REVIEWS

Platforms for antibiotic discovery

Kim Lewis

Abstract | The spread of resistant bacteria, leading to untreatable infections, is a major public health threat but the pace of antibiotic discovery to combat these pathogens has slowed down. Most antibiotics were originally isolated by screening soil-derived actinomycetes during the golden era of antibiotic discovery in the 1940s to 1960s. However, diminishing returns from this discovery platform led to its collapse, and efforts to create a new platform based on target-focused screening of large libraries of synthetic compounds failed, in part owing to the lack of penetration of such compounds through the bacterial envelope. This article considers strategies to re-establish viable platforms for antibiotic discovery. These include investigating untapped natural product sources such as uncultured bacteria, establishing rules of compound penetration to enable the development of synthetic antibiotics, developing species-specific antibiotics and identifying prodrugs that have the potential to eradicate dormant persisters, which are often responsible for hard-to-treat infections.

Persisters
Metabolically quiescent cells that neither grow nor die when exposed to bactericidal concentrations of antibiotics.

High-throughput screening
(HTS). An automated instrumental process for detecting the binding or activity of hundreds of thousands of compounds to an isolated receptor target or whole cells, thereby identifying worthwhile leads for development.

Rational drug design
A strategy by which drug molecules are developed based on the analysis of the three-dimensional structure of a protein interacting with a ligand.

Department of Biology and Antimicrobial Discovery Center, Northeastern University, Boston, Massachusetts 02115, USA.
e-mail: k.lewis@neu.edu
doi:10.1038/nrd3975

In the absence of an effective platform for antibiotic discovery, the rise and spread of resistant pathogens goes unchallenged. An increase in the occurrence of chronic infections — a side effect of medical intervention — presents an additional challenge: these diseases are often untreatable owing to the presence of antibiotic-tolerant persisters (BOX 1).

There is a very low probability of successfully developing an antibiotic from a lead compound identified in pre-clinical studies, and so it is essential to have platforms that are capable of reliably generating lead compounds. Nearly all antibiotics in use today are compounds that were discovered during the 1940s to 1960s — the golden era of antibiotic discovery — or their derivatives. Most of these compounds were discovered by screening soil-derived actinomycetes, but natural product discovery became impractical owing to the increasing difficulty of identifying new classes of antibiotics against the background of known compounds. Unfortunately, efforts that began in the 1990s to develop high-tech platforms as new sources of antibiotic leads — such as high-throughput screening (HTS) against defined targets and rational drug design — failed owing to issues such as the challenges associated with identifying synthetic compounds that could effectively penetrate bacterial cells.

Many gaps remain in our knowledge of antibiotics. For example, we only know the targets for a small number of the thousands of natural antibiotics that have been discovered (FIG. 1). It is unclear why some compounds penetrate well into bacterial cells in general, and how some can breach the more restrictive envelope of Gram-negative species. Indeed, the presence of an effective penetration barrier in bacteria and the paucity of novel penetrating compounds are the two problems largely responsible for the lack of progress in the field.

With such gaps and challenges in mind, this article analyses the lessons learned from the golden era of antibiotic discovery, considers factors that have been responsible for the failure of the numerous attempts to develop new platforms for antibiotic discovery and proposes how effective platforms could either be re-established or created. Such platforms could be greatly facilitated by formulating rules of compound penetration based on empirical measurements of molecules crossing the bacterial cell envelope, which would enable the construction of focused compound libraries for antibiotic discovery through HTS as well as structure-based rational design. We consider the use of prodrugs — molecules that are converted into reactive compounds inside bacterial cells and have the ability to kill dormant persisters — and the resuscitation of species-selective drug discovery, which was once a successful platform for anti-tuberculosis (TB) compounds. We also discuss reviving natural product discovery from untapped sources of antimicrobials such as uncultured bacteria, coupled with approaches to rapidly identify novel compounds against a background of known antibiotic classes.

Box 1 | Antibiotic resistance and tolerance

The rise and spread of antibiotic resistance presents a unique challenge to both science and medicine. Today, the crisis is epitomized by the spread of multidrug-resistant 'ESKAPE' organisms (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.)[83]. Indeed, in the case of some Gram-negative bacteria, such as *A. baumannii*, there are strains that are resistant to all currently available antibiotics[84].

Antibiotics shut down or subvert essential cellular functions, and resistance mechanisms appear to exploit every possible strategy of preventing a drug from hitting its target. The major types of clinically relevant resistance mechanisms have been studied for a long time and are generally well understood (reviewed in REFS 3, 4) and are shown in the figure. These include destruction of the antibiotic (for example, by β-lactamases); target modification (for example, mutation in the 30S ribosomal protein RpsL confers resistance to streptomycin); as well as restricted penetration and/or efflux of the drug (for example, efflux of linezolid by the AcrAB–TolC multidrug pump)[85,86].

The same cannot be said about tolerance. The main culprit responsible for the tolerance of pathogens to antibiotics is a specialized survivor — a persister[87,88]. Persisters are not mutants; they are phenotypic variants of actively dividing cells produced stochastically in the population, and their relative abundance rises — reaching 1% — at the late-exponential phase of growth[89]. Persisters are non-growing[90] dormant[91,92] cells, which explains their tolerance to bactericidal antibiotics that depend on the presence of active targets for killing the cell[93]. All of the pathogens examined so far form persisters[88], but the mechanisms underlying the formation of persisters is still largely unknown. Studies have shown, however, that in the model organism *Escherichia coli*, toxin–antitoxin modules are the principal mechanism of persister formation[92–95], and that pathways of persister formation are highly redundant[96]. Owing to this redundancy, a realistic target for drug discovery has yet to be identified.

The significance of persisters and drug tolerance in the clinical manifestation of disease was recently demonstrated when it was shown that elevated levels of persisters are selected for in the course of antimicrobial therapy in infections caused by *Candida albicans*[97] and *Pseudomonas aeruginosa*[98]. Persisters also have an important role in the development of conventional antibiotic-resistant mutants. Persisters are killed only slowly, if at all, and resume growth when antibiotic concentrations fall. The result is a relapsing infection with a large effective population size that favours the development of resistance[99]. The importance of persisters in the recalcitrance of infectious diseases raises the bar for drug discovery; there is an urgent need to develop therapies that effectively kill both actively dividing and dormant pathogens. The figure is reproduced, with permission, from REF. 100 © (2002) Macmillan Publishers Ltd. All rights reserved.
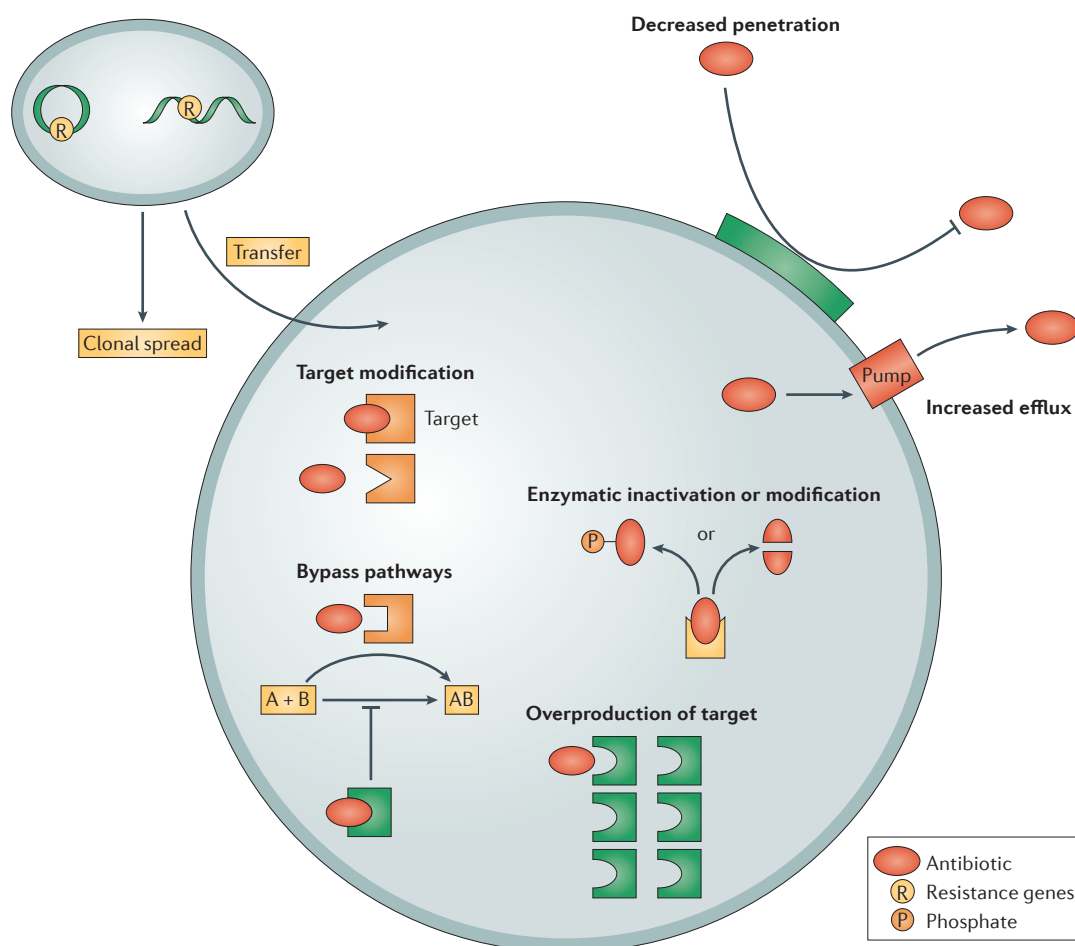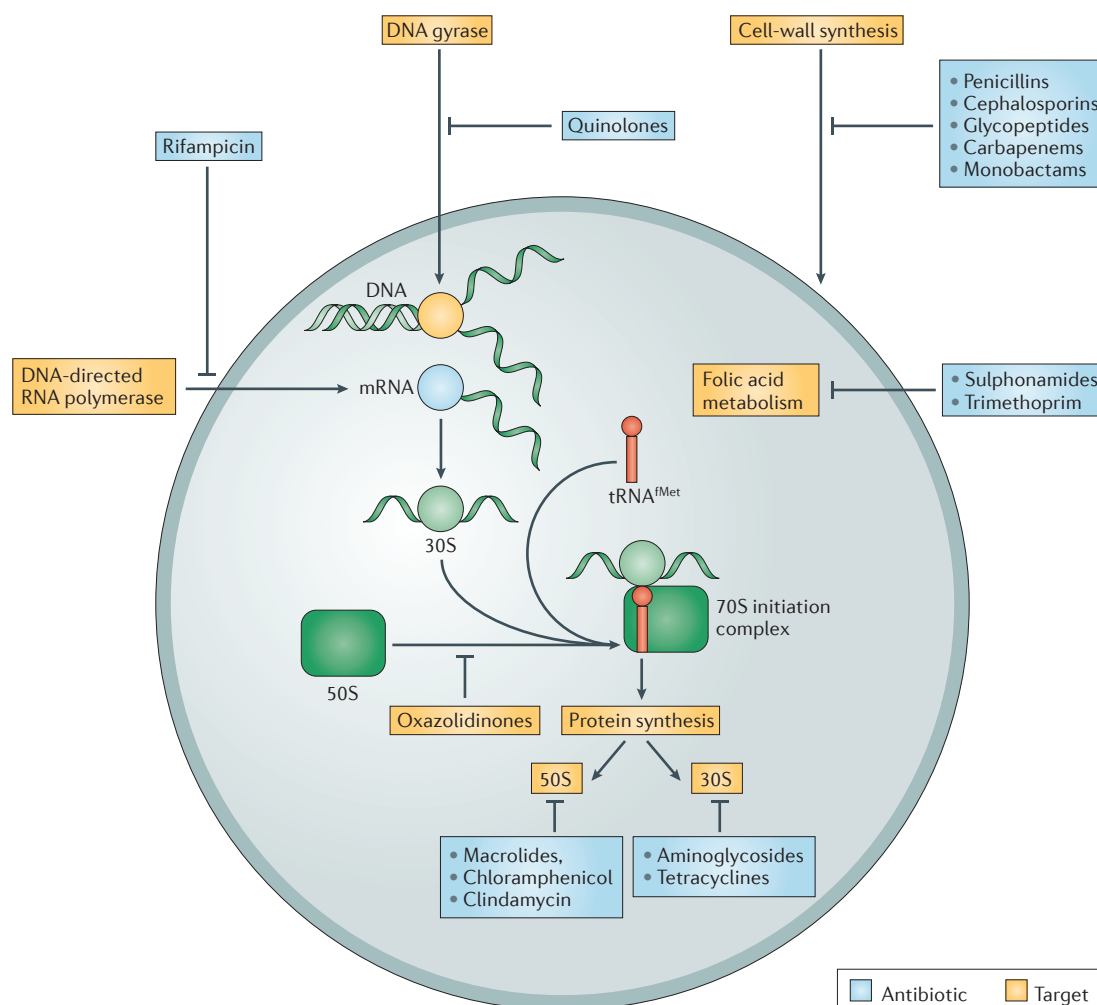
Figure 1 | **Targets of antibiotics.** There are approximately 200 conserved essential proteins in bacteria, but the number of currently exploited targets is very small. The most successful antibiotics hit only three targets or pathways: the ribosome (which consists of 50S and 30S subunits), cell wall synthesis and DNA gyrase or DNA topoisomerase. The figure is reproduced, with permission, from REF. 100 © (2002) Macmillan Publishers Ltd. All rights reserved.

