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ECOLOGICAL AND CLINICAL CONSEQUENCES OF ANTIBIOTIC SUBSISTENCE BY ENVIRONMENTAL MICROBES

GAUTAM DANTAS[1] AND MORTEN O. A. SOMMER[2]

[1]*Department of Pathology and Immunology, Center for Genome Sciences and Systems Biology, Washington University, St. Louis, Missouri*
[2]*Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark*

4.1 INTRODUCTION

Increasing multidrug resistance in clinical pathogens and declining rates of development of new antimicrobials is precipitating a worsening global health crisis (Fischbach and Walsh, 2009). Antibiotic resistance determinants encoded on mobilizable elements can readily transfer between diverse bacteria, allowing the accumulation and dissemination of resistance genes into a variety of interacting microbial communities (D'Costa et al., 2007; Davies, 1994; Davies and Davies, 2010; Wright, 2007). The genetic and biochemical mechanisms that govern the evolution and dissemination of drug resistance can be engineered into or be naturally acquired by many microbial pathogens, effectively annulling our primary chemotherapeutics against these disease-causing agents. For instance, a multidrug-resistant strain of the plague bacterium *Yersinia pestis* was recently isolated that had acquired a mobile genetic element that conferred resistance to six different drugs including tetracycline and chloramphenicol, the two first-line drugs against this pathogen (Pan et al., 2008). Consequently, there is an increasing interest in identifying and characterizing microbes from communities that may be accessible reservoirs of antibiotic resistance machinery (Allen et al., 2010; Wright, 2007).

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4.2 ENVIRONMENTAL ORIGINS OF RESISTANCE: THE PRODUCER HYPOTHESIS

Investigations into reservoirs of resistance are partly inspired by the ecological question of where antibiotic resistance originated or evolved—essentially, if pathogens are the recipient, who are the donors, both currently and over longer evolutionary time scales? Most antibiotics used in the clinic today are structural derivatives of compounds isolated from natural sources. Since antibiotics in nature represent one of the oldest forms of potential biochemical warfare, environmental bacteria would be expected to possess intrinsic mechanisms to detect, resist, and neutralize these threats to their persistence and, hence, represent a reservoir of accessible resistance machinery. Indeed, an intriguing hypothesis, put forward by Julian Davies and colleagues in the late 1970s, postulates that antibiotic-producing soil bacteria are one such primary "originator" of antimicrobial resistance genes (Benvenis and Davies, 1973). The theory intuits that the producers must minimally survive the toxicity of the antimicrobial compounds they produce, and, hence, the genetic antecedents of this self-immunity are de facto resistance genes. Additionally, enzymes involved in the microbial biosynthesis of these chemicals, capable of many specific chemical modifications of these compounds, might be repurposed to catalyze transformations in alternate genomic contexts that modulate or ablate their antimicrobial activity. Interestingly (and unfortunately), antibiotics are used in large quantities at subtherapeutic levels in animal agriculture, but the dosing is usually as crude lysates of the producer organisms, which in addition to the antimicrobial compound also contain genomic material from the producer (Webb and Davies, 1993). Hence, resistance genes natively contained in the producers would be available for lateral gene transfer in animal pathogens. Davies and colleagues' support for the "producer hypothesis" came from biochemical studies showing the activity of aminoglycoside resistance enzymes encoded by producers was identical to those found in pathogens (Benvenis and Davies, 1973). In 2006, Gerry Wright and colleagues provided further compelling evidence for producers being a substantial reservoir of antibiotic resistance genes (which they collectively termed the "resistome"), by phenotypically profiling the resistance of $\sim$400 soil *Streptomycete* isolates against 21 diverse antibiotics, which spanned all bacterial targets and most antibiotic chemical classes. Despite no specific selection for resistance during isolation, they found these microbes were resistant to 7–8 antimicrobials on average, and one microbe in the set was resistant to as many as 15 compounds (D'Costa et al., 2006, 2007). The conundrum with the producer hypothesis, however, comes from the finding that resistance gene sequences identified in the producers are phylogenetically distantly related to those identified in human commensals or pathogens (Marshall et al., 1998). Indeed, it appears these sets of producer versus pathogen resistance genes may have evolutionarily diverged before the anthropogenic antibiotic era (Aminov and Mackie, 2007).

