Digging for new solutions

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The magnitude of the increasing problem of resistance really takes all its meaning when appraised side-by-side with the paucity of new antimicrobials reaching the market (1). Several factors have contributed to making antimicrobial discovery less fashionable nowadays. The gigantic costs of bringing a new compound to market, from the identification of a promising target at the preclinical stages, to the final clinical trials and approval, are clearly a strong deterrent. This emphasizes the difficulty in realizing an interesting financial return, given that antimicrobials are used for diseases occurring on a very short timespan (compared with the treatment of chronic conditions) and that regulatory requirements are strict (2). In the United States, in an attempt to stimulate the discovery of new antimicrobials, the Generating Antibiotic Incentives Now (GAIN) Act has been passed by the Obama administration. Among the provisions of the Act, sponsors developing new antibiotics may benefit from the following incentives: five additional years of market exclusivity, priority review, fast-track approval and updated guidance (3). The impact of the GAIN Act is difficult to evaluate such a short time after its implementation, but considering the high costs of development and evaluation, five additional years of market exclusivity appears to be a small upgrade to really provide incentive to pharmaceutical companies to invest in this field.

Even if regulatory requirements are modified, leading to potential increases in investments in antimicrobial discovery, one major challenge remains: identifying new antimicrobials is an extremely challenging task and we are way past the golden era of antibiotic discovery.

From the 1940s to the 1960s, the majority of compounds or derivatives were obtained from natural sources (soil-derived actinomycetes). A majority of these compounds were the result of a once successful discovery platform, introduced by Selman Waksman in the 1940s (4). It was a very simple development platform, which consisted of screening soil-derived actinomycetes against susceptible microorganisms by detecting zones of inhibition on an overlay plate. It first led to the discovery of streptomycin and, eventually, was used by pharmaceutical companies for the following 20 years, leading to the development of several new classes of antibiotics. Eventually, the pipeline dried up and this approach was abandoned because of the increasing difficulty of identifying new 'unknown or unrelated' compounds (5).

Bacterial resistance to classic antibiotics led researchers to modify current antibiotics to produce active analogues or to develop combination treatment (eg, with the addition of ß-lactamase inhibitors) to make new versions of older compounds. Also, some important synthetic antibiotic classes were developed in the 1960s, the most important of which were the fluoroquinolones, as an optimized version of nalidixic acid. The ensuing decades were marked by the almost complete absence of new class discoveries; the last clinically useful antibiotic in a new class was daptomycin in 1986 (5). A significant number of recently developed and approved agents are based on old discoveries (eg, fidaxomicin, formerly known as lipiarmycin A3, was discovered in 1975) (6).

After the 1990s, the pharmaceutical industry responded to the rise in resistance by exploring new 'high-tech' approaches to create a new platform combining genomics, combinatorial chemistry, high-throughput screening (automated process to detect the activity of thousands of compounds to a receptor target or whole cells to identify potential leads for further development) and rational drug design (development based on the analysis of the three-dimensional structure of a protein interacting with a ligand) (7). To date, most of the compounds identified through these approaches were unable to sufficiently penetrate the bacterial cell wall and gain access to their targets, and/or did not possess a reasonable spectrum of activity.

One interesting approach has been the revival of attempting to find new natural antibiotics by screening untapped sources. This is not a new idea because it was the first strategy brought forward to extend the golden era of antibiotics in the 1960s, by prospecting for new compounds in the soils of the southern hemisphere. Unfortunately, this approach led to disappointing results; it appears that bacterial diversity in the soils across the world does not widely differ. Exploring deep waters of the oceans or other uncommon sites (eg, Canadian oil sands, Amazon basin, River Wiwi in Ghana) (8,9) has not led to an important discovery yet. For example, sporolides A and B are polycyclic macrolides from *Salinispora tropica* actinomycetes found in marine sediment (10). They had carried some interest because of their unique new structures but did not exhibit any significant antibacterial activity. Other untapped sources of antimicrobials include manipulating silent operons for antibiotic production (as silent operons harbour approximately 90% of natural product chemistry) (5), plants (two antimalarials are directly derived from plants: quinine from the *Cinchona* tree and artemisinin from Artemiannua) (11) and stimulating the growth of highly fastidious organisms by using different specialized media.