Antibiotic discovery: a brief history

Although not widely appreciated, the genesis of the antibiotic crisis was the breakdown of the once successful discovery platform introduced by Selman Waksman in the 1940s[1]. The platform was simple: soil-derived streptomycetes were screened for antimicrobial activity against a susceptible test microorganism by detecting zones of growth inhibition on an overlay plate[1]. The method is similar to the serendipitous discovery of penicillin by Alexander Fleming[2]. However, Waksman's application of a systematic screen is what made the difference between luck and a discovery platform, and this method of discovering antibiotics earned him a Nobel Prize. The screening of streptomycetes led to the discovery of streptomycin (FIG. 2), the first effective compound to act against TB and the first aminoglycoside. This 'Waksman platform' was widely adopted by the pharmaceutical industry and produced the major classes of antibiotics over the next 20 years (TABLE 1). But after 20 years

of success, the returns from the mining of soil-derived streptomycetes (and other actinomycetes) diminished owing to the rediscovery of known compounds, and so the platform was abandoned.

In parallel to their discovery, resistance to antibiotics by the target microorganisms was also emerging, but modifications to existing antibiotics produced active analogues. An excellent class of synthetic antibiotics, the fluoroquinolones (FIG. 2), was developed in a small focused project to optimize nalidixic acid — a rather unremarkable lead compound — in the 1960s, and the tempo of drug discovery seemed to be outpacing the spread of resistance. However, this did not last. Since the 1960s, no new class of broad-spectrum compounds has been discovered. Although several natural products or derivatives have recently been developed and approved, these are based on old discoveries (TABLE 1). The last clinically useful antibiotic in a new class to be discovered was daptomycin (in 1986), a lipopeptide that acts against
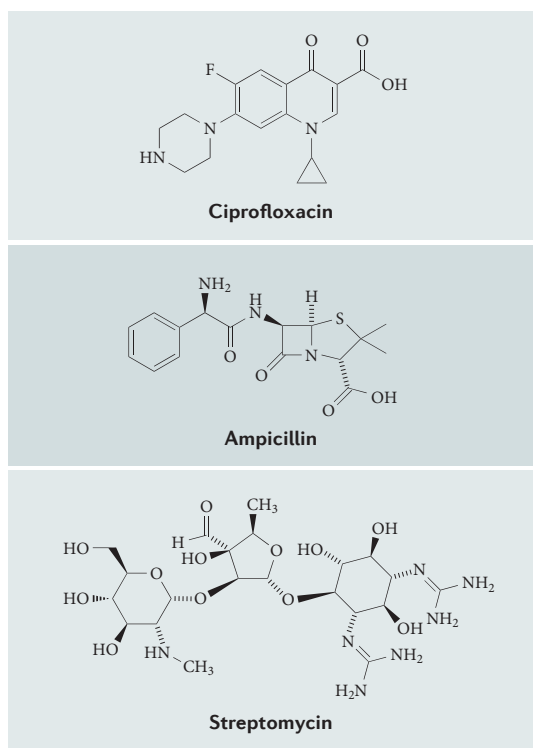
Figure 2 | The most successful antibiotic classes. Compounds representing the broad-spectrum and bactericidal antibiotic classes are shown; these compounds hit multiple targets, diminishing the probability of developing resistance. Ciprofloxacin is a fluoroquinolone antibiotic that targets the related DNA gyrase and DNA topoisomerase. Ampicillin is a semi-synthetic β-lactam that targets several penicillin-binding proteins that are responsible for peptidoglycan synthesis. Streptomycin is an aminoglycoside inhibitor of protein synthesis that binds primarily to 16S ribosomal RNA, which is encoded by several operons. Fluoroquinolones and aminoglycosides can kill non-growing cells, whereas β-lactams are only active against growing bacteria.

Lipinski's 'rule of five' guidelines
Guidelines (from Lipinski's analysis of the World Drug Index) identifying several key properties that should be considered for small molecules that are intended for oral delivery. These properties are: molecular mass <500 Da, number of hydrogen-bond donors <5; number of hydrogen-bond acceptors <10; and calculated octanol–water partition coefficient (an indication of the ability of a molecules to cross biological membranes) <5.

the bacterial cell membrane, but it was not approved until 2003. And until last year, when a new drug for TB, bedaquiline (Sirturo; Janssen Pharmaceuticals), was approved (see below), the last class of narrow-spectrum antibiotics (acting against a specific target) to be discovered were the streptogramins (in 1964), but these compounds were only introduced to the clinic ~30 years later.

By the 1990s, it became clear that our victory over bacterial pathogens was an illusion, with resistance spreading faster than the discovery of new antibiotics (a classic example is the rate at which resistance is emerging for β-lactams; see BOX 2), and we found ourselves in an alarming position of being on the losing side of this war. The pharmaceutical industry responded to the rise of antibiotic resistance by focusing on developing synthetic antibiotics, and various high-tech approaches were brought together to form a new platform based on

genomics, combinatorial chemistry, HTS and rational drug design. It was thought that essential, conserved bacterial proteins identified through genomics would serve as targets for HTS and rational drug design with the aim of producing novel antibiotics. However, not a single drug with a reasonable spectrum of activity against important pathogens emerged from this platform[3]. Although inhibitors of targets were readily identified through *in vitro* HTS, it proved very challenging to produce compounds that were able to sufficiently penetrate the bacterial cell wall to reach their targets, especially in Gram-negative bacterial species. The adoption of Lipinski's 'rule of five' guidelines by the industry also posed challenges — antimicrobials do not obey the rules. Antibiotics also have an enhanced inherent risk of toxicity owing to the high doses needed to achieve efficacy, and the returns on investment are relatively modest (BOX 3).

As a result, research and development on antibiotics went into severe decline and many larger pharmaceutical companies left the field altogether. There has nonetheless been a continued stream (albeit slow) of derivatives from old classes of antibiotics and there is no doubt that novel compounds would have an even better chance of being developed, given the growing recognition of the urgent need for new antibiotics. Platforms to produce such compounds — based on what we have learned from the golden era of antibiotic discovery, and since then — are presented below.

Why platforms?
Only two classes of synthetic antibiotics were developed in the past 50 years: fluoroquinolones and oxazolidinones (linezolid) (TABLE 1); only fluoroquinolones are broad-spectrum. All other similar efforts focused on a given series of compounds failed, which indicates that there is an extremely low probability of discovering a drug in the absence of a platform that is capable of churning out leads. The situation with natural compounds is very similar. Three novel natural products were introduced as systemic drugs during the past 40 years: daptomycin[4], quinupristin–dalfopristin[5] and fidaxomicin[6,7] (TABLE 1). All three were considered to be insufficiently attractive when they were first discovered, and the resurrection of these compounds decades later was necessitated by the lack of new leads.

This analysis suggests that if we want to efficiently discover and develop novel antibiotics, we need to develop reliable discovery platforms. There is another argument in favour of platforms: combination therapeutics. The first antibiotics to be introduced — penicillin and streptomycin — were used as monotherapies. The pathogens rapidly acquired resistance, necessitating the introduction of drug combinations. An inhibitor, clavulanic acid, was introduced to block the hydrolysis of β-lactams by β-lactamases. Streptomycin was highly effective against TB, until the pathogen acquired a mutation in the 30S ribosomal protein RpsL. As new anti-TB drugs were introduced, the mycobacterium acquired mutations in the targets for each of these drugs and this led to resistance[8]. It therefore became clear that a standard course of therapy had to include several drugs

Table 1 | **Timeline of the discovery and introduction of antibiotics**

Antibiotic class; example	Year of discovery	Year of introduction	Year resistance observed	Mechanism of action	Activity or target species
Sulfadrugs; prontosil	1932	1936	1942	Inhibition of dihydropteroate synthetase	Gram-positive bacteria
β-lactams; penicillin	1928	1938	1945	Inhibition of cell wall biosynthesis	Broad-spectrum activity
Aminoglycosides; streptomycin	1943	1946	1946	Binding of 30S ribosomal subunit	Broad-spectrum activity
Chloramphenicols; chloramphenicol	1946	1948	1950	Binding of 50S ribosomal subunit	Broad-spectrum activity
Macrolides; erythromycin	1948	1951	1955	Binding of 50S ribosomal subunit	Broad-spectrum activity
Tetracyclines; chlortetracycline	1944	1952	1950	Binding of 30S ribosomal subunit	Broad-spectrum activity
Rifamycins; rifampicin	1957	1958	1962	Binding of RNA polymerase β-subunit	Gram-positive bacteria
Glycopeptides; vancomycin	1953	1958	1960	Inhibition of cell wall biosynthesis	Gram-positive bacteria
Quinolones; ciprofloxacin	1961	1968	1968	Inhibition of DNA synthesis	Broad-spectrum activity
Streptogramins; streptogramin B	1963	1998	1964	Binding of 50S ribosomal subunit	Gram-positive bacteria
Oxazolidinones; linezolid	1955	2000	2001	Binding of 50S ribosomal subunit	Gram-positive bacteria
Lipopetides; daptomycin	1986	2003	1987	Depolarization of cell membrane	Gram-positive bacteria
Fidaxomicin (targeting *Clostridium difficile*)	1948	2011	1977	Inhibition of RNA polymerase	Gram-positive bacteria
Diarylquinolines; bedaquiline	1997	2012	2006	Inhibition of F_1F_O-ATPase	Narrow-spectrum activity (*Mycobacterium tuberculosis*)

in order to have a realistic chance of success (the current course of TB therapy is a combination of isoniazid, pyrazinamide and rifampicin). Moreover, the misuse of antibiotics has led to multidrug-resistant (MDR)- and extensively drug-resistant (XDR)-TB, with pan-resistance to almost all available compounds[9]. In retrospect, combining the three antibiotics above into a single pill as soon as they were approved would have probably kept them effective for decades and prevented or minimized the development of resistance.

Although we have the benefit of hindsight, there are currently no proposals to introduce combinations of several new antimicrobial compounds; this is unrealistic given the virtual absence of novel compounds. Platforms have the potential of changing this and largely solving the problem of resistance. Once there is a reliable method for discovering new compounds, this will enable the development of drugs acting against multiple as well as single targets — something that is currently beyond our reach owing to the development of resistance — and allow the production of combination therapies with lasting efficacy.

The lack of good starting compounds is the main problem, which underlies our suggestion to identify the rules of penetration to revive the high-tech platforms. But is it possible that existing libraries may harbour useful compounds that have been missed? Are there other approaches for rational drug discovery apart from designing ligands that bind to and block targets? Is it possible to resuscitate Waksman's platform? These questions are examined in the sections below.

The prodrug platform
It is useful to consider a theoretically ideal antibiotic from first principles and then decide whether it is realistic to produce it. The approaches that have been used so far do not address the daunting challenge of killing persisters or the need for broad-spectrum activity. With this in mind, one concept for an ideal antibiotic is a highly reactive compound that kills all cells, including persisters. In order to spare the host, the compound must be delivered as a prodrug, and then a bacteria-specific enzyme will activate it into a generally reactive

Box 2 | **History of the β-lactams**

The β-lactams — which encompass penicillins, cephalosporins, cephamycins, carbapenems and monobactams — are the most successful class of antibiotics developed so far, and the pace of their discovery and emergence of resistance to these antibiotics is particularly illuminating.