4.3 RESISTOME OF OTHER SOIL BACTERIA: RESPONSE TO THE PRODUCERS?

Antibiotic-producing microbes in the soil may still play a direct role with selection of resistance genes in environmental microbes that may serve as an accessible resistance reservoir for pathogens. The diverse microbial communities that inhabit the soil must

interact with the producers, and many of these bacteria likely respond by developing or acquiring resistance genes to enable coinhabitation of the ecological niches of the producers. Both culture-dependent and culture-independent investigations of soil (and aquatic) communities by numerous researchers have indeed revealed substantial reservoirs of diverse resistance genes (Aarestrup et al., 2001; Cavaco et al., 2008; Demanèche et al., 2008; Enne et al., 2008; Leng et al., 1997). These include areas with high anthropogenic contact, such as agricultural soils, as well as more pristine settings, such as on secluded islands in Alaska (Allen et al., 2009, 2010; Donato et al., 2010; Riesenfeld et al., 2004). From an ecological perspective, while resistance genes work to counteract antimicrobial activity, the biochemical processing of these compounds in the environment is unlikely to end here. Antimicrobials that have been cleaved or modified by a resistance gene could serve distinct cellular roles, such as the ability of anhydrotetracycline (a tetracycline precursor) to activate tetracycline efflux pumps (McCormick et al., 1968; Palmer et al., 2010), or could simply serve as substrates for microbial metabolism. In cases where a metabolic pathway is specific to degradation of the antimicrobial structure, it is even possible that multiple enzymes in that pathway could each serve de facto antimicrobial resistance roles in a new genomic context.

4.4 EARLY REPORTS OF ANTIBIOTIC CATABOLISM BY SOIL BACTERIA

A handful of reports, dating back to the early 1960s, have described soil isolates with the capacity to utilize a few antibiotics as the sole source of carbon and, in some cases, also the sole source of nitrogen. In 1961, Abd-El-Malek et al. reported on a *Streptomyces* sp. capable of utilizing chloramphenicol as a sole carbon and nitrogen source (Abd-El-Malek et al., 1961). Chloramphenicol solutions were percolated in multiple doses through sieved garden soil to enable in situ enrichment of subsisters. The enriched soil was plated on minimal media containing chloramphenicol and ammonium nitrate as the carbon and nitrogen sources, respectively, yielding colonies of a single morphotype after 15 days of incubation at 30°C. Further morphological and metabolic analysis suggested the bacterium was a *Streptomyces* species. The isolate was confirmed to also utilize chloramphenicol as the sole nitrogen source by serial subculture into chloramphenicol minimal media lacking nitrogen. Repeated subculturing was found to decrease the inactivation time and improve growth. The isolate was able to completely inactivate chloramphenicol within 13 days in media containing the compound at concentrations spanning 100 to 600 mg/L, but no growth was detected in 1000 mg/L chloramphenicol after 30 days postinoculation. Also in 1961, Kameda et al. reported on isolation of 8 strains from 4 different Japanese soils with the capacity of utilizing benzylpenicillin as the sole source of carbon (Kameda et al., 1961). The soil isolates (phylotype not described) could use both the parent compound and phenylacetatic acid as the sole carbon source, implicating penicillin acylase as a key step in catabolism. Scarce to no growth was observed with medium lacking ammonium chloride, suggesting that benzylpenicillin could not serve as a sole source of nitrogen. Similarly, 6-aminopenicillanic acid, the other moiety besides phenylacetate formed from penicillin acylase activity, was unable to support substantial growth of the isolates. Interestingly, the isolates seemed to be rod shaped in the phenylacetate medium but formed filaments in the benzylpenicillin medium.

