

# Weapons of Microbial Drug Resistance Abound in Soil Flora

Alexander Tomasz

Following the serendipitous discovery of penicillin in 1928 and streptomycin in 1943, the pharmaceutical industry has been screening thousands of soil samples for antimicrobial agents produced by inhabitant microbes. Chloramphenicol, clavulanic acid, erythromycin, gentamicin, rifampin, teichoplanin, tetracycline, and vancomycin represent only a few products of this spectacularly successful effort, and addition of these agents to the therapeutic arsenal has played a major role in controlling bacterial disease, the primary cause of human mortality in the preantibiotic era.

The study by D'Costa *et al.* on page 374 this issue (1) provides a fascinating view of the flip side of this story. The authors isolated 480 morphologically diverse spore-forming microbes from the soil and tested these not as producers of antimicrobial agents but rather as microbes that are resistant to existing antibiotics. Astonishingly, they found that every isolate was resistant to at least six to eight different antimicrobial agents and some to as many as 20! The antibiotics tested included both well-established and recently developed agents, natural products, semisynthetic derivatives, and fully synthetic antimicrobial agents.

With multidrug-resistant bacterial pathogens spreading globally and the enormous efforts to trace the source and mechanism of spread of drug-resistant genes and clones (2), the study by D'Costa *et al.* has particular poignancy. It illuminates the dark side of the antibiotic paradigm: Microbes that synthesize the sophisticated chemicals that have been key to humankind's success in controlling bacterial disease also possess equally sophisticated mechanisms to protect themselves against their own toxic products. Lifted out of this context, these self-protecting mechanisms represent formidable weaponry that could annul the successes of antimicrobial therapy if they were to find their way into human pathogens.

The microbes isolated and characterized by D'Costa *et al.* all belong to the genus *Streptomyces*, well known for producing multiple antimicrobial agents (3) that suppress the growth and/or kill other susceptible bacterial species in their vicinity. The 480 independent soil isolates examined presumably include producers of antimicrobial agents that also possess matching resistance mechanisms to protect against suicide in this chemical warfare (4).

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## Multidrug Resistance in *S. aureus*

Antibiotic	MSSA (1930)	MRSA (1994)	Resistance mechanism
Penicillin	S	R	+ (1945)
Streptomycin	S	R	+ (1948)
Tetracycline	S	R	+ (1950)
Methicillin	S	R	+ (1961) <i>mecA</i>
Oxacillin	S	R	+
Cephalothin	S	R	+
Cefotaxime	S	R	+
Imipenem	S	R	+
Chloramphenicol	S	R	+
Ciprofloxacin	S	R	A
Clindamycin	S	R	+
Erythromycin	S	R	+
Gentamycin	S	R	+
Rifampin	S	R	A
Vancomycin	S	S	A (1997) <i>VISA</i>
Vancomycin	S	S	+ (2002) <i>vanA</i>
Teichoplanin	S	S	+
Trimeth/Sulfa	S	R	A

### Emergence of multidrug resistance in *Staphylococcus aureus*.

The Brazilian clone of methicillin-resistant *S. aureus* (MRSA), isolated in 1994 (2), was resistant (R) to nearly all the antibiotics listed. Most of the resistance mechanisms were not adaptive (A), but acquired (+) from an extraspecies source. In contrast, an invasive strain of *S. aureus* (MSSA), recovered in 1930, was susceptible (S) to all the agents.

