with ORP), or that south polarity is characteristic of a particular species that occurs at a specific ORP.

The coexistence of magnetotactic bacteria with north and south polarity in the same chemical environment contradicts the current accepted model of magnetotaxis, which states that all magnetotactic bacteria in the Northern Hemisphere swim north (downward in situ) when exposed to oxidized conditions to reach their preferred microaerobic or anaerobic habitat. We observed that barbells with south polarity and cocci with north polarity coexist in microaerobic conditions in the water column. On the basis of this distributional pattern, south polarity is clearly not used to direct the barbell upward in the water column toward higher oxygen levels. The current model does not, therefore, provide any explanation that can account for the existence of south polarity.

This model implicitly assumes that polarity observed in the laboratory under atmospheric oxygen levels is equivalent to polarity in situ. Our results suggest that this assumption might be incorrect. Although the benefit of north polarity in situ is clear for microaerophilic magnetotactic bacteria, south polarity would have a clearly deleterious effect by directing the bacteria away from their preferred chemical environment. There are reasons to believe the behavior of magneotactic bacteria in situ could differ from behavior in the laboratory. Magnetotactic bacteria at the chemocline of a stratified water column rarely, if ever, experience atmospheric oxygen levels like those in the standard laboratory assay for polarity. They also experience chemical gradients (particularly of iron and sulfur species) not present in a drop of water exposed to air in the laboratory assay. It is also possible that bacteria with north and south polarity possess different chemo- or redox-sensors that have opposite responses to chemical concentrations out of the range they typically experience. On the basis of these results, new models are clearly needed to explain the adaptive significance of magnetotaxis by magnetotactic bacteria in the environment.

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Sampling the Antibiotic Resistome

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Microbial resistance to antibiotics currently spans all known classes of natural and synthetic compounds. It has not only hindered our treatment of infections but also dramatically reshaped drug discovery, yet its origins have not been systematically studied. Soil-dwelling bacteria produce and encounter a myriad of antibiotics, evolving corresponding sensing and evading strategies. They are a reservoir of resistance determinants that can be mobilized into the microbial community. Study of this reservoir could provide an early warning system for future clinically relevant antibiotic resistance mechanisms.

Most clinically relevant antibiotics originate from soil-dwelling actinomycetes (*1*). Antibiotic producers harbor resistance elements for self-protection that are often clustered in antibiotic biosynthetic operons (*2*, *3*). Genes orthologous to these have been identified on mobile genetic elements in resistant pathogens in clinical settings. It has been suggested that aminoglycoside-modifying kinases (*4*) and the alternate peptidoglycan biosynthetic machinery that confers resistance to vancomycin (*5*) probably originated in soil-dwelling antibiotic producers.

The presence of antibiotics in the environment has promoted the acquisition or independent evolution of highly specific resistance elements in the absence of innate antibiotic production [such as vancomycin resistance in *Streptomyces coelicolor*, *Paenibacillus*, and *Rhodococcus* (*6*, *7*)]. The soil could thus serve as an underrecognized reservoir for resistance that has already emerged or has the potential to emerge in clinically important bacteria. Consequently, an understanding of resistance determinants present in the soil—the soil resistome—will provide information not only about antibiotic resistance frequencies but also about new mechanisms that may emerge as clinical problems.

We isolated a morphologically diverse collection of spore-forming bacteria from soil samples originating from diverse locations (urban, agricultural, and forest). Strains that resembled actinomycetes both morphologically and microscopically were serially subcultured to apparent homogeneity. Amplification and sequencing of 16*S* ribosomal DNA from a subset of strains indicated that they belonged to the actinomycete genus *Streptomyces*, whose species synthesize over half of all known antibiotics (*1*). We constructed a library of 480 strains that was subsequently screened against 21 antibiotics, including natural products (such as vancomycin and erythromycin), their semisynthetic derivatives (such as minocycline and cephalexin), and completely synthetic molecules (such as ciprofloxacin and linezolid). The antibiotics encompassed all major bacterial targets (*8*) and included drugs

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that have been on the market for decades as well as several that have only recently been clinically approved (such as telithromycin and tigecycline).

The screen was conducted at high antibiotic concentrations (Fig. 1), and strains of interest were analyzed by determination of the minimal inhibitory concentration (MIC). A subset of isolates was characterized on the basis of mode of resistance in order to distinguish resistance arising from antibiotic alteration or modification from that arising from nondestructive mechanisms (such as efflux, altered target, or transport) (Table 1).

