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 magnetotactic 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.

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 treatments 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 16S 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 cephalaxin), and completely synthetic molecules (such as ciprofloxacin and linezolid). The antibiotics encompassed all major bacterial targets (8) and included drugs

References and Notes

11. S. L. Simmons, K. J. Edwards, manuscript in preparation.
15. S. L. Simmons, K. J. Edwards, unpublished observations.
18. Materials and methods are available as supporting material on Science Online.
23. We thank P. Canovas and O. Rafie for field and lab assistance; R. Frankel, E. Webb, and S. Sievert for input on the manuscript; and K. Canter for use of a magnetometer. S.L.S. was partially supported by a National Defense Science and Engineering Graduate Fellowship. This work was partially funded by grants to S.L.S. and K.J.E. from the WHOI Reinhardt Coastal Research Center, the WHOI Ocean Venture Fund, and the WHOI Ocean Life Institute. D.A.B. is supported by National Science Foundation grant EAR-0311950. GenBank sequence accession numbers for the sequences reported in this study are as follows. Group 1: BL_G6, DQ322653; BL_H2, DQ322654; BL_H1, DQ322655; BL_H4, DQ322662; BL_H5, DQ322656; BL_H6, DQ322657; BL_C5, DQ322658. Group 2, south-seeking bacterium: BL_C2c, DQ322659; BL_C2c, DQ322660; BL_G11c, DQ322661.
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).

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 Ca2+-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 β subunit.

Synercid, which was FDA-approved in 1999 for the treatment of drug-resistant bacte remia, 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

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.
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 lipo-glycopeptide teicoplanin (MICs of 1 to 4 μg/ml), AA#4 was resistant to both (MICs of vancomycin and teicoplanin of 256 μg/ml).

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 Ser83 and Asp87, using the *Escherichia coli* numbering system), as well as novel sites within the QRDR (such as Met100 and Ser110). Among these strains, the isolate with the highest MIC displayed a mutation at a novel location (Ser110). The high incidence of mutations in the absence of obvious environmental selective pressures is consistent with

### 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.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number of strains</th>
<th>Complete inactivation:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Screened for inactivation</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>442</td>
<td>16</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>107</td>
<td>46</td>
</tr>
<tr>
<td>Daptomycin</td>
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<tr>
<td>Erythromycin</td>
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<td>128</td>
</tr>
<tr>
<td>Novobiocin</td>
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<td>12</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Smercid</td>
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<td>71</td>
</tr>
<tr>
<td>Telithromycin</td>
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<td>83</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>478</td>
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</tr>
<tr>
<td>Vancomycin</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

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

[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 fluorquinolones 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 macrolide resistance was particularly in B. cereus and the semisynthetic telithromycin (FDA-approved in 2004, 17%).

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.

References and Notes
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Supporting Online Material
www.sciencemag.org/cgi/content/full/311/5759/374/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S5
References
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