Picture Library / Alamy

More than 70 percent of all antibiotics sold in the United States are used to keep pigs, cows, chickens, turkeys, and other meat animals from getting sick and help them grow larger.

and disease, involves some interaction with the microbiota and microbiome.

Because one of the microbiota's main jobs is to keep pathogens from invading the gut, and the fact that any invading pathogen is more likely to interact with bacterial cells than human cells, the commensal resistome is often in a position to exchange resistance genes with nearby pathogens. The increasing levels of antibiotic exposure the microbiota has been subjected to since the beginning of the antibiotic era provides ample selection pressure to maintain a robust and diverse

resistome to participate in these exchanges.

The earliest insights into the human commensal resistome come from culture-based studies of these bacteria. Studies in the 1990s by the University of Illinois's Abigail Salyers on the normally mutualistic gut microbe *Bacteroides* established that tetracycline and macrolide resistance genes were being passed back and forth between the resistomes of pathogenic and nonpathogenic forms of the bacteria. Their analysis of archived *Bacteroides* samples also established that the levels of these
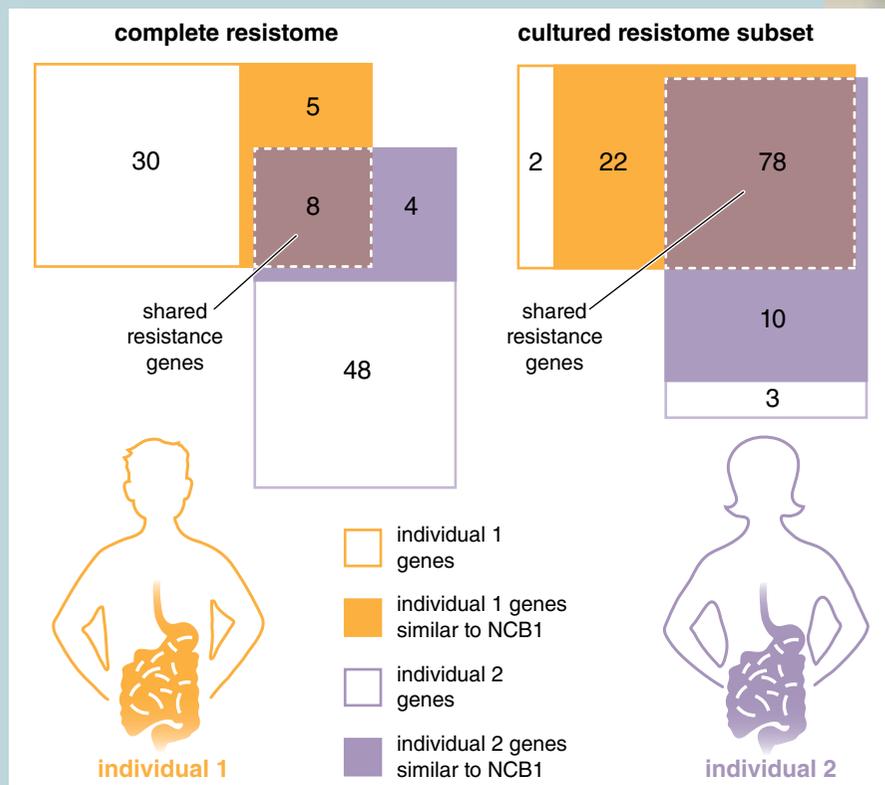
resistance genes had steadily increased over the course of two decades.

The implication from these studies—that increased antibiotic use was leading to higher levels of resistance—was supported by numerous other studies on other commensal microbes. Martin Blaser and colleagues at New York University found increases in macrolide resistance in commensal *Enterococci*, a bug associated with urinary tract infections and meningitis, as a collateral response to therapy targeting another bacterium, *Helicobacter pylori*, found in some ulcers. They also observed that this enriched resistance persisted for years after therapy ceased, challenging the conventional wisdom that antibiotic resistance encoded a fitness cost to the host bacterium and hence would evolve away or be outcompeted by antibiotic-susceptible strains in the absence of the antibiotic.

These results are not unique to the gut ecosystem; for example, persistently antibiotic resistant *Staphylococcus epidermis,* a major player in hospital-acquired infections, have been isolated from the nostrils of antibiotic-treated patients. Numerous studies in animal models and in humans show the com-

> ## The most resounding message that comes through from every new resistome study is that the pool of resistance genes, and the mechanisms of resisting antibiotics, available to bacteria are effectively limitless.