While β-lactamase activity was not directly assayed, it is unlikely to have played a role in benzylpenicillin catabolism by these isolates since the β-lactam ring is contained within the 6-aminopenicillanic acid moiety. Benzylpenicillin degradation was detected after as little as 3 days after inoculation of the isolates. In 1977, Johnsen reported on isolation of a *Pseudomonas fluorescens* strain from the sediment surface of a German lake that could also utilize benzylpenicillin as the sole source of carbon (Johnsen, 1977). This isolate differed from the Kameda strain in a number of ways. It was able to use benzylpenicillin as a sole source of both carbon and nitrogen. It did not possess penicillin acylase activity but did show β-lactamase activity. The degradation pathway proposed by Johnsen involves destruction of the β-lactam ring, to produce benzylpenicilloic acid, followed by decarboxylation to benzylpenilloic acid, which may provide the needed carbon for growth. Interestingly, growth on pure benzylpenicilloic acid by the strain was retarded to 25% of the growth on benzylpenicillin, which might indicate that efficient coexpression of multiple genes required for benzylpenicilloic acid catabolism might require the initial expression of the β-lactamase gene, induced to detoxify the antibiotic activity of benzylpenicillin. In 1979, Beckman and Lessie reported on a number of *Pseudomonas cepacia* (*P. cepacia*), *P. marginata*, and *P. caryophylli* strains with a similar ability to utilize benzylpenicillin as a sole source of carbon, with concomitant expression of high levels of β-lactamase activity (Beckman and Lessie, 1979). These strains exhibited resistance to benzylpenicillin, and its derivatives ampicillin, carbenicillin, and cephalosporin C, but were unable to utilize these other compounds as growth substrates, indicating that cleavage of the β-lactam ring alone was not sufficient for catabolism. Furthermore, other *Pseudomonas* species tested, including strains of the genetically related *P. picketii*, as well as strains of the less related *P. aeruginosa*, *P. putida*, and *P. fluorescens*, were unable to utilize benzylpenicillin as the sole carbon source despite having nearly equal levels of β-lactamase activity and resistance profiles to the various β-lactams. Intriguingly, *P. cepacia* mutants auxotrophic for lysine were also unable to utilize or resist benzylpenicillin, and the utilization phenotype was not rescued with genetic complementation with a β-lactamase containing plasmid from *P. aeruginosa*. Accordingly, the authors speculated that *P. cepacia* genes involved with lysine biosynthesis and for benzylpenicillin resistance and catabolism may reside in an extrachromosomal gene cluster, though attempts to isolate such plasmids were unsuccessful. In 1981, Johnsen reported on isolation of another *Pseudomonas* sp. from activated sludge with the ability to utilize benzylpenicillin as the sole source of carbon (Johnsen, 1981). Unlike the previously reported benzylpencillin-utilizing isolates, this strain was found to express both penicillin acylase and β-lactamase activities. However, much like the Kameda strain, the phenylacetic acid moiety produced by acylase activity served as the carbon source, and the β-lactamase activity in this case may be unrelated to the catabolism phenotype. These early studies demonstrated that soil microbes with antibiotic subsistence phenotypes for a couple of natural antibiotics could be isolated from geographically diverse locations, through expression of diverse catabolic mechanisms.

4.5 THE ANTIBIOTIC SUBSISTOME: WHO AND HOW MUCH?

We recently isolated a few hundred soil strains capable of utilizing 1 of 18 antibiotics as their sole source of carbon (Dantas et al., 2008). Soils were collected from 11 sites

in the United States, with varying degrees of anthropogenic contact, inoculated into a minimal medium containing an antibiotic as the carbon source, and allowed to grow at room temperature over 7 days. Cultures were serially passaged three times, followed by clonal selection on antibiotic–agar plates, and a final passage in liquid antibiotic minimal media. The 18 antibiotics were chosen to target all major bacterial targets, 8 distinct chemical classes, even representations of natural, semisynthetic, and synthetic origins, and a range of ages of clinical deployment. The final growth assays yielded microbes subsisting on antibiotics in 85% of the 11 soil by 18 antibiotic conditional grid (Fig. 4.1), with no statistically significant differences in the ability to culture these microbes from the various soil sources and with the various antibiotics. We refer to the aggregate of all mechanisms to deploy antibiotic as energy source as the antibiotic subsistome. We used 16S ribosomal ribonucleic