A number of the resistance mechanisms described by D'Costa *et al.* have not been previously characterized. Almost half of the test strains could enzymatically inactivate rifampin (often used against mycobacterial tuberculosis), in contrast to clinical isolates in which resistance is based on point mutations in a bacterial gene. Several strains could detoxify the semisynthetic drug telithromycin (for respiratory tract infections) by a glucosylation reaction not seen before among clinical isolates. Most strains were resistant to daptomycin, an agent only recently introduced for skin and soft-tissue infections. Surprisingly, many of the soil organisms also showed resistance against fully synthetic antibiotics such as ciprofloxacin (frequently used in urinary tract infections) and linezolid (for infections by drug-resistant enterococci, staphylo-

Bacteria found in soils show robust resistance to many antibiotics. These protective mechanisms may offer clues for generating a new arsenal of therapeutic drugs.

cocci, and pneumococci). In the case of ciprofloxacin resistance, the authors identified mutations in the gene encoding gyrase, an enzyme involved in DNA replication. Some mutations were the same as those seen in ciprofloxacin-resistant pathogens, but others involved unfamiliar mutational changes elsewhere in the gene. It is unknown whether active residues of the enormous amounts of antimicrobial agents deployed yearly in human and veterinary medicine and agriculture find their way back into the soil where they may participate in the selection for antibiotic resistance.

The obvious concern is that some of these resistance mechanisms may be exported from the "underground" world to the genomes of human pathogens. Actually, the majority of the most effective antibiotic-resistance mechanisms in human pathogens are acquired (see the figure). The superiority of such acquired mechanisms is illustrated by the contrast between *Staphylococcus aureus* strains that have decreased susceptibility to vancomycin through mutations (so-called VISA strains) as compared to VRSA strains, *S. aureus* that acquired a complete vancomycin-resistance gene complex via the transposon Tn1546 (5). The VISA strains have low-level resistance (the minimal inhibitory concentration of vancomycin is 6 to 12  $\mu\text{g/ml}$ ), are often associated with reduced oxacillin resistance, and show abnormal cell wall synthesis (6); the multiple transcriptional changes documented by DNA microarray analysis reflect the complexity of this mechanism (7). In contrast, in VRSA strains, the Tn1546-based mechanism produces high-level vancomycin resistance (with a minimal inhibitory concentration of more than 500  $\mu\text{g/ml}$ ) that does not interfere with oxacillin resistance, and cell wall synthesis proceeds with a depsipeptide cell wall precursor specific to these strains (8).

The exact nature of the bridges that connect the underground and aboveground microbial flora through which resistance genes may find their way into human pathogens is not known.

The mechanism of resistance to aminoglycoside antibiotics in human pathogens may be traced to aminoglycoside producers in the soil flora (9). Also, the critical genetic determinants of vancomycin resistance—*vanH*, *vanA*, and *vanX*—appear to be very similar to the self-protection mechanism in the vancomycin producer *Actinomyces* strains (10). Clearly, mobilization of a resistance mechanism must involve “packaging” into a plasmid, phage, or some transposable element. However, the number of stages in the movement of such a mobilized resistance mechanism before its emergence in a human pathogen driven by the selective pressure of antibiotic use is unknown. One of the first stages in the emergence of the Tn1546-based vancomycin-resistance gene complex from the underground world may have occurred on European farms where a derivative of vancomycin—avoparcin—had been in extensive use until recently. The fecal flora of animals

from such farms contained enterococcal strains that were highly resistant to vancomycin through the acquisition of the *vanA* gene complex (11). The next stages through which this transposon made its way into human strains of enterococci is unclear, but by the early 1990s, epidemic spread of vancomycin-resistant enterococcal (VRE) strains in U.S. hospitals was documented. It took another decade before the vancomycin-resistance genes found their way into the more dangerous human pathogen *S. aureus*. Here, the critical step may have been physical contact between a VRE donor and an *S. aureus* recipient, both of which were recovered from the highly immunocompromised infection site of a diabetic wound where the first VRSA isolates were identified (12).

The remarkable variety of mechanisms described by D’Costa *et al.* in their analysis of the microbial soil “resistome” may provide the medicinal chemist with precious clues in the

design of new antimicrobial agents. Hopefully, these will be less at risk in confronting an already formidable resistance mechanism.