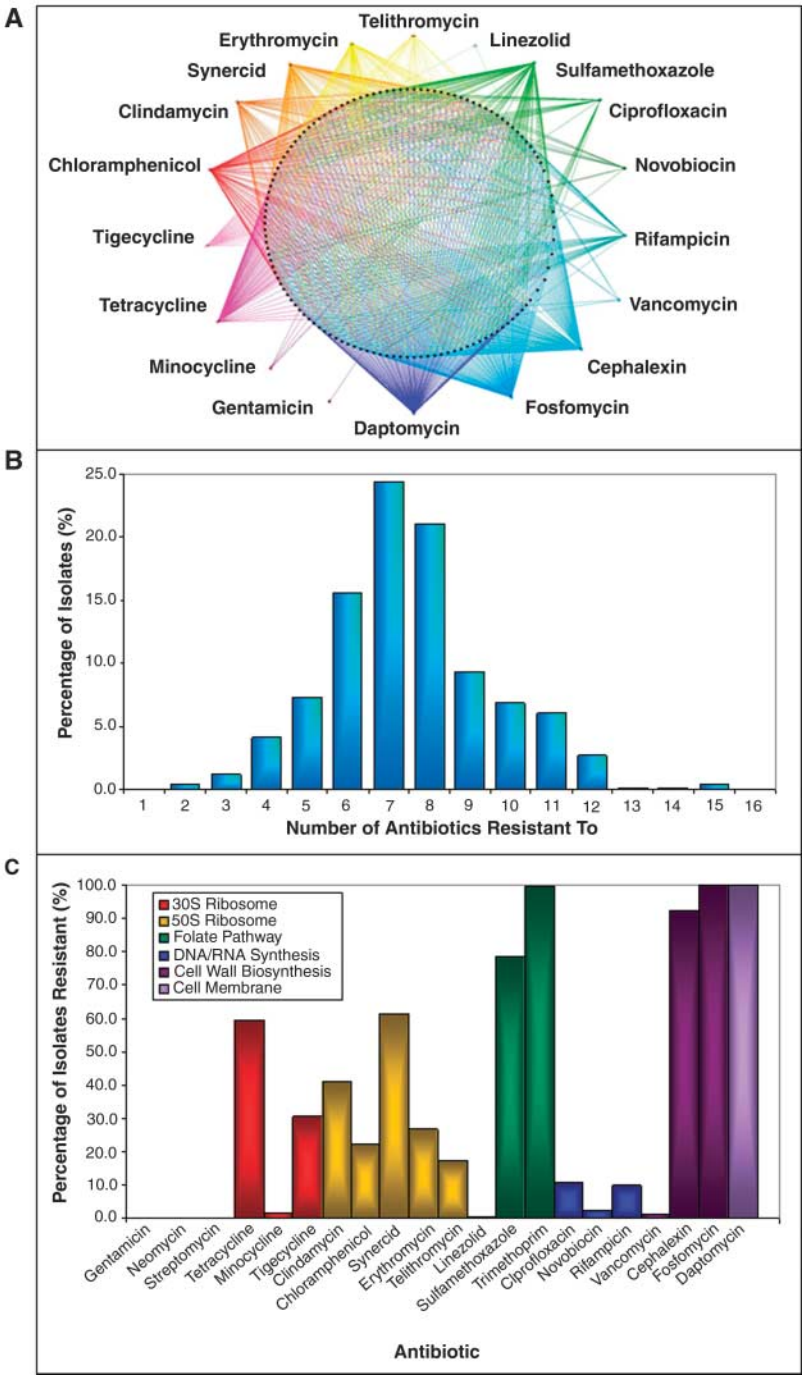

Fig. 1. Antibiotic resistance profiling of 480 soil-derived bacterial isolates. (**A**) Schematic diagram illustrating the phenotypic density and diversity of resistance profiles. The central circle of 191 black dots represents different resistance profiles, where a line connecting the profile to the antibiotic indicates resistance. (**B**) Resistance spectrum of soil isolates. Strains were individually screened from spores on solid *Streptomyces* isolation media (SIM) against 21 antibiotics at 20 μg of antibiotic per ml of medium (μg/ml). Resistance was defined as reproducible growth in the presence of antibiotic. (**C**) Resistance levels against each antibiotic of interest.

Without exception, every strain in the library was found to be multi-drug resistant to seven or eight antibiotics on average, with two strains being resistant to 15 of 21 drugs (Fig. 1B). Reproducible resistance to most of the antibiotics, regardless of origin, was observed, and almost 200 different resistance profiles were seen (Fig. 1, A and C), exemplifying the immense genetic and phenotypic diversity of the collection of bacteria.