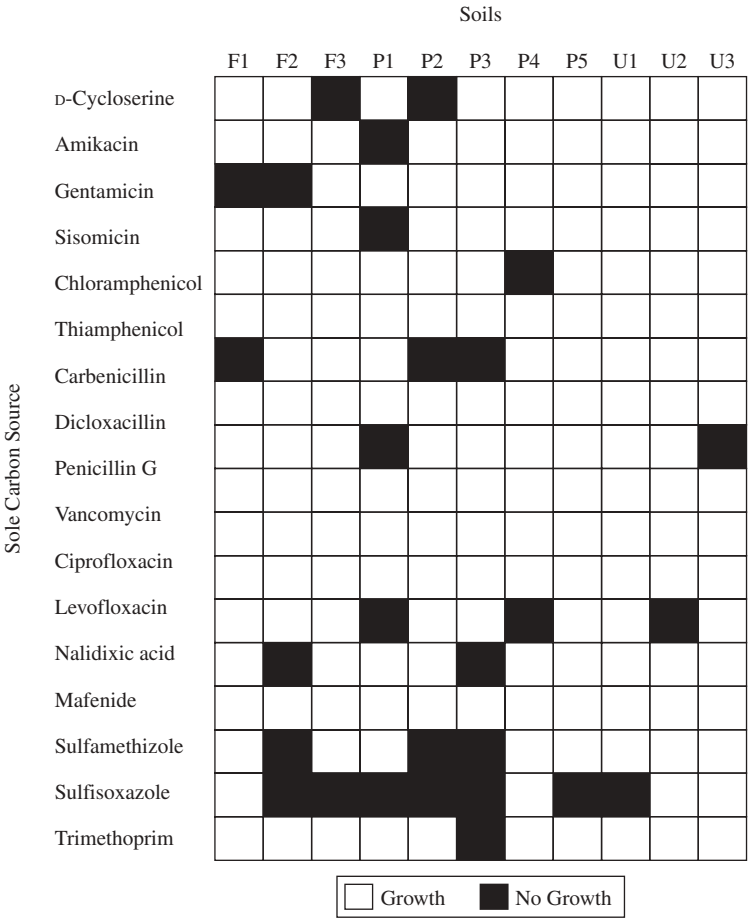

FIGURE 4.1 Clonal bacterial isolates subsisting on antibiotics. Heat map illustrating growth results from all combinations of 11 soils by 18 antibiotics, where white squares represent successful isolation of bacteria from a given soil that are able to utilize that antibiotic as sole carbon source at 1 g/L. Soil samples labeled F1–3 are farm soils and U1–3 are urban soils. Soil samples P1–5 are pristine soils, collected from non urban areas with minimal human exposure over the last 100 years.

acid (rRNA) gene sequencing to determine the phlyogenies of the isolates and found the set included 3 of the 4 major phyla of the soil—dominated by the Proteobacteria (87%), and including isolates from the Actinobacteria (7%) and the Bacteroidetes (6%) (Fig. 4.2). The missing major soil phylum was the Acidobacteria (Janssen, 2006). Interestingly, the 3 observed phyla are also 3 of the 4 major phyla of the human gut microflora, with the Firmicutes not represented (Eckburg et al., 2005; Qin et al., 2010). A set of 75 isolates were then profiled for their resistance to the same 18 antibiotics at both 20 mg/L [approximate minimal inhibitory concentration (MIC) for these antibiotics] and 1 g/L (concentrations used in the subsistence assays). We found these isolates to be extensively multidrug resistant at both sets of concentrations. At the lower concentration, nearly 60% of the set were pan-resistant to the