An FDA microbiologist holds tomatoes being tested for salmonella *(above).* Genetic sequences of all bacteria *(far left)* and all cultured bacteria *(middle left)* from two healthy individuals are compared with sequences cataloged by the National Center for Biotechnology Information. The cultured resistome overlaps greatly with the NCBI sequences and with resistance genes in common pathogens. (Adapted from M. O. A. Sommer et al., *Virulence* 2010 1:299.)

mensal resistome can readily participate in exchanges within and between ecosystems. Anette Hammerum and colleagues at Statens Serum Institut in Denmark recently demonstrated the transfer of vancomycin resistance genes between human and swine hosts.

Just Scratching the Surface

All of this evidence indicates that antibiotic treatment selects for genes conferring antibiotic resistance, that these increases in resistance can persist for many years, and that these resistance genes can be exchanged within the commensal microbiota as well as with foreign microbes. As with the soil, we and others in our field have also begun to appreciate that this portrait of the human commensal resistome is a vast underestimate due to an over-reliance on culture-based methods.

In 2009 we reported on the first application of culture-independent functional metagenomic selections to study the commensal gut resistomes of two healthy, unrelated individuals, who had been antibiotic therapy free for at least one year. We characterized the resistomes of both a subset of bacteria cultured from the subjects' fecal samples (the cultured resistome) as well as that of the entire uncultured bacteria (the complete resistome) from the same samples (Figure 6). We found the cultured resistome largely consisted of genes found in public sequence databases, and most of these genes were identical to resistance genes found in common pathogens.

This result confirmed that the resistome of cultured bacteria from the human gut have been in recent exchange with pathogenic microbes. In stark contrast, the majority of genes we identified in the complete resistomes from these same fecal samples were novel, meaning they had little similarity to any genes in public sequence databases. Note that every one of these novel genes enabled the model pathogen *E. coli* to resist clinically relevant antibiotics, highlighting that this uncharacterized genetic reservoir is fully functional and must be considered when evaluating resistome exchanges.

A couple of recent microbial censuses indicate we have just scratched the surface; deeper interrogation of the resistomes of many more individuals is required to begin to approach a comprehensive view of the human commensal resistome. Earlier this year,

research groups led by Peer Bork at the European Molecular Biology Laboratory in Heidelberg and Baoli Zhu at the Institute of Microbiology in Beijing reported on computationally predicted resistomes from sequencing data of human gut microbiomes from 207 and 162 individuals, respectively, representing multiple nationalities and cultural traditions. Collectively, these studies predict the existence of thousands of resistance genes across the analyzed commensal microbiome. The abundance of resistance genes correlates with the available data on the amount of human and agricultural antibiotic use, as well as with how long ago those antibiotics were introduced.

Although we are beginning to gain a glimpse into the genetics of such complex ecosystems, we will need improved culture-based and culture-independent techniques to better understand these reservoirs of antibiotic resistance. Long-term studies of various healthy and perturbed groups of human microbiota will enable us to transition from snapshots to movies of commensal resistomes. Where possible, matched samples are being collected from microbial habitats touched by human activity to map out the ecology and transmission dynamics of resistome exchange. Community-wide microbiome surveys, such as the Earth Microbiome Project and the Hospital Microbiome Project, are profiling the genetic sequences of specialized environmental microbiomes and providing a framework for mapping resistome interactions. Methods such as PARFuMS will enhance the ability of functional metagenomics to uncover novel resistance mechanisms.

The investigation of resistomes in diverse microbial habitats, including the many not discussed here, serves many purposes. There is clearly the basic science interest in understanding the principles that govern the ecology, evolution, and dynamics of antibiotic resistance. Such knowledge will also assist the critical goal of slowing down the spread of multidrug resistance in the clinic and extending the therapeutic lives of antibiotics. But no amount of resistome characterization will end the fight against infectious diseases.

What is most desperately needed are new antibiotics, and as many of them as possible. The most resounding message that comes through from

every new resistome study is that the pool of resistance genes, and the mechanisms of resisting antibiotics, available to bacteria are effectively limitless. To stay ahead of the game we must take a multipronged approach, looking for new ways to keep pathogens in check, while also searching for even newer antipathogen strategies. Even when therapies appear to be highly effective during their initial deployment, it is only a matter of time before pathogens tap into the enormous resistomes of their many neighbors and once again thwart our very best drugs.

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