Resistance to penicillin was recorded shortly after its introduction in 1945, and traced to hydrolysis of the antibiotic by β-lactamase[101]. A naturally produced β-lactamase inhibitor, clavulanic acid, was discovered in 1976 (REF. 102) and combined with β-lactams. One of the most successful antibiotics currently on the market is augmentin, which is a combination of amoxicillin and clavulanic acid. Pathogens, however, continuously develop resistance by modifying or replacing the target — penicillin-binding proteins — and acquiring new β-lactamases, probably from soil-derived microorganisms[84,103,104]. As a result, we are reaching the fourth generation of semi-synthetic β-lactams. Novexell and AstraZeneca are also developing a new β-lactamase inhibitor, avibactam, which is active against most β-lactamases[105] and is currently in Phase III trials (see the 18 November 2011 press release on the AstraZeneca website). However, bacteria are counteracting these efforts; metallo-β-lactamases (for which we do not have an inhibitor) have recently emerged[106], as has the NMD-1 'New Dehli' plasmid encoding a β-lactamase that provides resistance to all β-lactams[107].

molecule that will covalently bind to unrelated targets (FIG. 3). Importantly, this mechanism creates an irreversible sink, largely resolving the issue of MDR efflux in Gram-negative species, so the antibiotic is automatically a broad-spectrum compound.

So, is there a realistic opportunity of identifying such compounds? Several existing antibiotics— isoniazid, pyrazinamide, ethionamide and metronidazole — closely match the properties of this idealized prodrug antibiotic. The first three drugs target *Mycobacterium tuberculosis*, whereas metronidazole is a broad-spectrum compound that targets anaerobic bacteria. All four compounds are converted into active molecules inside bacterial cells and covalently bind to their targets. It seems no accident that prodrug antibiotics make up the core of the anti-TB drug arsenal, as an ability to kill the pathogen — and not just inhibit replication — is crucial for treating the disease. *M. tuberculosis* evades the immune system by hiding within macrophages, which makes it very hard to eradicate the infection.

A perfect prodrug is nonspecific once activated, but preferred targets have been identified for isoniazid, ethionamide[10,11] and pyrazinamide[12], which indicates that the activated drugs have a relatively limited reactivity. Metronidazole is the only prodrug that forms a highly reactive compound, which binds to different targets (such as proteins and DNA) and indeed has broad-spectrum activity. Its use is, however, limited to treating infections with *Helicobacter pylori*, *Clostridium difficile* and other pathogens that live under microaerophilic and/or anaerobic conditions, in which nitroreductases — the activating enzymes for metronidazole — are expressed.

It is interesting to note that all prodrugs were discovered in the 1950s, when the global library comprised less than 10,000 compounds. So, it seems that we once had a discovery platform for prodrugs but then lost it, in spite of an approximately 1,000-fold increase in the number of available compounds. Why did this happen from the 1960s onwards? One possible reason why we lost the art of finding prodrugs is because of the introduction of advanced methods to eliminate nuisance compounds such as detergents, redox agents and generally active antiseptics that do not have a specific target. A common validation step is the specificity test, whereby different labelled precursors of biopolymers (such as amino acids, nucleotides, fatty acids and sugars) are added individually to bacterial cells and the effect of a drug on label incorporation is measured. A specific inhibitor rapidly blocks label incorporation only into a given biopolymer or, if the target is unknown, it has no effect. Nuisance compounds block all label incorporation simultaneously. According to this test, metronidazole is a nuisance compound. This analysis suggests that screening for prodrugs is a validated platform that is waiting to be revived.

The species-specific platform

The history of anti-TB drug discovery provides a compelling case for the development of species-specific antibiotics. The disease is caused by a single pathogen, the diagnosis is unambiguous and testing random compounds against *M. tuberculosis* has produced antimicrobials that act primarily against mycobacteria. The first anti-TB compound to be discovered was streptomycin, a broad-spectrum natural antibiotic. Subsequent screening of synthetic compounds against *M. tuberculosis* resulted in the discovery of isoniazid, pyrazinamide, ethionamide and ethambutol[13], and all of these compounds turned out to be selective against mycobacteria. Increasing resistance of the pathogen owing to target modification mutations necessitated the continuous addition of new antibiotics to treatment regimens, and these were then borrowed from the growing arsenal of compounds that had a broader spectrum of activity against microorganisms, such as other aminoglycosides (apart from streptomycin), rifampicin and moxifloxacin.

Direct screening against *M. tuberculosis* was largely abandoned owing to this practice of borrowing broad-spectrum compounds. However, a screen against a rapidly growing surrogate, *Mycobacterium smegmatis*, was performed relatively recently by Janssen Pharmaceuticals and resulted in a compound that is also selective against mycobacteria. The compound, bedaquiline, turned out to be an inhibitor of F_1F_0-ATPase, which is conserved from bacteria to mammals[14].

Several important lessons can be learned from this empirical quest for TB therapies. It appears that a screen against a pathogen primarily produces compounds that act selectively against this pathogen (or against closely related members of a group). These compounds can work in either of two ways. One type hits group-specific targets. For example, ethambutol is an inhibitor of arabinosyl transferase[15], which is part of a biosynthetic pathway for mycolic acids that are specific to mycobacteria. The other type affects conserved enzymes but is only effective against mycobacteria. For example, a minor difference in the sequence of the C subunit of mycobacterial ATP synthase compared to ATP synthases

---

MDR efflux
Multidrug-resistant (MDR) efflux; an active transport system for the removal of several structurally non-related antibiotics from cells. The major facilitator (MF) family of MDRs are drug or proton antiporters present in all bacteria that are primarily responsible for efflux of hydrophobic cations and have some role in protecting bacteria from disinfectants, but not from systemically used antibiotics. The resistance nodulation cell division (RND) MDRs of Gram-negative bacteria are very broad-spectrum and will extrude most amphipathic compounds. These MDRs span the entire cell envelope and extrude compounds across the outer membrane — the main penetration barrier for antibiotics.

Box 3 | Additional challenges for antibiotic discovery

Gram-negative bacteria

Gram-negative bacteria in particular are highly efficient at keeping out drugs. Their outer membrane is a barrier for amphipathic compounds, and essentially all drugs are amphipathic as they need to be soluble and be able to cross the cytoplasmic membrane. The multidrug-resistant (MDR) pumps extrude any compounds that leak in through the outer membrane[108,109] and recognize chemically unrelated molecules based mainly on polarity, preferring amphipathic molecules. The inner membrane restricts the penetration of hydrophilic substances, resulting in a perfect barrier. Porins in the outer membrane and transporters in the inner membrane allow the uptake of specific nutrients.

Antibiotics and Lipinski's 'rule of five' guidelines

The vastly negative experience with antibiotic discovery during the past half-century clearly indicates that there is a bottleneck owing to the paucity of compounds that can penetrate into bacterial cells. High-throughput screening has been very successful in identifying hits against targets in (and outside) human cells. Compound libraries are typically focused by filters such as Lipinski's rules on desirable physicochemical properties to improve the likelihood of oral bioavailability[31]. However, these rules are not useful for identifying good antibiotic lead compounds, which need to satisfy a different requirement: penetration into prokaryotes. Nevertheless, this does not mean that a random unfiltered library has promising starting compounds; applying Lipinski's filters simply exacerbates the problem, as it may remove compounds that have some capability to penetrate bacterial membranes. It is noteworthy in this respect that early screening libraries did not have these filters. According to the ZINC database, upwards of three million compounds are commercially available[110,111], and around ten million have been screened by the pharmaceutical industry in search of antibiotics. However, this is still very small compared to the theoretical chemical space, which is made up of molecules of comparable size and composition to known drugs and has been estimated to contain 10^{60} molecules[112]. It may be possible to discover new or under-explored regions of this chemical space with properties that are more suited to antibiotic development if we can, for example, establish rules for effective bacterial penetration.

Inherent risk of toxicity

Antibiotics are effective at micromolar concentrations that are two to three orders of magnitude higher than for a typical drug acting against a eukaryotic target. The main reason for this disparity derives from poor penetration: the binding constants of antibiotics to purified targets are comparable to those observed with other drugs. The need to deliver high amounts of a compound substantially increases toxicity, diminishing the probability of developing good lead compounds.

Clinical trials

New compounds are tested in clinical trials against acute infections that last for only several days. The main reason why new antibiotics are introduced is to combat resistant pathogens, but most patients are infected with drug-susceptible pathogens. Placebos cannot be used for infected patients owing to ethical considerations. Such factors make it more challenging to define clear end points for clinical trials of new antibiotics. However, it seems possible that the regulatory environment could soon begin to better reflect such challenges. For example, the recent adoption of the GAIN (Generating Antibiotic Incentives Now) Act in the United States, which is aimed at encouraging the development of antibiotics, promises meaningful reforms to clinical trials and regulatory assessment for new antibiotics (see the 24 September 2012 press release on the US Food and Drug Administration (FDA) website for further details). These reforms could, for instance, be based on the development of pathogen-specific regulatory pathways[113].

Modest return on investment

Apart from the above obstacles, which science may ultimately overcome, there are also challenges related to market realities that are specific to antibiotics. Antibiotic therapy is typically short-term, lasting only several days. Resistance to any antibiotic will eventually develop, limiting its useful lifetime. By contrast, patients with a typical chronic disease require drug treatment every day for a long period of time, often for the rest of their life. The best-selling cholesterol-lowering drug atorvastatin, for example, is taken daily and had annual sales of US$12 billion before losing patent protection, whereas the best-selling antibiotic levofloxacin is taken only for a few days, and has annual sales of $2.5 billion. However, a $2.5 billion market is attractive enough, provided there is a realistic chance of producing a drug to be sold in that market.

---

in other organisms underlies the selective binding of bedaquiline, which has no activity against other bacteria. Similarly, isoniazid (a prodrug) is activated by a well-conserved catalase, and the active substance then forms an adduct with NADH. The catalase is essential only in mycobacteria, and the adduct is an effective inhibitor of the NADH-dependent enoyl-ACP reductase InhA, a mycolic acid biosynthetic enzyme[10].

It makes sense that identifying a compound that inhibits a single protein is easier than finding a compound that inhibits many members of an orthologous group. As noted above, HTS campaigns did not lead to the discovery of a single antibiotic with a reasonable activity against a range of important pathogens[3]. Bedaquiline was obtained through an HTS of a commercially available library and was recently approved by the
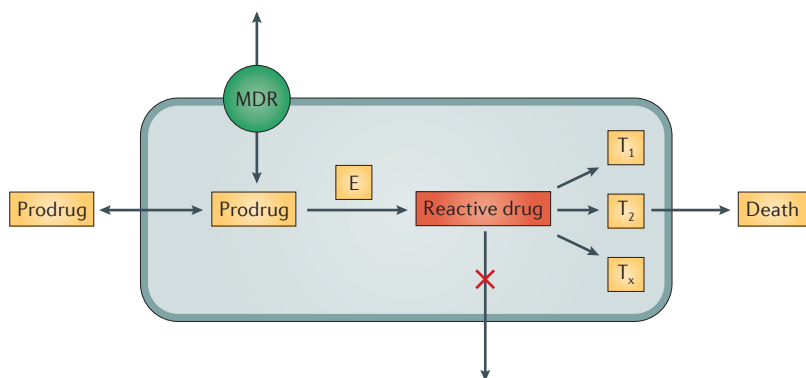
Figure 3 | **An ideal antibiotic from first principles.** A prodrug enters the cell, where it is converted into a reactive compound by a bacteria-specific enzyme (E). The reactive moiety covalently attaches to unrelated targets (T_1, T_2 to T_x), killing both actively dividing and dormant cells, thus sterilizing an infection. Covalent binding to targets provides an irreversible sink, leading to effective accumulation of the active drug over time and ensuring a broad specificity of action. MDR, multidrug-resistant.

US Food and Drug Administration (FDA) for MDR-TB treatment[16]. This is the first antibiotic that was discovered through an HTS approach and so perhaps it is not surprising that the compound is narrowly selective.