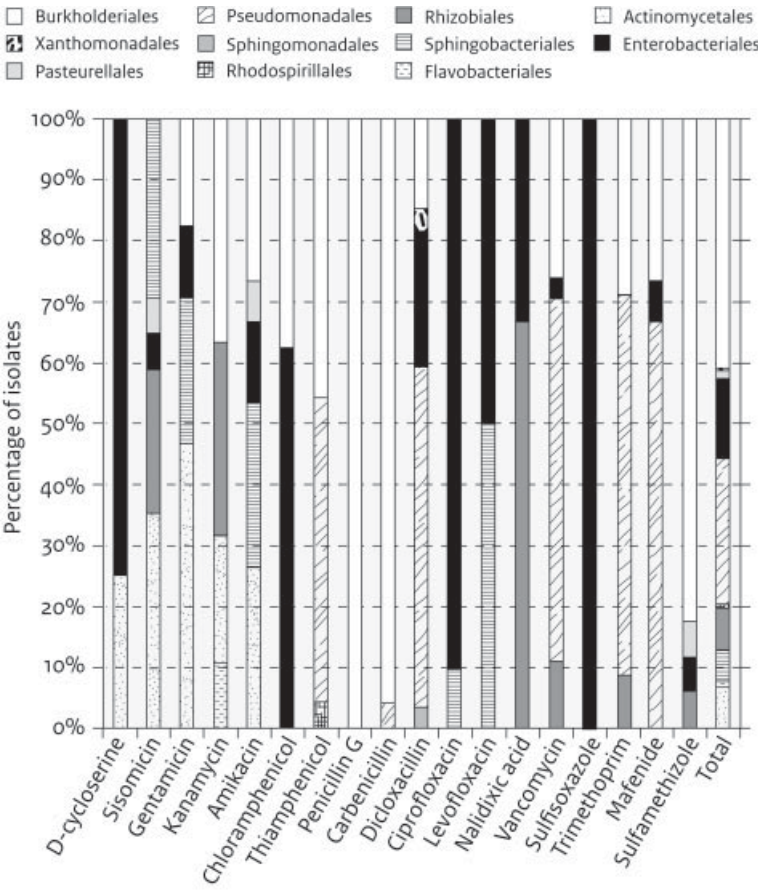

FIGURE 4.2 Phylogenetic distribution of bacterial isolates subsisting on antibiotics. 16S ribosomal DNA (rDNA) was sequenced from antibiotic catabolizing clonal isolates using universal bacterial rDNA primers. High-quality, nonchimeric sequences were classified using Greengenes (DeSantis et al., 2006), with consensus annotations from RDP (Cole et al., 2007) and NCBI taxonomies (Wheeler et al., 2000). Histogram displays the fraction of bacteria subsisting on different antibiotics that belong to particular bacterial orders as determined from the consensus classifications.

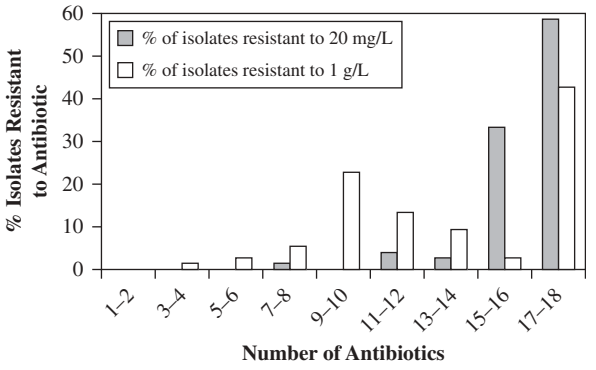

FIGURE 4.3 Antibiotic resistance profiling of 75 representative clonal isolates capable of subsisting on antibiotics. The histogram displays percentage of clonal isolates resistant to each of the 18 antibiotics at 20 mg/L (black bars) and 1 g/L (white bars).