Several antibiotics, including the synthetic dihydrofolate reductase (DHFR) inhibitor trimethoprim and the new lipopeptide daptomycin, were almost universally ineffective against the library. The genomes of *S. coelicolor* and *S. avermitilis* do not contain annotated DHFR genes, which is consistent with insensitivity to trimethoprim (*9*). However, extensive daptomycin resistance was not anticipated. Recently approved by the Food and Drug Administration (FDA), daptomycin is highly active against Gram-positive bacteria, including multi-drug–resistant pathogens (*10*, *11*). A member of a large antibiotic class commonly produced by actinomycetes, daptomycin is thought to act by insertion into the bacterial cell membrane in a Ca^{2+}-dependent manner (*12*, *13*).

Eighty percent of the resistant strains assayed inactivated daptomycin after 48 hours of cell growth, while the remaining strains retained active antibiotic in the culture media (Table 1). This finding is notable not only because it is only the second documented occurrence of daptomycin inactivation (*14*) but because of its unprecedented high frequency. Furthermore, it suggests that there are multiple mechanisms of daptomycin resistance in soil organisms.

We uncovered a wealth of inactivating enzymes produced by soil bacteria. Of the 11 antibiotics screened, bacterial isolates were detected that putatively metabolized 6 drugs (Table 1), including rifampicin and Synercid.

Rifampicin, a semisynthetic derivative of a natural *Amycolatopsis mediterranei* product, is central to the treatment of mycobacterial infections. Forty percent of resistant isolates were capable of inactivating the drug, which is intriguing because clinically, the most prevalent mechanism of rifampin resistance is through point mutations in the target: RNA polymerase's β subunit.

Synercid, which was FDA-approved in 1999 for the treatment of drug-resistant bacteremia, is a combination of two semisynthetic derivatives of *Streptomyces* metabolites, each with a distinct mode of action. Eighteen percent of resistant isolates tested were able to detoxify both antibiotics. These findings collectively reinforce the importance of enzymatic antibiotic inactivation as a means of resistance (*4*).

The screen yielded five strains that were highly resistant to the glycopeptide vancomycin (MICs of 128 to 256 μg/ml). Resistance in both clinically significant and glycopeptide-producing

bacteria is the result of the biosynthesis of an altered peptidoglycan terminating in D-alanine-D-lactate rather than D-alanine-D-alanine, resulting in a poor binding affinity to vancomycin. This mechanism is encoded by a cluster of three genes, *vanH-vanA-vanX*, which can be readily identified by polymerase chain reaction analysis in resistant *Streptomyces* (*5*). Using this strategy, the cluster was amplified in 80% of resistant strains (fig. S1). The outlying strain AA#4 appeared to be resistant by a nondestructive mechanism and displayed a distinct glycopeptide resistance profile. Although the *vanHAX* strains demonstrated vancomycin resistance (MICs of 128 to 256 μg/ml) but sensitivity to the lipoglycopeptide teicoplanin (MICs of 1 to 4 μg/ml), AA#4 was resistant to both (MICs of vancomycin and teicoplanin of 256 μg/ml).

Table 1. Antibiotic inactivation screen of the soil library. Cultures of liquid SIM supplemented with antibiotic (20 μg/ml) were grown from spore suspensions. Supernatants were used as samples in disk diffusion assays, and putative inactivating strains were identified by the absence of a zone of inhibition.

Antibiotic	Number of strains		Complete inactivation: %	
	Resistant	Screened for inactivation	Of isolates screened	Of library
Cephalexin	442	16	18.8	N/A*
Ciprofloxacin	52	52	0.0	0.0
Clindamycin	107	46	0.0	N/A
Daptomycin	480	80	80.0	N/A
Erythromycin	128	128	7.0	1.9
Novobiocin	12	12	0.0	0.0
Rifampicin	49	49	40.8	4.2
Synercid	294	71	18.3	N/A
Telithromycin	83	83	4.8	0.8
Trimethoprim	478	80	0.0	N/A
Vancomycin	5	5	0.0	0.0

*Not applicable. Statistic cannot be determined, because all resistant isolates were not assayed.

Synthetic fluoroquinolones target DNA gyrase A (GyrA)–dependent supercoiling and topoisomerase IV–dependent decatenation of bacterial DNA, inhibiting DNA replication and segregation (*15*, *16*). Clinical resistance occurs primarily through point mutations in the N-terminal region of *gyrA*, termed the quinolone resistance–determining region (QRDR) (*17*); however, resistance has also been documented through antibiotic efflux (*17*) and plasmid-mediated protection of DNA gyrase (*18*).