The above analysis suggests that we may have a validated discovery platform that could be applied to other species. Indeed, there is a growing consensus that species-selective compounds are the future of antibiotic discovery[17]. Species selectivity removes an important uncertainty at the beginning of the discovery process: nuisance compounds that present a major obstacle are automatically eliminated. Indeed, if a compound is active only against a particular pathogen, it is unlikely to be a detergent or a DNA intercalator. Species-selective compounds will also have a lower probability of being toxic to humans, as a target harboured by a particular bacterial species is unlikely to be present in mammals.

Another important advantage of species-selective compounds is that they minimize possible adverse effects on our gut symbionts. We are becoming increasingly appreciative of the potential role of our microbiota in health and disease. The composition of the microbiome seems to affect everything from gastrointestinal diseases, diabetes and obesity to the general state of the immune system and mental health[18–21]. Taking broad-spectrum antibiotics may result not only in antibiotic-induced diarrhoea (which is unpleasant but treatable) but also have more problematic and lasting consequences: the microbiome recovers poorly from antibiotic treatment[22]. An additional important advantage of selective compounds relates to resistance. Resistance to a broad-spectrum compound may emerge in any bacterium, including commensal bacteria (each of us carries 10^{14} bacterial cells), and then move into a pathogen. This likelihood is greatly minimized with a narrow-spectrum compound that only targets a specific pathogen.

The development of species-selective compounds will require the introduction of diagnostics that are able to rapidly identify the disease-causing pathogen so that the

Microbiota
The entire collection of microorganisms (bacteria, archaea and fungi, as well as protozoa and viruses) that are resident on or in the host.

appropriate drug can be given. This is a developing field, with molecular diagnostics replacing traditional plating techniques. A welcome example is the recent approval of a rapid diagnostic to detect methicillin-resistant *Staphylococcus aureus* (MRSA) by MicroPhage[23], which will enable the development of selective agents against this clinically important pathogen.

What is the best discovery approach?
Rules of penetration and resuscitation of the high-tech platforms. It appears that libraries composed of random compounds are unlikely to contain molecules that have a reasonable range of antibacterial activity and good bacterial penetration properties. The penetration problem is also the main obstacle for smaller, focused medicinal chemistry projects and for rational drug design based on ligand–target binding. One approach to overcome this problem is to take out the major component of the penetration barrier: MDR pumps of Gram-negative bacteria.

Microcide discovered an effective inhibitor of the resistance nodulation cell division (RND) superfamily of MDR pumps, which are primarily responsible for the high intrinsic resistance of Gram-negative species to amphipathic compounds[24,25]. The inhibitor, MC-207110, discovered in an HTS of commercial compounds, is highly active against different RND pumps of various bacteria. Clinical isolates of *Pseudomonas aeruginosa* overexpressing the MexAB–OprM pump (consisting of the membrane fusion protein MexA, the multidrug-efflux transporter MexB and the outer membrane protein OprM) are resistant to fluoroquinolones and other antibiotics, and MC-207110 was able to restore susceptibility to antibiotics both *in vitro* and in an animal model of infection. However, polycationic MC-207110 and its derivatives proved to be nephrotoxic, which is a common liability of cationic compounds that accumulate in lysosomes. The positive charges appeared to be essential for MDR inhibitors with a good coverage of various RND pumps.

Although having an effective and safe MDR inhibitor would be very useful, there undoubtedly remains a need for novel self-penetrating compounds. The crystal structures of RND pumps were obtained[26–29] but they did not prove to be particularly instructive for designing better inhibitors or self-penetrating molecules. The large, poorly structured binding sites of the *Escherichia coli* AcrAB–TolC pump can accommodate a variety of chemically unrelated compounds. It therefore seems that a meaningful path forward is to produce focused libraries based on rules of penetration. These could be obtained empirically[30], similarly to Lipinski's 'rule of five' guidelines[31], which have been highly valuable in selecting orally bioavailable compounds that hit mammalian targets.

Lipinski's guidelines were formulated by extracting the useful properties from a large set of known drugs and drug candidates[31]. Rules of penetration for antibiotics could be similarly established, based on data from a sufficiently large number of compounds that effectively penetrate into cells of Gram-negative bacteria, as well as those that do not. The missing piece is the availability of such a set of compounds, particularly as the existing panel of compounds that effectively penetrate Gram-negative

bacteria is very small. Nevertheless, obtaining such information is entirely feasible, as the penetration of compounds from a random library can be measured using mass spectrometry and other analytical methods. Once compounds are ranked by penetration, the rules can be deduced from the set.

Some insights into the rules of penetration can be gleaned even from the small set of good permeators that we currently have. One simple consideration is that MDR pumps have their preferences: hydrophobic cations are the preferred substrates[32–34] and highly hydrophobic compounds in general are also well recognized, whereas anions are not good substrates. Relatively hydrophilic compounds with a mass <600 Da are also favoured for penetration[35,36], probably owing to their ability to pass through porins of the outer membrane. Furthermore, it seems that the inclusion of atoms that are not frequently found in natural compounds (such as fluorine and boron) is good for penetration, possibly as MDR pumps have not been exposed to them. Another consideration regards targets: the broad-spectrum β-lactams are fairly hydrophilic, enter through porins and do not need to be amphipathic, as their target is in the periplasm. Fluoroquinolones, the only truly successful class of synthetic antibiotics, are small, hydrophilic and carry both an anionic group and a fluorine atom. The boron-containing compounds being developed by Anacor penetrate well into Gram-negative bacteria. The company's lead compound is a very small oxaboral ring molecule that inhibits tRNA[Leu] (tRNA recognizing the triplet codon for leucine) synthase of Gram-negative species[37]. The fact that these useful insights into permeability are based on a very small set of available compounds bodes well for the formulation of proper rules of penetration based on the evaluation of a large sample of molecules.

Once such rules are established, they are likely to breathe new life into the failed high-tech platforms. Libraries that are specifically compiled for antimicrobial discovery could be synthesized that would follow the rules of penetration. This would be a major undertaking, but an achievable one. The better-penetrating compounds from a screen of a commercial library could also serve as a basis for expanding the chemical space around these scaffolds and building a library that follows the rules of penetration. Similarly, these rules would be very helpful in resuscitating rational design based on the knowledge of the crystal structure of targets (for both traditional and fragment-based design strategies), as ligands could be optimized not only for good binding but also for penetration.

The in situ *screening platform: back to Domagk.* Prodrugs lacking a specific target are only one type of antimicrobial compounds that would be missed by modern screening and validation approaches. Is it not peculiar that the first useful antibiotic, the sulphanilamide drug prontosil[38], was discovered by Gerhard Domagk in the 1930s from a small screen of available dyes (probably no more than several hundred), whereas screens of the current libraries, which include ~10^7 compounds overall, have produced nothing at all?

As the libraries grew, various innovations were introduced that were aimed at improving the screening outcome; these include *in vitro* screening, targeted screens, Lipinski's 'rule of five' guidelines and specificity tests. It is possible that every time we tried to improve the outcome, valuable compounds were discarded. By contrast, Domagk tested compounds against mice infected with *Streptococcus* spp. An *in vitro* screen would have missed prontosil, as the compound is cleaved in the intestine by gut bacteria, releasing the active sulphonamide moiety, which inhibits dehydropteroate synthase in the folate pathway. There are obvious advantages to testing compounds *in situ*, as this automatically eliminates the considerable burden of toxic molecules and demonstrates efficacy, again automatically eliminating compounds that have problems such as serum binding, instability or poor tissue distribution *in vivo*. In addition, different types of compounds may be uniquely uncovered, such as those that require activation *in situ* and those that act on targets that are only important in an infection but not *in vitro*.

Although this would theoretically be a highly effective approach to discover viable antibiotic leads, screening compounds in 10^7 mice is not feasible for several reasons, including ethical considerations and because large amounts of the test compound would be required. We therefore considered an intermediate between *in vitro* testing and a mammalian model: the worm *Caenorhabditis elegans*, which can be dispersed in microtitre wells. *C. elegans* can be infected with human pathogens by simply ingesting them, and we found that these worms can be cured by common antibiotics such as tetracycline and vancomycin, at the concentrations typically observed in human plasma[39]. Dead worms infected with a pathogen such as *Enterococcus faecalis* can be detected using typical eukaryotic vital dyes. An automated approach based on vital staining was developed, and a pilot screen produced hits, some of which had no activity *in vitro*[39,40]. Although *C. elegans* lacks serum and other important properties, this platform has at least some of the benefits of the approach applied by Domagk.

Reviving the Waksman platform

The crisis in antibiotic discovery, especially the disappointing results obtained from the massive effort aimed at developing synthetic compounds, has prompted many experts to call for a revival of natural product drug discovery[17,41,42]. Prospecting for natural products is, in essence, a 'screen of screens': active compounds produced by bacteria are the result of natural selection over billions of years. There is little doubt that the screen performed by nature vastly outnumbers what we have in our compound libraries. The enormous background of known and/or nuisance compounds, however, presents a serious barrier to antibiotic discovery. Accessing the chemical diversity that has been hidden — by prospecting in the bottom of the ocean, growing previously uncultured organisms or turning on silent operons that could contain genes encoding the biosynthesis of novel natural products (see below) — could lead to novel antibiotics, but the background of known compounds remains an almost insurmountable obstacle.

Operons
Loci consisting of two or more genes that are transcribed as a unit and expressed in a coordinated manner.

Advances in analytical methods such as mass spectrometry and NMR allow dereplication as well as determination of an accurate mass and general structure to find an exact match to a known compound or at least make a reasonable guess as to the potential novelty of a compound. However, this often requires the analysis of a pure fraction and is far removed from an initial screen by an agar overlay, which can be performed with tens of thousands of bacterial colonies that are potentially producing antibiotics a week. For now, it seems that a biological approach is our best prospect for resolving the enormous bottleneck of known compounds.

Transcription profiling of antibiotic compounds enables a fairly good assignment into those compounds that are known; those that are unknown but hitting a known target; and those that are unknown and hitting a novel target[43]. This capability has existed for some time now, but the methodology based on hybridization was slow and costly for a high-throughput project. As a result of the dramatic (and continuing) decrease in costs, the sequencing of a large number of mRNA (reverse-transcribed) samples is now realistically possible. Once the profile is obtained, the identity of the target can be verified by comparing the activity of the extract against a wild-type strain with its activity against a strain in which a particular gene is knocked down (for example, this can be achieved via the expression of antisense RNA for the gene). Higher activity against the knockdown strain will confirm the identity of the target.

Elitra Pharmaceuticals and Merck developed a collection of 245 *S. aureus* strains, each with a knockdown in an essential gene; screening against a strain deficient in the 3-oxoacyl-acyl carrier protein synthase FabF led to the discovery of a novel class of antibiotics — the platensimycins[44]. Merck has made this collection available to researchers. A similar but complete collection is the ASKA overexpression *E. coli* library available from the Japan National Institute of Genetics[45]. The activity of a compound will be lower against an *E. coli* strain overexpressing a target than against the wild-type strain. Permeabilizing *E. coli* cells by adding polymyxin B nonapeptide to the medium will allow the testing of narrow-spectrum compounds as well[46].

An important question is how many producing strains to test. In an influential paper, Richard Baltz estimated that 10^7 strains would need to be examined in order to discover the next novel class of useful antibiotics[47]. This estimate was made on the basis of a simple assumption: the probability of finding a compound depends on its frequency of occurrence in nature and on the total number of strains screened. The frequency of occurrence of a compound in nature is available for some of the most common antibiotics: nearly 10% of streptomycetes produce streptothricin, 1% make streptomycin and 0.1% produce tetracycline. Waksman would have discovered streptomycin by screening the first 100 or so strains in his collection. Knowing the total number of strains screened and the year of discovery can provide an estimate of frequency for the rest of the antibiotics. For example, erythromycin was discovered in 1949, and the estimated number of strains screened by that time is

2×10^5, resulting in a probability of 5×10^{-6}. The estimated probability of daptomycin, one of the last antibiotics to be discovered and introduced into the clinic is 2×10^{-7}. A novel compound class is therefore probably somewhere among 10^7 different microorganisms.

It is possible that this calculation encouraged the termination of the natural product discovery efforts at large pharmaceutical companies, but it may also need to be adjusted in the light of recent research. The typical size of a strain collection at a large pharmaceutical company is relatively modest: ~50,000 isolates. The recent discovery of platensimycin[44] and phomallenic acid[48] was made by scientists at Merck using their old collection that had been previously screened for growth-inhibitory activity against *S. aureus*. Platensimycin and phomallenic acid were discovered through a targeted screen for compounds that were more active against a strain of *S. aureus* with a knockdown in the FabF and FabB elongation-condensing enzymes in the fatty acid synthase II (FASII) biosynthetic pathway. Although the development of these compounds is uncertain owing to their poor *in vivo* stability and solubility, and because fatty acids may be available *in vivo*, they can certainly be considered as novel and potentially useful compounds.