18 antibiotics. At the higher concentration, nearly 40% of the total set were pan-resistant (Fig. 4.3).

Analysis of the degradation products in media of benzylpenicillin-subsisting bacteria using liquid chromatography and mass spectrometry was consistent with a pathway involving first the hydrolysis of the β-lactam ring, followed by a decarboxylation of benzylpenicilloic acid to yield benzylpenilloic acid, similar to the pathway proposed for *P. fluorescens* degradation of penicillin by Johnsen (1977). The proposed catabolic pathway for benzylpenicillin involves the action of a β-lactamase as a first step, providing a potential link between a very common resistance gene seen in pathogenic isolates and antibiotic subsistence by these soil bacteria (Davies and Davies, 2010).

4.6 ANTIBIOTIC SUBSISTENCE AS A SCAVENGER PHENOTYPE

We and others have demonstrated that bacteria subsisting on both synthetic and natural product antibiotics can be identified from a variety of sources. Since many of the antibiotics that can sustain growth of bacteria are produced by soil-dwelling bacteria and can be found at up to milligram per gram quantities in the soil (Thiele-Bruhn, 2003), it is not surprising that certain bacteria have evolved the capacity to subsist on these naturally occurring antibiotics as they represent a carbon source that can be utilized. The fact that organic molecules present in nature are typically biotically recycled could explain the catabolism of natural product antibiotics; however, we see almost equal microbial catabolism of human-made synthetic antibiotics such as the sulfonamides (Fig. 4.1). One explanation for the widespread catabolism of synthetic antibiotics may relate to their organic substructures, which are found in nature. Metabolic mechanisms exist for processing those substructures and may allow for the utilization of the parent synthetic antibiotic molecule. However, it should be noted that more than half of the bacterial isolates we identified belong to the orders Burkholderiales and Pseudomonadales (Fig. 4.2). These bacterial orders often have large genomes 6–10 Mb, which has been suggested

to be positively correlated to their metabolic diversity and multiple antibiotic resistance (Projan, 2007). Indeed *Burkholderiales* and *Pseudomonadales* can be thought of as scavengers, capable of utilizing a large variety of single carbon sources as food.

We hypothesize that some enzymatic antibiotic resistance mechanisms may have originated from such metabolically diverse scavenging organisms, which are capable of subsisting on a variety of naturally occurring molecular substructures. As these organisms were confronted with antibiotics in their habitat, they were likely able to repurpose those parts of their diverse catabolic arsenal that recognized the natural substructures, to allow them to utilize this new carbon source. This catabolic capability would give the scavengers a substantial selective advantage over other microbes, since they would be both tolerant to these microbial toxins while simultaneously cornering a specialized catabolic environmental niche. Lateral transfer of even a single gene involved in antibiotic enzymatic processing (and consequent inactivation) to other organisms could allow for the conferral of antibiotic resistance on the recipient organism disseminating the antibiotic resistance genes from the bacteria subsisting on antibiotics.

4.7 ECOLOGICAL CONSEQUENCES OF THE ANTIBIOTIC SUBSISTOME

While we have observed bacterial antibiotic subsistence in a variety of environmental samples, we have yet to determine whether microbes with this phenotype have evolved specifically for this purpose or if this is simply a by-product of a diverse and plastic metabolism. Nevertheless, having this machinery distributed in the environment has some interesting ecological consequences. In the case where these toxins can accumulate to inhibitory concentrations, perhaps the most obvious consequence is detoxification of the microenvironment inhabited by these bacteria, which could additionally benefit other susceptible microbes sharing that microenvironment. Given the complex and poorly understood interactions between the complex communities in the soil, such altruistic actions by one bacterium might enable survival of otherwise susceptible bacterial communities upon which the antibiotic-subsisting bacterium relies. In this way, antibiotic subsistence may represent an alternative population-based resistance mechanism, as was recently demonstrated in lab evolution experiments with *Escherichia. coli* by Collins and colleagues (Lee et al., 2010). Another consequence of antibiotic subsistence may be signal degradation or interception, as antibiotics at subinhibitory concentrations have been hypothesized to serve as signaling molecules (Davies et al., 2006; Yim et al., 2007). Efficient signaling requires signal degradation on appropriate time scales, and bacteria subsisting on antibiotics may serve such roles through interactions with producers. Alternatively, subsisters may also simply hijack or "eavesdrop" on signaling between producers by utilizing their signaling molecules. Finally, the ability to subsist on compounds that are toxic to most other microbes has the consequence of reduced competition for this specialized food niche. Since the above-described scenarios are by no means mutually exclusive, it is quite likely that all of these ecological roles may be at play in different environmental niches and community interactions.