Despite a lack of known prior exposure to fluoroquinolones or bacterially synthesized analogs, 11% of strains demonstrated intrinsic resistance to ciprofloxacin (Fig. 1C, MICs of 6 to 128 μg/ml). Of the 52 resistant strains, none eliminated fluoroquinolone antibacterial activity, indicating that enzymatic inactivation was unlikely (Table 1). To investigate the possibility of QRDR mutation, this region was cloned and sequenced from 38 resistant isolates (Fig. 2). Eleven different amino acid substitutions were identified at nine QRDR locations in 24% of strains sequenced. These included locations commonly associated with clinical ciprofloxacin resistance (such as Ser^{83} and Asp^{87}, using the *Escherichia coli* numbering system), as well as novel sites within the QRDR (such as Met^{100} and Ser^{110}). Among these strains, the isolate with the highest MIC displayed a mutation at a novel location (Ser^{110}). The high incidence of mutations in the absence of obvious environmental selective pressures is consistent with

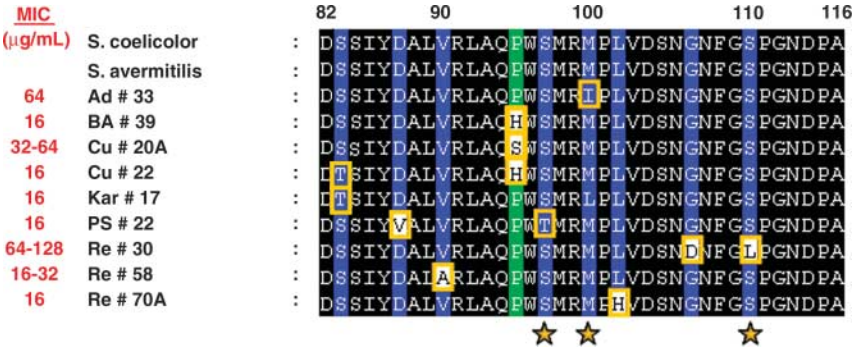

Fig. 2. Protein sequence alignment of the QRDR of ciprofloxacin-resistant strains exhibiting mutations. A 266–base pair region of *gyrA* was amplified in resistant strains and sequenced. Mutations are labeled in orange, and residues are numbered according to the *E. coli* system. Black sites are completely conserved among the 38 strains sequenced, blue sites display 80 to 99% identity, and green sites demonstrate 60 to 80% sequence identity. A white background represents amino acids not displaying similarity with their wild-type counterpart. Sites labeled with a star are novel with respect to mutations. The corresponding MICs of ciprofloxacin are indicated at the left.

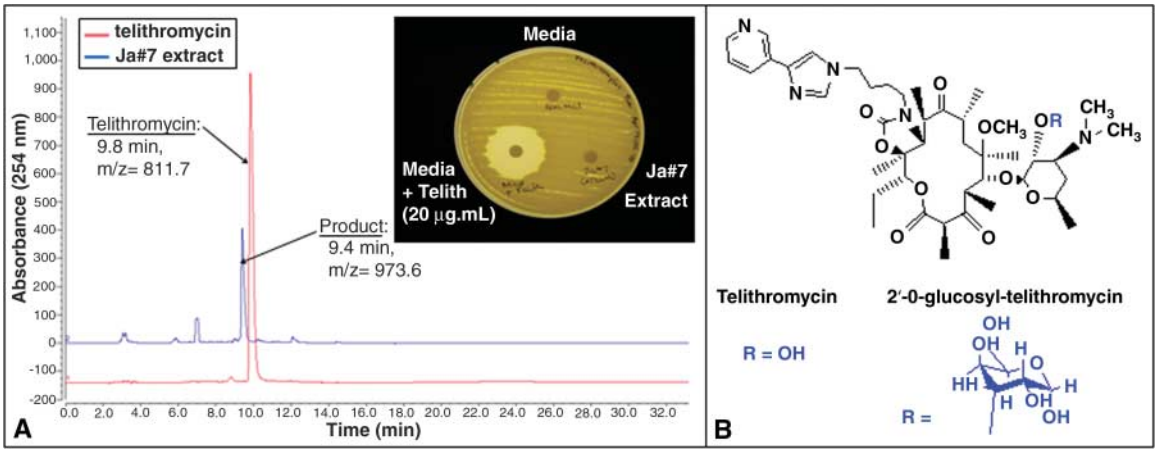

Fig. 3. Modification of telithromycin by strain Ja#7. (**A**) Culture media of Ja#7 grown in the presence of 20 μg/ml telithromycin were analyzed by high-performance liquid chromatography with in-line electrospray mass spectrometry analysis. The modification of telithromycin was accompanied by a shift in retention time, an increase in mass-to-charge (*m/z*) ratio, and the loss of antimicrobial activity against the indicator organism *Micrococcus luteus*. (**B**) Structures of telithromycin and the Ja#7 inactivation product.

previous studies that found natural sequence variation within this domain in soil bacteria (*19*). Knowledge of such natural variations could complement studies on clinical isolates to guide the rational development of next-generation fluoroquinolones that will be active against resistant strains.