So, why were platensimycin and phomallenic acids missed in previous screens for compounds inhibiting growth? They were probably not missed but instead ignored; the activity of the producing strains is relatively low, and these compounds do not make large zones of inhibition on test plates. A certain cut-off point for activity is typically used in the discovery process. Unless you know that a compound is potentially interesting, as was the case in the targeted screen for FASII inhibitors, you will choose to focus on producing strains with high activity. Importantly, the Merck screen led to the rediscovery of all known inhibitors of FabF and FabB enzymes as well as the associated FabH enzyme (the initiation-condensing enzyme of the same pathway): cerulenin, thiolactomycin, thiotetromycin and Tu3010. This suggests that the screen was comprehensive and the discovery of the two novel classes was not an accident. This example also suggests that novel screening approaches may uncover new compounds in old libraries at probabilities of around 10^{-5} to 10^{-4} rather than 10^{-7}. Furthermore, if the dereplication problem can be solved by transcription profiling, then natural products could once again become an excellent source of new antibiotics. The probability of antibiotic discovery will be further increased if we turn to new sources of natural products.

Untapped sources of natural compounds. Chemical diversity follows biological diversity; this is a common and probably correct view among scientists working in the field of natural products. It would be beneficial to have a more solid basis for this view than the opinion of experts in the field, and this is one of the essential pieces of information that would emerge if we build a database of known antibiotics (approximately 3,000), their producing strains and their targets. Placing antibiotics on a taxonomic relatedness tree of producers will reveal the relationship between biological and chemical novelty.

Dereplication
The rapid identification of known compounds to avoid the duplication of efforts (also called counterscreening).

Transcription profiling
Large-scale studies of the expression of genes at the mRNA level, typically with microarray technology.

Polymyxin B nonapeptide
A small cationic peptide that disrupts the outer membrane of Gram-negative bacteria by binding to lipopolysaccharide.

The first antibiotic producer was discovered in a laboratory in St. Mary's Hospital in London, UK, after a *Penicillium rubens* spore landed on a partially opened Petri dish[2]. The next producers were obtained in a focused isolation from the soil of Rutgers University in New Jersey, USA[1]. As the discovery efforts broadened, so did prospecting, and companies would ask their employees to bring soil samples from their travels. Some of the strains that produce currently used antibiotics come from far-flung locations — the producer of vancomycin was from Southeast Asia, for example. After the golden era of antibiotic discovery ended, a more concerted effort was made to prospect outside North America and Europe, but this did not pay off; it seems that the diversity of secondary metabolites in soils of the Southern hemisphere or Asia does not differ much from that in soil samples from the better-explored locations of the Northern hemisphere.

Exploring the oceans has led to the discovery of a plethora of secondary metabolites, especially compounds from marine invertebrates such as sponges and corals. Interestingly, it appears that most of these compounds are actually made by bacteria colonizing these organisms. Some of the novel compounds are antimicrobials. For example, sporolide A and sporolide B are compounds from *Salinispora tropica* actinomycetes living in the marine sediment[49]. However, toxicity has been a problem for secondary metabolites derived from the sea, which has so far precluded their development as antibiotics.

Another untapped source of antimicrobials is the silent operons of producing microorganisms. Sequencing of the first genomes of actinomycetes showed that their potential for producing secondary metabolites far surpasses their known ability. Enzymes producing antimicrobials — the polyketide synthases and non-ribosomal peptide synthases — are encoded by large operons that are homologous to known genes. Sequencing the genome of *Streptomyces coelicolor* — the model actinomycete — showed that it encodes 20 secondary metabolites, although it is known to produce only three antimicrobial compounds[50]. *Streptomycetes avermitilis*, the industrial producer of the anti-helminthic avermectin, does not produce antimicrobial compounds at all (at least not in the laboratory), but according to its genome sequence it harbours 30 operons for secondary metabolites[51]. Many of the additional actinomycete genomes that have since been sequenced similarly contain numerous silent operons.

Trying to stimulate the production of antimicrobials by varying growth conditions is a standard approach to discovery; initial small-scale fermentations are usually carried out in several different media, and activity is tested by taking samples over time. This approach is helpful but not enough, as evidenced by the unrealized genomic potential. At least some silent operons do encode antimicrobial compounds.

One approach is to simply try many different growth media. Ecopia BioSciences was an early pioneer in genome mining for biosynthetic clusters, and producer strains selected by bioinformatics were then fermented in 40 different media[52]. This resulted in the discovery of several novel secondary metabolites, including new enediynes — highly reactive compounds that were advanced as anticancer agents but ultimately failed. One obvious reason for the failure was the limited ability of empirically composed fermentation conditions to stimulate the production of the hidden wealth of secondary metabolites; indeed, the fact that we contemplate using tomato juice or cornmeal to elicit the production of antibiotics from an isolate, 60 years after Waksman's work, indicates that this aspect of the science of antibiotic discovery is not in great shape.

Nevertheless, there have been notable advances in our understanding of what stimulates bacteria to produce antibiotics. Antibiotic production usually peaks when the culture approaches the stationary state. The first quorum sensing factor, butyrolactone, was actually discovered in a study that was aimed at identifying a factor to activate the production of streptomycin by *Actinomyces streptomycin* (also known as *Streptomyces griseus*)[53]. Since this early study, butyrolactones were found to induce the production of various antibiotics in several actinomycetes, and a regulatory pathway linking butyrolactone to streptomycin synthesis was identified in *S. griseus*[54,55]. However, starvation and quorum sensing factors are not universal elicitors of antibiotic synthesis, and it is not yet known what the missing factors are.

One straightforward approach to circumvent this lack of knowledge is to force the production of encoded secondary metabolites by cloning the silent operons in a suitable host or by engineering expression in the original producer by disrupting putative repressors or overexpressing activators[51,56–58]. Every year, several compounds are reported in studies that are focused on manipulating silent operons[59]. This is interesting and important research, but the current throughput cannot support a drug discovery pipeline. The large-scale induction of silent operons for antibiotic production remains a considerable challenge, but could provide a new platform for antibiotic discovery if it can be successfully achieved.

Uncultured bacteria are perhaps the most promising untapped source of secondary metabolites[41,42]. Based on metagenomic analyses of soil and marine samples, 99% of all microbial species on the planet are 'uncultured'; that is, they do not grow under laboratory conditions[60,61]. Several groups in academia and the industry, realizing that this new source of potential antibiotics could revive the Waksman platform, established metagenomic approaches to access the enormous hidden potential of these uncultured microorganisms. Biotechnology companies such as TerraGen Discovery, Xoma, Diversa and Millennium Pharmaceuticals as well as at least one large pharmaceutical company, Rhône-Poulenc Rorer, searched for antimicrobial compounds from uncultured bacteria. There was an appealing elegance in this approach: the metabolites of uncultured microorganisms would be accessed by cloning the producing operons from soil DNA, without the need to know to which organisms those sequences belonged to[62]. Cloned DNA was transformed into *E. coli*, *S. coelicolor* or another suitable host. Proof of principle was established for this approach and several antimicrobial compounds resulting from soil DNA were described[63].

Quorum sensing
A system by which bacteria communicate. Signalling molecules — chemicals that are similar to pheromones that are produced by an individual bacterium — can affect the behaviour of surrounding bacteria.

But, as with silent operons, the throughput never surpassed several compounds per year, and so the approach was abandoned.

Growing bacteria that have previously not been cultured is an obvious alternative, and a general method for this has been developed[64–66]. The essence of the approach is to grow bacteria in their natural environment. This is done by introducing a marine sediment or soil sample diluted with agar between two semipermeable membranes, and placing this 'diffusion chamber' back into the natural environment (or in a simulated environment such as a marine aquarium or a bucket of soil). Compounds diffuse freely through the chamber, and bacteria are tricked into perceiving it as their natural environment. This approach dramatically improves growth recovery; up to 40% of cells seeded into the chamber produce colonies.

A common cause of 'uncultivatability' is a need for growth factors produced by neighbouring bacteria. Iron-chelating siderophores have been identified as an important group of such factors in the marine sediment environment[67]. Repeated reinoculation from chamber to chamber results in the 'domestication' of a considerable proportion of uncultured microorganisms, which enables their growth on a Petri dish or in a fermenter[68]. At least one company, NovoBiotic Pharmaceuticals, has been using *in situ* cultivation for antibiotic discovery.

Fungi have been an important source of antimicrobials and secondary metabolites in general. Penicillin and later another class of β-lactams, cephalosporins, have been isolated from fungi. Fungi apparently 'stole' these antibiotic-coding genes from actinomycetes, and — perhaps not surprisingly — the discovery of useful antibiotics from fungi has been limited to these two classes (penicillins and cephalosporins). Fungi are very good at making generally toxic antimicrobial compounds and secondary metabolites that attack eukaryotes, including other fungi. Echinocandins, inhibitors of fungal cell wall synthesis that target the β-glucan synthase, were discovered in an extract of *Aspergillus nidulans*. Caspofungin, a potent antifungal, was derived from echinocandin[69]. Caspofungin is an excellent broad-spectrum antifungal compound that kills growing cells; it is, in many respects, analogous to antibacterial β-lactams. Humans lack the caspofungin target, so the drug is well tolerated. Caspofungin is also the only natural antifungal product originating from fungi. It is unclear at present whether there are additional useful antifungals to be discovered from this source; fungi, unlike bacteria, share many conserved targets with mammals, and so finding a specific antifungal compound is difficult.

Conversely, the homologous targets present an opportunity to use fungal secondary metabolites for treating human diseases. Fungi were the source of the first statins[70], a class of highly successful cholesterol-lowering compounds, as well as the immunosuppressants cyclosporine[71] and rapamycin[72]. Inhibitors of heat shock protein 90 derived from fungi are also currently in development as cancer chemotherapeutics[73]. It seems likely that exploiting fungi will lead to the discovery of additional useful drugs, but it is currently unclear whether fungi are a good source of antimicrobial compounds.

Plants, which were historically the source of remedies to treat any disease, are another potential source of antimicrobial compounds. Numerous drugs are of plant origin and derived from toxins that evolved to prevent grazing by animals, and these toxins are useful when taken in moderation. For example, glucoside inhibitors of sodium/potassium ATPase are used to treat cardiac arrhythmias and certain kinds of heart failure[74]. There are also two plant-derived antimicrobial therapeutics, both of which are borrowed from ethnobotany and are antimalarial: quinine and artemisinin. Quinine comes from the bark of the Cinchona tree[75] and was used by Native Americans, whereas artemisinin is produced by *Artemiannua*[76] and is used in traditional Chinese medicine as an antimalarial compound.

However, there are no plant-derived antibiotics, which is surprising given the very large effort aimed at mining this source and the fact that plants have numerous chemical defences against their microbial pathogens[77]. It appears that plants do not generally produce highly potent inhibitors of specific microbial targets. The known exception is coumarins, which are inhibitors of bacterial DNA gyrase[78]. But even in this case, novobiocin, a systemic therapeutic produced by streptomycetes, is a more potent inhibitor of the same class.

Microorganisms rapidly acquire resistance, which probably forces plants to rely mainly on nonspecific and therefore toxic compounds[34]. A good example is berberine alkaloids, which are membrane-acting hydrophobic cations and also DNA intercalators. They accumulate inside the cell, driven by the membrane potential, and hit two immutable targets: the cell membrane and DNA. The only defence against these compounds is efflux by MDR pumps, and it is probably not an accident that hydrophobic cations are the preferred substrates of MDR pumps[32]. Plants harbour various MDR pump inhibitors, producing an effective synergistic combination with berberine[33,34]. The known inhibitors, however, act only against the major facilitator (MF) family of MDR pumps of Gram-positive species; efforts to find inhibitors of RND MDR pumps of Gram-negative species have not been successful (K.L., unpublished observations), and there are no known small-molecule plant-derived antimicrobial compounds with high activity against Gram-negative species. The generally toxic plant phenols, catechols and tannins are more widespread and less sophisticated than berberines. Even the antimalarial products do not appear to have a target: quinine and artemisinin are iron chelators. The exact mechanism by which they inhibit the growth of the malarial parasite remains unclear; however, it is thought that they interfere with haem sequestration[79].