4.8 INVESTIGATING CONNECTIONS BETWEEN SUBSISTOMES AND RESISTOMES

Thus far, the genes involved in antibiotic subsistence for any compound have not been identified; however, the identity and relationships of these genes to common resistance genes will illuminate the relationship between soil subsistence and antibiotic resistance. It should be noted that over a quarter of the isolates capable of subsisting on antibiotics have human pathogenic isolates as their closest relative based on 16S profiling (Dantas et al., 2008). Considering that lateral gene transfer occurs more readily between closely related species, the bacteria subsisting on antibiotics could indeed serve as an accessible reservoir of antibiotic resistance genes to human pathogenic isolates.

4.9 METAGENOMIC FUNCTIONAL SELECTIONS FOR DISCOVERING GENES ENABLING ANTIBIOTIC SUBSISTENCE AND RESISTANCE

A promising approach for discovery of antibiotic degradation genes is *metagenomic functional selections*, wherein genomic or metagenomic DNA (deoxyribonucleic acid) from a microbial strain or community can be shotgun cloned in an expression system in a bacterial host lacking the phenotype of interest (in this case, antibiotic catabolism), followed by subjecting the metagenomic transformants to a survival selection (Sommer et al., 2009, 2010). In the case of antibiotic subsistence, only genetic fragments enabling utilization of the antibiotic would survive on media containing the antibiotic as a sole carbon source. We use the phrase metagenomic functional selections to specifically refer to the idea of subjecting metagenomic DNA to an experimental functional assay, generally through shotgun expression in a heterologous host, in an attempt to distinguish this approach from broader definitions of *functional metagenomics*, which include sophisticated computational approaches for annotation of functions in shotgun metagenomic sequence data, but without direct experimental validation (Dinsdale et al., 2008). Metagenomic functional selections have been successfully applied to identify a variety of enzymatic functions from cultured and uncultured microbes, including genes and pathways for degradation of or resistance toward numerous xenobiotics [recently reviewed in Uchiyama and Miyazaki (2009)]. Since we postulate that antibiotic catabolism in at least some of our isolates involves the initial expression of resistance genes, we highlight below the power of metagenomic functional selections by describing our application of this method to investigate the antibiotic resistome of human intestinal microflora (Sommer et al., 2009).

It is quite possible that environmental resistomes may use the commensal human microflora as a conduit for eventual dissemination of resistance genes to pathogens, especially considering the enrichment of resistomes in the microflora of food animals through extensive use of antimicrobials in agriculture (Aarestrup et al., 2001). The microbes that inhabit the human body are likely the most directly accessible reservoir of resistance genes for pathogens, due to their high likelihood of genetic interaction during disease progression. Accordingly, we recently investigated the resistome harbored by the intestinal microflora of two healthy adult humans who had been