Resistance to macrolide antibiotics in pathogens of clinical significance has increased considerably over recent decades and is commonly a result of antibiotic efflux and ribosomal protection mechanisms (*20*, *21*). Substantial levels of macrolide resistance were also detected in our soil isolate library, both to the natural product erythromycin (introduced in 1952, 27%), and the semisynthetic telithromycin (FDA-approved in 2004, 17%). The high frequency of telithromycin resistance was particularly intriguing, because telithromycin is known for its activity against macrolide-resistant bacteria.

Five percent of library isolates detoxified telithromycin in culture media (Table 1). One of these, *Streptomyces* strain Ja#7 (MIC of 32 μg/ml), completely modified telithromycin to an inactive hydrophilic product with a mass of 973.6 daltons (Fig. 3). This addition of 162 daltons to telithromycin (811.7 daltons) is a signature indicator of monoglycosylation. Large-scale purification of the product, followed by multidimensional and multinuclear magnetic resonance analysis, confirmed that the inactive product was 2′-*O*-glucosyl-telithromycin (table S1).

Modification of the cladinose 2′-OH of erythromycin is known to result in antibiotic resistance (*22*, *23*). However, Ja#7, despite its ability to inactivate telithromycin, was unable to completely inactivate erythromycin or its derivative clarithromycin under identical conditions. Thus, a distinct mechanism seems to be operating. Given the abundance of resistance determinants in streptomycetes that are homologous to those in clinically significant pathogens (*5*, *24*, *25*), it is evident that once this mechanism is fully characterized, it should be monitored as telithromycin use increases clinically and resistant organisms inevitably emerge.

This study provides an analysis of the antibiotic resistance potential of soil microorganisms. The frequency of high-level resistance seen in the study to antibiotics that have for decades served as gold-standard treatments, as well as those only recently approved for human use, is remarkable. No class of antibiotic was spared with respect to bacterial target or natural or synthetic origin. Although this study does not provide evidence for the direct transfer of resistance elements from the soil resistome to pathogenic bacteria, it identifies a previously underappreciated density and concentration of environmental antibiotic resistance. The level and diversity of resistance uncovered in this work is only partially reflective of the true extent of the environmental resistome, because this study was restricted exclusively to culturable spore-forming bacteria, which represent only a fraction of soil-dwelling bacteria. For example, a recent soil metagenome analysis uncovered several aminoglycoside resistance genes in uncultured organisms (*26*). Furthermore, the primary screen was conducted at high antibiotic concentrations, thereby excluding phenotypes exhibiting low to intermediate resistance. The level of resistance genes in the environment is therefore very likely to be substantially higher and the antibiotic resistome much more extensive than this study reveals.

The survey of antibiotic resistance mechanisms can assist the elucidation of novel mechanisms that may emerge clinically, as well as serve as a foundation for new antibiotic development. In addition, the study of enzymatic inactivation could lead to the development of inhibitors for combination therapies to restore antimicrobial activity.

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Supporting Online Material

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Vaccinia Virus–Induced Cell Motility Requires F11L-Mediated Inhibition of RhoA Signaling

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RhoA signaling plays a critical role in many cellular processes, including cell migration. Here we show that the vaccinia F11L protein interacts directly with RhoA, inhibiting its signaling by blocking the interaction with its downstream effectors Rho-associated kinase (ROCK) and mDia. RNA interference–mediated depletion of F11L during infection resulted in an absence of vaccinia-induced cell motility and inhibition of viral morphogenesis. Disruption of the RhoA binding site in F11L, which resembles that of ROCK, led to an identical phenotype. Thus, inhibition of RhoA signaling is required for both vaccinia morphogenesis and virus-induced cell motility.

The spatial and temporal regulation of cell adhesion and motility is essential during development and throughout the lifetime of multicellular organisms (*1*, *2*). Deregulation of these two fundamental cellular processes frequently occurs during pathological situations such as tumor cell metastasis (*3*, *4*). Dramatic changes in cell migration and adhesion, as well as loss of contact inhibition, are also observed during many viral infections, including that of vaccinia virus (*5*, *6*). In contrast to the wild-type Western-Reserve (WR) virus,