One important missing piece from the studies of plant-derived antimicrobial compounds is their mode of action. There have been numerous publications describing the isolation and purification of plant-derived antimicrobials, in which activity against a limited panel of microorganisms has served as the end point of the study. In a small number of cases, compounds with high submicromolar activity against *S. aureus* have been described, pointing to a possible target-specific mode of action
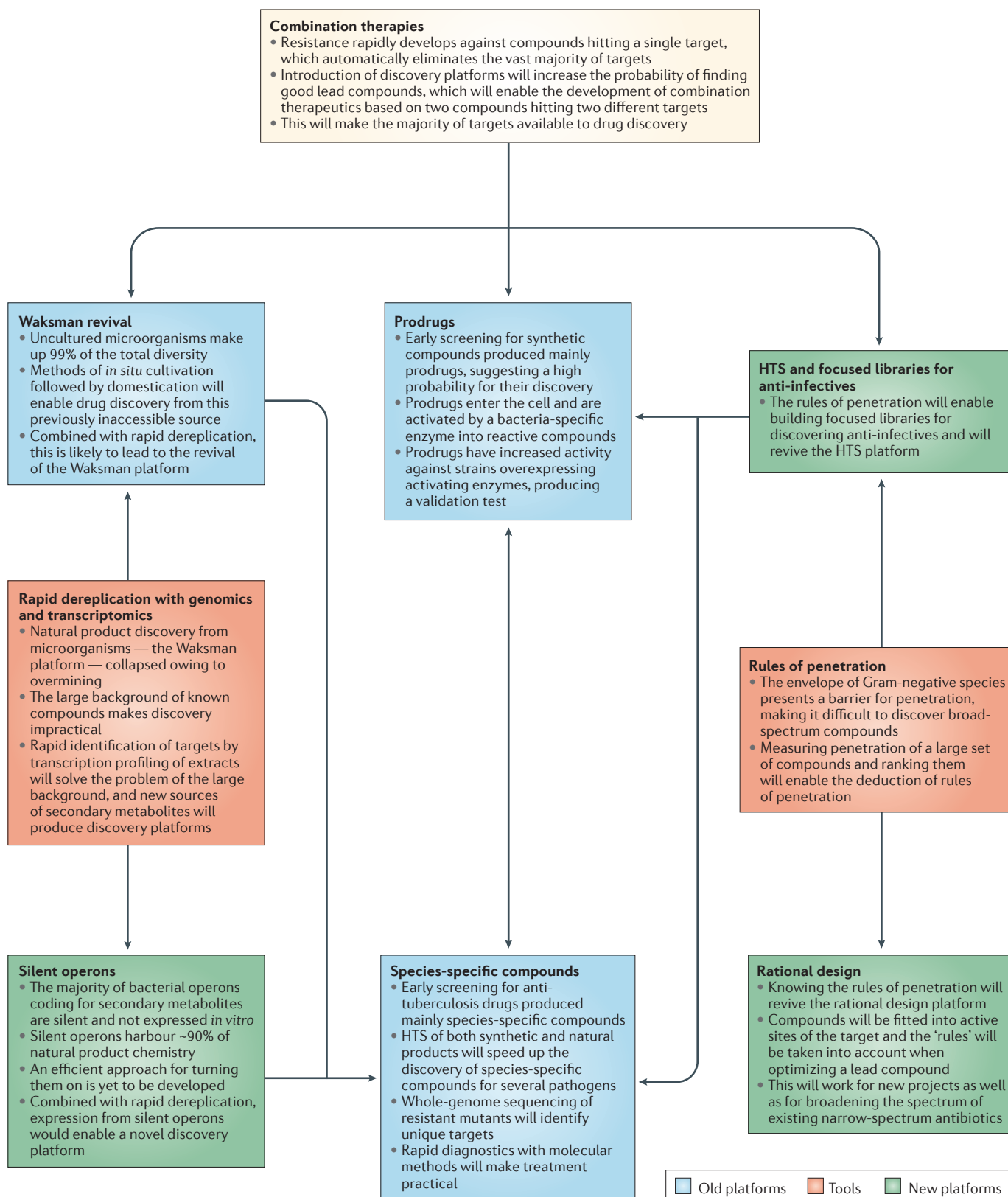
Combination therapies
- Resistance rapidly develops against compounds hitting a single target, which automatically eliminates the vast majority of targets
- Introduction of discovery platforms will increase the probability of finding good lead compounds, which will enable the development of combination therapeutics based on two compounds hitting two different targets
- This will make the majority of targets available to drug discovery

Waksman revival
- Uncultured microorganisms make up 99% of the total diversity
- Methods of *in situ* cultivation followed by domestication will enable drug discovery from this previously inaccessible source
- Combined with rapid dereplication, this is likely to lead to the revival of the Waksman platform

Prodrugs
- Early screening for synthetic compounds produced mainly prodrugs, suggesting a high probability for their discovery
- Prodrugs enter the cell and are activated by a bacteria-specific enzyme into reactive compounds
- Prodrugs have increased activity against strains overexpressing activating enzymes, producing a validation test

HTS and focused libraries for anti-infectives
- The rules of penetration will enable building focused libraries for discovering anti-infectives and will revive the HTS platform

Rapid dereplication with genomics and transcriptomics
- Natural product discovery from microorganisms — the Waksman platform — collapsed owing to overmining
- The large background of known compounds makes discovery impractical
- Rapid identification of targets by transcription profiling of extracts will solve the problem of the large background, and new sources of secondary metabolites will produce discovery platforms

Rules of penetration
- The envelope of Gram-negative species presents a barrier for penetration, making it difficult to discover broad-spectrum compounds
- Measuring penetration of a large set of compounds and ranking them will enable the deduction of rules of penetration

Silent operons
- The majority of bacterial operons coding for secondary metabolites are silent and not expressed *in vitro*
- Silent operons harbour ~90% of natural product chemistry
- An efficient approach for turning them on is yet to be developed
- Combined with rapid dereplication, expression from silent operons would enable a novel discovery platform

Species-specific compounds
- Early screening for anti-tuberculosis drugs produced mainly species-specific compounds
- HTS of both synthetic and natural products will speed up the discovery of species-specific compounds for several pathogens
- Whole-genome sequencing of resistant mutants will identify unique targets
- Rapid diagnostics with molecular methods will make treatment practical

Rational design
- Knowing the rules of penetration will revive the rational design platform
- Compounds will be fitted into active sites of the target and the 'rules' will be taken into account when optimizing a lead compound
- This will work for new projects as well as for broadening the spectrum of existing narrow-spectrum antibiotics

☐ Old platforms   ☐ Tools   ☐ New platforms

Figure 4 | **Platforms for discovering novel antibiotics.** New tools (red boxes) will enable the development of effective discovery platforms. Old successful platforms (blue boxes) can be revived and new ones (green boxes) can be developed. Once platforms start to churn out lead compounds reliably, this will enable the development of combination therapies (white box). The relationships between tools and platforms are shown as a network. For example, a revived Waksman platform would enable the discovery of species-specific antibiotics. HTS, high-throughput screening.

Box 4 | Other possible platforms for developing antibiotics

Several additional approaches to antibiotic discovery have been pursued, including targeting virulence factors, antimicrobial peptides and phage therapy, and these are summarized briefly below. Monoclonal antibodies (for example, to neutralize bacterial toxins)[114] have also been investigated, but immunotherapies and vaccines are not considered in this article.

Virulence factors
The rationale behind targeting virulence factors is based on the ostensible lack of resistance development towards a therapeutic that will not inhibit cell growth. By contrast, there is a high frequency of resistance for compounds that act on a single target, and this presents a barrier that will eliminate most compounds (and targets) from consideration. If blocking a virulence factor will not lead to resistance, this class of targets becomes fairly attractive.

   However, it is also possible that any decrease in fitness — in this case a decrease in the ability to survive and proliferate effectively in a host environment — will lead to the selection of bacteria that have resistance against an anti-virulence agent. However, we do not know whether this does occur, and it is surprising that this hypothesis has not been tested. Various experimental compounds have been reported that inhibit virulence factors and show *in vivo* efficacy in animal models of disease[115,116], which means that the tools for testing the development of resistance are available. Anti-virulence programmes were put in place at several large pharmaceutical companies 15–20 years ago, but are no longer in existence.

   Potential problems associated with this approach include: narrow-spectrum activity; lack of a convenient end point for animal studies; uncertainty associated with the ability of such factors to cure rather than prevent an infection; and an untested path for clinical studies. This approach continues to be popular in academia, but a compound has yet to reach clinical trials. Experimental evidence for no (or low) resistance will probably reinvigorate this approach.

Antimicrobial peptides
Animals, plants and bacteria all produce antimicrobial peptides (AMPs)[117–119], and these are probably as numerous and varied as small-molecule antibiotics. Although these compounds are not homologous, they share several similarities: they are primarily small (3–5 kDa), alpha-helical, amphipathic, positively charged membrane-acting molecules. Their mode of action presents the main problem — toxicity. Humans produce numerous AMPs, including defensins (found in the bloodstream), which are an important component of non-adaptive immunity. One would imagine that defensins should be potent and non-toxic, given their location. However, this is not the case; defensins show considerable cytotoxicity at the concentrations that are needed to effectively target a broad range of pathogens.

   A concerted effort over the past few decades, aimed at developing natural, semi-synthetic and synthetic AMPs, has not resulted in a therapeutic. Nevertheless, daptomycin — a membrane-acting antibiotic — could be classified as an AMP; it is a cyclic amphipathic peptide produced by *Streptomyces roseosporus*. Daptomycin increases the potassium conductance of bacterial membranes and has a reasonably good selectivity, sparing human cells[4], but the compound does have toxicity issues and is only used against serious infections as a second-line therapeutic. The mechanistic basis for daptomycin's selectivity is not well understood. The example of daptomycin suggests, however, that there may be promise for AMPs.

Phage therapy
Phages are specific and rapidly propagate, which should help to fight an infection. There are good *in vitro* data suggesting that phage therapy is effective against the difficult to treat biofilms[120,121]. Phages will also enter dormant persisters and kill them when they reactivate[122]. Several biotech companies have been developing phage therapies for over a decade. There are many barriers to the development of therapeutic phages: development of resistance is easy, necessitating the need for a cocktail of phages, which in turn makes it difficult to manufacture standard lots of a product; the immunogenicity of a phage can be problematic; and there is an untested path to clinical studies[123]. There is also progress in this challenging field, as a topical phage therapy — Biophage-PA — is in clinical trials in the United Kingdom against antibiotic-resistant *Pseudomonas aeruginosa* in chronic otitis[124].

(reviewed in REF. 80). It is worth taking a second look at these compounds to determine their mode of action and test their cytotoxicity. Perhaps plants will turn out to be a useful source of species-selective compounds acting against Gram-positive pathogens.

Concluding remarks
The antibiotic discovery platforms proposed in this article are summarized in FIG. 4 (other platforms that have been investigated are discussed briefly in BOX 4). Some of these platforms — species-specific approaches and prodrug discovery — follow directly from old practices and can be improved by applying modern tools of validation. Reviving the Waksman platform will require

the development of new biology-based dereplication tools, which is entirely feasible on the basis of what we currently know. Establishing the rules that govern the penetration of molecules across the bacterial envelope will enable the failed high-tech platforms — target-based HTS and rational design — to be pursued effectively. The only platform for which we do not currently have a straightforward path for development is the activation of the silent operons of microorganisms that could potentially produce antibiotics.

   It is worth remembering that early successes in antibiotic discovery and development were based on very modest knowledge; most drugs were introduced well before we had any idea of their mode of action or how

Biofilms
A cell–cell or surface-adherent assemblage of microorganisms that are encased in an extracellular matrix of self-produced polymers and exhibit distinctive phenotypes.

they are made by the producing microorganisms. Since the end of the golden age of antibiotic discovery in the 1960s, microbiology has made enormous advances in understanding the fundamental questions regarding bacterial cells and their interactions with humans. We now know that microbes have complex developmental programmes determining cell fate; bacteria form multicellular societies based on members that communicate with each other; pathogens subvert their hosts by injecting effectors into their cells; and a microbial cell is not a bag of enzymes, but a complex structure with a cytoskeleton and precise placement of hereditary and sensory components[81]. We have also acquired specific knowledge about the targets of currently used antibiotics and the mechanisms of resistance and tolerance.