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## CELL BIOLOGY

# Serving Up a Plate of Chromosomes

Rebecca Heald

Chromosomal DNA encodes the blueprint required to maintain eukaryotic cell and organism viability. Chromosomes replicate during each round of the cell division cycle and remain as pairs of sister chromatids until a bipolar apparatus, called the mitotic spindle, precisely segregates them into two sets, each destined for a new daughter cell (1). Accuracy in this process of mitosis is imperative, as transmission of a faulty blueprint can cause cell death or contribute to cancer. On page 388 of this issue, Kapoor *et al.* (2) address a long-standing question in the field: How do chromosomes efficiently achieve the right kind of spindle attachments so that they can be properly distributed?

To prepare for segregation, sister chromatids connect at their kinetochores to spindle microtubule bundles, called kinetochore fibers (K-fibers), that emanate from opposite spindle poles. The sisters are thus “bi-oriented” and poised to go their separate ways. Chromosomes gradually “congress” to the central region of the spindle, called the metaphase plate. The cell monitors this process and dissolves the glue holding sisters together only when every chromosome is properly attached

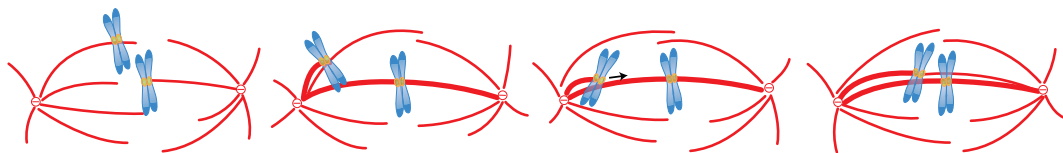
and aligned at this plate. At anaphase of the cell division cycle, sister chromatids move to opposite poles as their attached K-fibers depolymerize, completing segregation.

The question of how chromosomes achieve the prerequisite bi-orientation has intrigued cell biologists for decades. The common view has been that before congressing, each sister chromatid of a pair connects to a K-fiber that is asso-

Before being pulled into their respective daughter cells, duplicated chromosomes line up at the center of the cell. Video microscopy and high-resolution electron microscopy show how this precise arrangement is set up by the cell.

ciated with the opposite spindle pole (3). However, spindle attachment is a stochastic process that depends on the interaction of microtubules with a subset of proteins localized at the kinetochore. As soon as one sister kinetochore “captures” a microtubule emanating from one spindle pole, the chromosome (that is, the pair of sister chromatids) is transported toward that pole, becoming “mono-oriented.” How then do microtubules from the other spindle pole make contact with the unattached sister? This is a puzzle, because structural analyses of the spindle indicate that microtubules from the distal pole rarely penetrate far enough to be captured by a chromosome that has already moved toward the opposite pole (4). Yet chromosomes still congress to the plate and become bi-oriented.

Using a combination of sophisticated microscopy techniques, Kapoor *et al.* have documented kinetochore behavior that solves this



**A mechanism of chromosome congression that promotes bi-orientation.** Microtubules forming a mitotic spindle (red) that contains two chromosomes is shown, each with paired sister chromatids (blue) and kinetochores (yellow). Thicker red lines represent bundled kinetochore fibers. In the scenario depicted, microtubules growing from both spindle poles have been captured by sister kinetochores of one chromosome, and the chromosome is oscillating (congressed) at the metaphase plate. The other chromosome attaches initially to only one pole and becomes mono-oriented in a position where microtubules from the opposite pole are unlikely to make contact. By attaching to and sliding along the kinetochore fiber of the congressed chromosome, the unattached sister kinetochore moves toward the center of the spindle, where it makes microtubule connections to bi-orient.

conundrum. By following congression in living cells through video microscopy and then rapidly preparing the cells for high-resolution electron microscopy, the authors observed mono-oriented chromosomes with the kinetochore of one sister chromatid attached to the ends of microtubules extending from a proximal pole. Interestingly, its sister was laterally associated with the K-fiber of another chromosome that was already bi-oriented and congressed. Visualizing fluorescent kinetochores and micro-

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