free of antibiotic therapy for over one year prior to sampling (Sommer et al., 2009). We applied a metagenomic functional selection approach to capture and sequence hundreds of antibiotic resistance genes from genomic DNA of aerobic bacteria cultured from the individuals' fecal samples, as well as from direct culture-independent metagenomic sampling of the same samples (Sommer et al., 2009). The genes from cultured isolates were closely related to genes previously described, including many that were identical to resistance genes described in human pathogens. These include the CTX-M-15 β-lactamase, recently identified in epidemic plasmids in disease isolates from around the globe. This work confirmed that resistance gene exchange between commensals and pathogens has likely occurred in our recent past (Salyers et al., 2004). In stark contrast, the genes we uncovered with culture-independent sampling were largely novel, with less than 65% average nucleotide identity to any genes in the National Center for Biotechnology Information (NCBI) nonredundant gene database. Phylogenetic analysis of these uncultured genes confirmed their genomic sources as the Bacteroidetes and Firmicutes, the dominant members of the human microflora, which are genetically distinct from the proteobacterial host (*E. coli*) used from functional selection. These results highlighted the severe previous undersampling of the resistome of the human microbiota due to reliance on culturing. However, they also demonstrated the utility of this method for capturing fully functional genes for xenobiotic resistance from phylogenetically diverse sources. We are currently applying these methods to interrogate the genetic antecedents of antibiotic utilization and resistance in the antibiotic subsisters.

4.10 ANTIBIOTIC SUBSISTENCE BY PATHOGENIC BACTERIA

Antibiotic subsistence on a variety of distinct antibiotics was recently identified in several hundred isolates of the pathogenic proteobacterium *Salmonella* (Barnhill et al., 2010). These strains included multiresistant and antibiotic-sensitive isolates derived from various food animals, in clinical, nonclinical, and food samples. Almost a third of the isolates were *Salmonella enterica* subspecies *enterica* serovar Typhimurium, which harbors the Salmonella genomic island 1 (SGI1) integron encoding multidrug resistance. The authors speculated a connection between the subsistence phenotype and the SGI1 integron, since more than half the isolates harboring the integron exhibited an antibiotic subsistence phenotype. Of the 572 isolates profiled, nearly a quarter subsisted on at least one antibiotic, while about 7% could utilize more than one antibiotic. Of the 12 antibiotics tested, only tetracycline was unable to support subsistence for any of the isolates profiled. Intriguingly, the authors found several cases in which it appeared that the ability to subsist on an antibiotic was unrelated to the mechanism of resistance. For instance, most isolates capable of subsisting on sulfisoxazole contained the *sul1* gene, which encodes a nonsusceptible version of dihydropteroate synthase. This resistance gene confers high-level resistance to sulfonamide antibiotics and would not be expected to be involved in the catabolism of sulfisoxazole. This work represents the first ever demonstration of antibiotic subsistence in a human pathogen, and highlights the importance of this phenotype, initially identified in the environment, in clinical settings. The fact that *Salmonella* is primarily a food-borne pathogen is particularly troubling when one considers that the amounts of antibiotics used in food animals, largely for nontherapeutic reasons,

outweigh human therapeutic use by manyfold (Silbergeld et al., 2008). Hence, mechanistic elucidation of antibiotic catabolism by these *Salmonella* isolates and their relationship to resistance requires urgent attention. Since *Salmonella* is closely related to *E. coli,* metagenomic functional selections in *E. coli* should be ideally suited to identify the catabolic pathways from these isolates, which would enable a more clear view of how this phenotype has been acquired by pathogenic *Salmonella* isolates.

4.11 CONCLUDING REMARKS

Investigations into microbial antibiotic subsistence and degradation are still clearly in their infancy, with substantial work to be done on the mechanisms that enable these phenotypes, and the potential evolutionary advantages these may confer on the microbes that harbor them. The role these phenotypes might play in microbial community ecology remain a complete mystery, both in environments like the soil where antibiotic producers exist in appreciable numbers, as well as within animal and human microflora, where there is a heightened potential for genetic exchange with pathogens. New systemswide molecular methods are allowing researchers to systematically investigate antibiotic resistance as a property exchanged within and between diverse communities. Application of these methods to the subsistome will allow us to answer the fascinating ecological and clinical questions that the discovery of these antibiotic subsistence phenotypes have posed.

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