Microbiology has also been the birthplace of major revolutions in science, including molecular biology (such as recombinant DNA and gene cloning) and genomics (*Haemophilus influenzae* was the first organism with a sequenced genome). The field continues to incubate revolutions in the making, such as synthetic biology, systems biology and the synthesis of new organisms[82]. But for several reasons, the relatively simple questions that are most relevant to the discovery of antibiotics — something microbiology as a discipline should contribute to society — have been neglected. For example, what exactly allows compounds to penetrate into bacterial cells? What are the good targets that have been identified by nature, beyond the small number of clinically important compounds? What are the properties of the thousands of antibiotics that have been discovered but were never properly examined? It is high time we build a solid scientific base for the platforms that will transform the lottery of prospecting for antibiotics into a science of discovery.

1. Schatz, A., Bugie, E. & Waksman, S. A. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. *Proc. Soc. Exp. Biol. Med.* **55**, 66–69 (1944).
2. Fleming, A. G. Responsibilities and opportunities of the private practitioner in preventive medicine. *Can. Med. Assoc. J.* **20**, 11–13 (1929).
3. Payne, D. J., Gwynn, M. N., Holmes, D. J. & Pompliano, D. L. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nature Rev. Drug Discov.* **6**, 29–40 (2007).
   This is a revealing account, from the industry perspective, of the failure to develop synthetic antimicrobials by rational design or HTS.
4. Eliopoulos, G. M. *et al. In vitro* and *in vivo* activity of LY 146032, a new cyclic lipopeptide antibiotic. *Antimicrob. Agents Chemother.* **30**, 532–535 (1986).
5. Nord, C. E., Lindmark, A. & Persson, I. Susceptibility of anaerobic bacteria to the new streptogramin RP 59500 *in vitro*. *Eur. J. Clin. Microbiol. Infect. Dis.* **8**, 1064–1067 (1989).
6. Talpaert, M., Campagnari, F. & Clerici, L. Lipiarmycin: an antibiotic inhibiting nucleic acid polymerases. *Biochem. Biophys. Res. Commun.* **63**, 328–334 (1975).
7. McDonald, K. M. *et al.* Closing the quality gap: a critical analysis of quality improvement strategies. (Vol. 7: Care Coordination; Technical Review No. 9) *US Agency for Healthcare Research and Quality* [online], http://www.ahrq.gov/downloads/pub/evidence/pdf/caregap/caregap.pdf (2007).
8. Gillespie, S. H. Evolution of drug resistance in *Mycobacterium tuberculosis*: clinical and molecular perspective. *Antimicrob. Agents Chemother.* **46**, 267–274 (2002).
9. Almeida Da Silva, P. E. & Palomino, J. C. Molecular basis and mechanisms of drug resistance in *Mycobacterium tuberculosis*: classical and new drugs. *J. Antimicrob. Chemother.* **66**, 1417–1430 (2011).
10. Banerjee, A. *et al.* InhA, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science* **263**, 227–230 (1994).
11. Morlock, G. P., Metchock, B., Sikes, D., Crawford, J. T. & Cooksey, R. C. ethA, inhA, and katG loci of ethionamide-resistant clinical *Mycobacterium tuberculosis* isolates. *Antimicrob. Agents Chemother.* **47**, 3799–3805 (2003).
12. Shi, W. *et al.* Pyrazinamide inhibits *trans*-translation in *Mycobacterium tuberculosis*. *Science* **333**, 1630–1632 (2011).
    This paper uncovered the molecular target for a 50-year old drug.
13. Sneader, W. *Drug Discovery: A History* (Wiley, 2005).
14. Andries, K. *et al.* A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* **307**, 223–227 (2005).
    This paper reported the first successful antibiotic lead from an HTS of synthetic compounds, a species-specific anti-TB agent.
15. Takayama, K. & Kilburn, J. O. Inhibition of synthesis of arabinogalactan by ethambutol in *Mycobacterium smegmatis*. *Antimicrob. Agents Chemother.* **33**, 1493–1499 (1989).
16. Diacon, A. H. *et al.* The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N. Engl. J. Med.* **360**, 2397–2405 (2009).
17. Fischbach, M. A. & Walsh, C. T. Antibiotics for emerging pathogens. *Science* **325**, 1089–1093 (2009).
18. Larsen, N. *et al.* Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PloS ONE* **5**, e9085 (2010).
19. Turnbaugh, P. J. *et al.* A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484 (2009).
20. Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L. & Gordon, J. I. Human nutrition, the gut microbiome and the immune system. *Nature* **474**, 327–336 (2011).
21. Neufeld, K. M., Kang, N., Bienenstock, J. & Foster, J. A. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol. Motil.* **23**, 255–e119 (2011).
22. Dethlefsen, L. & Relman, D. A. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc. Natl Acad. Sci. USA* **108** (Suppl. 1), 4554–4561 (2011).
    This paper shows that broad-spectrum antibiotics harm the symbiotic flora.
23. Schofield, D. A., Sharp, N. J. & Westwater, C. Phage-based platforms for the clinical detection of human bacterial pathogens. *Bacteriophage* **2**, 105–121 (2012).
24. Renau, T. E. *et al.* Inhibitors of efflux pumps in *Pseudomonas aeruginosa* potentiate the activity of the fluoroquinolone antibacterial levofloxacin. *J. Med. Chem.* **42**, 4928–4931 (1999).
25. Lomovskaya, O. *et al.* Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. *Antimicrob. Agents Chemother.* **45**, 105–116 (2001).
    This paper describes a class of inhibitors for MDR pumps, providing proof of principle for targeting them.
26. Murakami, S., Nakashima, R., Yamashita, E. & Yamaguchi, A. Crystal structure of bacterial multidrug efflux transporter AcrB. *Nature* **419**, 587–593 (2002).
27. Yu, E. W., McDermott, G., Zgurskaya, H. I., Nikaido, H. & Koshland, D. E. Jr. Structural basis of multiple drug-binding capacity of the AcrB multidrug efflux pump. *Science* **300**, 976–980 (2003).
28. Symmons, M. F., Bokma, E., Koronakis, E., Hughes, C. & Koronakis, V. The assembled structure of a complete tripartite bacterial multidrug efflux pump. *Proc. Natl Acad. Sci. USA* **106**, 7173–7178 (2009).
29. Nakashima, R., Sakurai, K., Yamasaki, S., Nishino, K. & Yamaguchi, A. Structures of the multidrug exporter AcrB reveal a proximal multisite drug-binding pocket. *Nature* **480**, 565–569 (2011).
30. Lewis, K. in *Antibiotic resistance: implications for global health and novel intervention strategies — workshop summary* (eds Choffnes, E. R., Relman, D. A. & Mack, A.) 202–223 (National Academies Press, 2010).
31. Lipinski, C. A., Lombardo, F., Dominy, B. W. & Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **46**, 3–26 (2001).
    This paper reports the first successful attempt to formulate rules for orally bioavailable drug candidates based on an empirical analysis of compounds.
32. Hsieh, P. C., Siegel, S. A., Rogers, B., Davis, D. & Lewis, K. Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. *Proc. Natl Acad. Sci. USA* **95**, 6602–6606 (1998).
33. Stermitz, F. R., Lorenz, P., Tawara, J. N., Zenewicz, L. A. & Lewis, K. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor. *Proc. Natl Acad. Sci. USA* **97**, 1433–1437 (2000).
34. Lewis, K. & Ausubel, F. M. Prospects for plant-derived antimicrobials. *Nature Biotech.* **24**, 1504–1507 (2006).
35. O'Shea, R. & Moser, H. E. Physicochemical properties of antibacterial compounds: implications for drug discovery. *J. Med. Chem.* **51**, 2871–2878 (2008).
    This is the first study to systematically link the physicochemical properties of compounds to their ability to penetrate into bacteria.
36. Silver, L. L. Are natural products still the best source for antibacterial discovery? The bacterial entry factor. *Expert Opin. Drug Discov.* **3**, 487–500 (2008).
37. Rock, F. L. *et al.* An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site. *Science* **316**, 1759–1761 (2007).
38. Domagk, G. Ein beitrag zur chemotherapie der bakteriellen infektionen. *Deutsche Medizinische Wochenschrift* **61**, 250–253 (in German) (1935).
39. Moy, T. I. *et al.* Identification of novel antimicrobials using a live-animal infection model. *Proc. Natl Acad. Sci. USA* **103**, 10414–10419 (2006).
    This is the first description of a live-animal screen for antimicrobials.
40. O'Rourke, E. J., Conery, A. L. & Moy, T. I. Whole-animal high-throughput screens: the *C. elegans* model. *Methods Mol. Biol.* **486**, 57–75 (2009).
41. Clardy, J., Fischbach, M. A. & Walsh, C. T. New antibiotics from bacterial natural products. *Nature Biotech.* **24**, 1541–1550 (2006).
42. Demain, A. L. Prescription for an ailing pharmaceutical industry. *Nature Biotech.* **20**, 331 (2002).
43. Hutter, B. *et al.* Prediction of mechanisms of action of antibacterial compounds by gene expression profiling. *Antimicrob. Agents Chemother.* **48**, 2838–2844 (2004).

44. Wang, J. *et al.* Platensimycin is a selective FabF inhibitor with potent antibiotic properties. *Nature* **441**, 358–361 (2006).
This paper reports the discovery of a new class of antimicrobials based on a targeted whole-cell screen of natural products.

45. Kitagawa, M. *et al.* Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *E. coli* K-12 ORF archive): unique resources for biological research. *DNA Res.* **12**, 291–299 (2005).

46. Morris, C. M., George, A., Wilson, W. W. & Champlin, F. R. Effect of polymyxin B nonapeptide on daptomycin permeability and cell surface properties in *Pseudomonas aeruginosa*, *Escherichia coli*, and *Pasteurella multocida*. *J. Antibiot.* **48**, 67–72 (1995).

47. Baltz, R. H. Antibiotics from actinomycetes: will a renaissance follow the decline and fall? *SIM News* **55**, 186–196 (2005).

48. Young, K. *et al.* Discovery of FabH/FabF inhibitors from natural products. *Antimicrob. Agents Chemother.* **50**, 519–526 (2006).

49. Buchanan, G. O. *et al.* Sporolides A and B: structurally unprecedented halogenated macrolides from the marine actinomycete *Salinispora tropica*. *Org. Lett.* **7**, 2731–2734 (2005).

50. Bentley, S. D. *et al.* Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417**, 141–147 (2002).
This is the first genomic study to show the unrealized potential of silent operons for secondary metabolites.

51. Ikeda, H. *et al.* Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. *Nature Biotech.* **21**, 526–531 (2003).

52. Zazopoulos, E. *et al.* A genomics-guided approach for discovering and expressing cryptic metabolic pathways. *Nature Biotech.* **21**, 187–190 (2003).

53. Khokhlov, A. S. *et al.* The A-factor, responsible for streptomycin biosynthesis by mutant strains of *Actinomyces streptomycini*. *Dokl. Akad. Nauk SSSR* **177**, 232–235 (1967).

54. Ohnishi, Y., Kameyama, S., Onaka, H. & Horinouchi, S. The A-factor regulatory cascade leading to streptomycin biosynthesis in *Streptomyces griseus*: identification of a target gene of the A-factor receptor. *Mol. Microbiol.* **34**, 102–111 (1999).

55. Horinouchi, S. A microbial hormone, A-factor, as a master switch for morphological differentiation and secondary metabolism in *Streptomyces griseus*. *Front. Biosci.* **7**, 2045–2057 (2002).

56. Baltz, R. H. *Streptomyces* and *Saccharopolyspora* hosts for heterologous expression of secondary metabolite gene clusters. *J. Ind. Microbiol. Biotechnol.* **37**, 759–772 (2010).

57. Gomez-Escribano, J. P. & Bibb, M. J. Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters. *Microb. Biotechnol.* **4**, 207–215 (2011).

58. Komatsu, M., Uchiyama, T., Omura, S., Cane, D. E. & Ikeda, H. Genome-minimized *Streptomyces* host for the heterologous expression of secondary metabolism. *Proc. Natl Acad. Sci. USA* **107**, 2646–2651 (2010).

59. Baltz, R. H. Strain improvement in actinomycetes in the postgenomic era. *J. Ind. Microbiol. Biotechnol.* **38**, 657–666 (2011).

60. Rappe, M. S. & Giovannoni, S. J. The uncultured microbial majority. *Annu. Rev. Microbiol.* **57**, 369–394 (2003).

61. Schloss, P. D. & Handelsman, J. Status of the microbial census. *Microbiol. Mol. Biol. Rev.* **68**, 686–691 (2004).

62. Seow, K. T. *et al.* A study of iterative type II polyketide synthases, using bacterial genes cloned from soil DNA: a means to access and use genes from uncultured microorganisms. *J. Bacteriol.* **179**, 7360–7368 (1997).

63. Gillespie, D. E. *et al.* Isolation of antibiotics turbomycin A and B from a metagenomic library of soil microbial DNA. *Appl. Environ. Microbiol.* **68**, 4301–4306 (2002).

64. Kaeberlein, T., Lewis, K. & Epstein, S. S. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science* **296**, 1127–1129 (2002).
This paper describes a general method to grow uncultured bacteria.

65. Gavrish, E., Bollmann, A., Epstein, S. & Lewis, K. A trap for *in situ* cultivation of filamentous actinobacteria. *J. Microbiol. Methods* **72**, 257–262 (2008).

66. Nichols, D. *et al.* Use of ichip for high-throughput *in situ* cultivation of "uncultivable" microbial species. *Appl. Environ. Microbiol.* **76**, 2445–2450 (2010).

67. D'Onofrio, A. *et al.* Siderophores from neighboring organisms promote the growth of uncultured bacteria. *Chem. Biol.* **17**, 254–264 (2010).
This is the first description of growth factors for uncultured bacteria.

68. Bollmann, A., Lewis, K. & Epstein, S. S. Incubation of environmental samples in a diffusion chamber increases the diversity of recovered isolates. *Appl. Environ. Microbiol.* **73**, 6386–6390 (2007).

69. Kurtz, M. B. *et al.* Increased antifungal activity of L-733,560, a water-soluble, semisynthetic pneumocandin, is due to enhanced inhibition of cell wall synthesis. *Antimicrob. Agents Chemother.* **38**, 2750–2757 (1994).

70. Alberts, A. W. *et al.* Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl Acad. Sci. USA* **77**, 73957–3961 (1980).

71. Borel, J. F., Feurer, C., Gubler, H. U. & Stahelin, H. Biological effects of cyclosporin A: a new antilymphocytic agent. *Agents Act.* **6**, 468–475 (1976).

72. Vezina, C., Kudelski, A. & Sehgal, S. N. Rapamycin (AY-22,989), a new antifungal antibiotic. Taxonomy of the producing streptomycete and isolation of the active principle. *J. Antibiot.* **28**, 721–726 (1975).

73. Pacey, S. *et al.* A Phase II trial of 17-allylamino, 17-demethoxygeldanamycin (17-AAG, tanespimycin) in patients with metastatic melanoma. *Invest. New Drugs* **30**, 341–349 (2012).

74. Hallberg, P., Michaelsson, K. & Melhus, H. Digoxin for the treatment of heart failure. *N. Engl. J. Med.* **348**, 661–663 (2003).

75. Bryskier, A. (ed.) *Antimicrobial Agents: Antibacterials and Antifungals* (ASM Press, 2005).

76. Miller, L. H. & Su, X. Artemisinin: discovery from the Chinese herbal garden. *Cell* **146**, 855–858 (2011).

77. Dixon, R. A. Natural products and plant disease resistance. *Nature* **411**, 843–847 (2001).

78. Gellert, M., O'Dea, M. H., Itoh, T. & Tomizawa, J. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. *Proc. Natl Acad. Sci. USA* **73**, 4474–4478 (1976).

79. Kappe, S. H. I., Vaughan, A. M., Boddey, J. A. & Cowman, A. F. That was then but this is now: malaria research in the time of an eradication agenda. *Science* **328**, 862–866 (2010).

80. Gibbons, S. Anti-staphylococcal plant natural products. *Nat. Prod. Rep.* **21**, 263–277 (2004).

81. Slonczewski, J. & Foster, J. W. *Microbiology: An Evolving Science* 2nd edn (W.W. Norton, 2011).

82. Gibson, D. G. *et al.* Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* **329**, 52–56 (2010).

83. Boucher, H. W. *et al.* Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **48**, 1–12 (2009).

84. Higgins, P. G., Dammhayn, C., Hackel, M. & Seifert, H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* **65**, 233–238 (2010).

85. Walsh, C. *Antibiotics: Actions, Origins, Resistance* (ASM Press, 2003).

86. Alekshun, M. N. & Levy, S. B. Molecular mechanisms of antibacterial multidrug resistance. *Cell* **128**, 1037–1050 (2007).

87. Lewis, K. Persister cells, dormancy and infectious disease. *Nature Rev. Microbiol.* **5**, 48–56 (2007).

88. Lewis, K. Persister cells. *Annu. Rev. Microbiol.* **64**, 357–372 (2010).

89. Keren, I., Kaldalu, N., Spoering, A., Wang, Y. & Lewis, K. Persister cells and tolerance to antimicrobials. *FEMS Microbiol. Lett.* **230**, 13–18 (2004).

90. Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L. & Leibler, S. Bacterial persistence as a phenotypic switch. *Science* **305**, 1622–1625 (2004).

91. Shah, D. *et al.* Persisters: A distinct physiological state of *E. coli*. *BMC Microbiol.* **6**, 53–61 (2006).

92. Dorr, T., Vulic, M. & Lewis, K. Ciprofloxacin causes persister formation by inducing the TisB toxin in *Escherichia coli*. *PLoS Biol.* **8**, e1000317 (2010).
This study describes the first characterized molecular mechanism of persister formation.

93. Keren, I., Shah, D., Spoering, A., Kaldalu, N. & Lewis, K. Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J. Bacteriol.* **186**, 8172–8180 (2004).

94. Schumacher, M. A. *et al.* Molecular mechanisms of HipA-mediated multidrug tolerance and its neutralization by HipB. *Science* **323**, 396–401 (2009).

95. Maisonneuve, E., Shakespeare, L. J., Jorgensen, M. G. & Gerdes, K. Bacterial persistence by RNA endonucleases. *Proc. Natl Acad. Sci. USA* **108**, 13206–13211 (2011).

96. Hansen, S., Lewis, K. & Vulic, M. The role of global regulators and nucleotide metabolism in antibiotic tolerance in *Escherichia coli*. *Antimicrob. Agents Chemother.* **52**, 2718–2726 (2008).

97. LaFleur, M. D., Qi, Q. & Lewis, K. Patients with long-term oral carriage harbor high-persister mutants of *Candida albicans*. *Antimicrob. Agents Chemother.* **54**, 39–44 (2010).

98. Mulcahy, L. R., Burns, J. L., Lory, S. & Lewis, K. Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis. *J. Bacteriol.* **192**, 6191–6199 (2010).

99. Levin, B. R. & Rozen, D. E. Non-inherited antibiotic resistance. *Nature Rev. Microbiol.* **4**, 556–562 (2006).

100. Coates, A. *et al.* The future challenges facing the development of new antimicrobial drugs. *Nature Rev. Drug Discov.* **1**, 895–910 (2002).

101. Bellamy, W. D. & Klimek, J. W. Some properties of penicillin-resistant staphylococci. *J. Bacteriol.* **55**, 153–160 (1948).

102. Brown, A. G. *et al.* Naturally-occurring beta-lactamase inhibitors with antibacterial activity. *J. Antibiot.* **29**, 668–669 (1976).

103. Livermore, D. M. & Woodford, N. The beta-lactamase threat in Enterobacteriaceae, *Pseudomonas* and *Acinetobacter*. *Trends Microbiol.* **14**, 413–420 (2006).

104. Garza-Ramos, U. *et al.* Metallo-beta-lactamase IMP-18 is located in a class 1 integron (In96) in a clinical isolate of *Pseudomonas aeruginosa* from Mexico. *Int. J. Antimicrob. Agents* **31**, 78–80 (2008).

105. Stachyra, T. *et al.* Mechanistic studies of the inactivation of TEM-1 and P99 by NXL104, a novel non-beta-lactam beta-lactamase inhibitor. *Antimicrob. Agents Chemother.* **54**, 5132–5138 (2010).

106. Yum, J. H. *et al.* Molecular characterization of metallo-β-lactamase-producing *Acinetobacter baumannii* and *Acinetobacter* genomospecies 3 from Korea: identification of two new integrons carrying the bla_{VIM-2} gene cassettes. *J. Antimicrob. Chemother.* **49**, 837–840 (2002).

107. Kumarasamy, K. K. *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* **10**, 597–602 (2010).

108. Lomovskaya, O. & Lewis, K. Emr, an *Escherichia coli* locus for multidrug resistance. *Proc. Natl Acad. Sci. USA* **89**, 8938–8942 (1992).

109. Li, X. Z. & Nikaido, H. Efflux-mediated drug resistance in bacteria. *Drugs* **64**, 159–204 (2004).

110. Irwin, J. J. & Shoichet, B. K. ZINC — a free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.* **45**, 177–182 (2005).

111. Irwin, J. J. Using ZINC to acquire a virtual screening library. *Curr. Protoc. Bioinformatics* **Ch. 14**, Unit 14.6 (2008).
112. Bohacek, R. S., McMartin, C. & Guida, W. C. The art and practice of structure-based drug design: a molecular modeling perspective. *Med. Res. Rev.* **16**, 3–50 (1996).
113. Rex, J. H. *et al*. A comprehensive regulatory framework to address the unmet need for new antibacterial treatments. *Lancet Infect. Dis.* **13**, 269–275 (2013).
114. Lowy, I. *et al*. Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *N. Engl. J. Med.* **362**, 197–205 (2010).
115. Hung, D. T., Shakhnovich, E. A., Pierson, E. & Mekalanos, J. J. Small-molecule inhibitor of *Vibrio cholerae* virulence and intestinal colonization. *Science* **310**, 670–674 (2005).
116. Oh, K. B. *et al*. Therapeutic effect of (Z)-3-(2,5-dimethoxyphenyl)-2-(4-methoxyphenyl) acrylonitrile (DMMA) against *Staphylococcus aureus* infection in a murine model. *Biochem. Biophys. Res. Commun.* **396**, 440–444 (2010).
117. Garcia-Olmedo, F., Molina, A., Alamillo, J. M. & Rodriguez-Palenzuela, P. Plant defense peptides. *Biopolymers* **47**, 479–491 (1998).
118. Sahl, H. G. & Bierbaum, G. Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from Gram-positive bacteria. *Annu. Rev. Microbiol.* **52**, 41–79 (1998).
119. Zasloff, M. Antimicrobial peptides of multicellular organisms. *Nature* **415**, 389–395 (2002).
120. Ahiwale, S. *et al*. *In vitro* management of hospital *Pseudomonas aeruginosa* biofilm using indigenous T7-like lytic phage. *Curr. Microbiol.* **62**, 335–340 (2011).
121. Hughes, K. A., Sutherland, I. W. & Jones, M. V. Biofilm susceptibility to bacteriophage attack: the role of phage-borne polysaccharide depolymerase. *Microbiol.* **144**, 3039–3047 (1998).
122. Pearl, S., Gabay, C., Kishony, R., Oppenheim, A. & Balaban, N. Q. Nongenetic individuality in the host-phage interaction. *PLoS Biol.* **6**, e120 (2008).
123. Projan, S. Phage-inspired antibiotics? *Nature Biotech.* **22**, 167–168 (2004).
124. Wright, A., Hawkins, C. H., Anggard, E. E. & Harper, D. R. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clin. Otolaryngol.* **34**, 349–357 (2009).

Competing financial interests
The author declares competing financial interests: see Web version for details.

FURTHER INFORMATION
Kim Lewis's homepage: http://www.northeastern.edu/biology/people/faculty/kim-lewis
"AstraZeneca and Forest Laboratories to initiate Phase III clinical trials for ceftazidime/avibactam to treat serious Gram-negative bacterial infections" (18 November 2011 press release; AstraZeneca): http://www.astrazeneca.com/Partnering/Article/20111118--astrazeneca-and-forest-laboratories
"New FDA task force will support innovation in antibacterial drug development" (24 September 2012 press release; FDA): http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm320643.htm
ZINC database: http://zinc.docking.org

ALL LINKS ARE ACTIVE IN THE ONLINE PDF