Growing bacteria with the potential to produce antimicrobials that are deemed 'uncultivable' is a promising new approach that may revive the Waksman platform. Based on a metagenomics analysis of different soils, 99% of all microbial species are 'uncultivable' (12). In 2002, Kaeberlein et al (13) published a breakthrough approach to make this possible by developing a novel method that enables growing bacteria in their natural environment. In recent years, this initially cumbersome method was adapted for high-throughput testing by integrating microfluidics methods in a device known as the iChip (14).

The iChip is a miniaturized plate with multiple through-holes. The first step consists of dipping the device in a suspension of bacteria targeted for cultivation. Each hole will capture a volume of the suspension and several bacteria proportional to the concentration of bacteria in liquid agar-based medium (on average, one cell per hole). Cells are individually trapped in each hole while the agar solidifies. The next step involves the application of membranes to both sides of the device, to prevent the migration of bacteria in and out of agar plugs. Subsequent incubation for a period of two weeks is performed by inserting the iChip in a solution consisting of diluted soil samples that

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served as sources of the cells. The microorganisms grown in iChips are identified using 16S ribosomal RNA gene sequencing. The growth recovery using this method approaches 50% (14).

In January 2015, Ling et al (15) used this technology to identify a new cell wall inhibitor, teixobactin. Extracts from 10,000 isolates obtained by growth in iChips were screened for antimicrobial activity on plates overlaid with *Staphylococcus aureus*. It led to the identification of a new species of ß-proteobacteria related to *Aquabacteria*, provisionally named *Eleftheria terrae*, with good antibacterial activity. From culture supernatant of *E terrae*, they obtained a partially purified active fraction containing a compound, teixobactin. It is an unusual depsipeptide that contains enduracididine, methylphenylalanine and four amino acids.

Teixobactin is particularly interesting because it possesses a different mode of action from other known antibacterials. In *S aureus*, it inhibits the synthesis of the cell wall by binding to a highly conserved motif of both lipid II and lipid III. Lipid II is an amphipathic peptidoglycan precursor and lipid III a teichoic acid precursor. Teichoic acids anchor autolysins preventing uncontrolled hydrolysis of peptidoglycans. Inhibition of teichoic acid synthesis would liberate autolysins and contribute to the lytic activity of teixobactin. Teixobactin has excellent in vitro activity against Gram-positive pathogens such as streptococci, enterococci (including vancomycin-resistant strains), *S aureus* (including methicillin-resistant strains), *Bacillus anthracis*, *Clostridium difficile* and *Mycobacterium tuberculosis*. It demonstrates excellent bactericidal activity against *S aureus* strains, superior to vancomycin in late exponential phase populations, including strains with intermediate resistance to vancomycin. Teixobactin has no biological activity against Gram-negative pathogens. In vivo efficacy was demonstrated in two mouse models (mouse *S aureus* septicaemia and thigh models) with favourable results. No resistant *S aureus* was isolated at 4× the minimum inhibitory concentration and after serial passage in subinhibitory concentrations.

Resistance to this agent could eventually emerge from horizontal transmission from close microbial neighbours resistant to the antibiotic, or from the producing microorganism (otherwise it would be suicidal for the bacteria). However, previous testing with vancomycin has shown that with this type of cell wall agent it can take years before resistance appears.

Innovative methods to identify new classes of antibiotics are essential to counter the emergence of bacterial resistance worldwide. Uncultivable bacteria are perhaps one of the most promising untapped sources of potential compounds. Teixobactin is the first antibacterial with a new mechanism of action identified using this approach; unfortunately, it only targets Gram-positive organisms, similar to many other recent 'new' drugs. Resistance in Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae* is worrisome, as demonstrated in the WHOs report (1). Even if some new compounds may appear interesting (eg, aspergillomarasmine A) (16), the difficulty in efficiently killing these bacteria (because of their cell wall structure, efflux pumps and extended-spectrum ß-lactamase) have forced clinicians to try to resuscitate almost extinct compounds with various results (eg, temocillin) (17). In the future, it will also be interesting to witness the impact of the GAIN Act on antibiotic development; however, it appears to offer a relatively small incentive in our opinion. Teixobactin may never reach the market; however, its discovery brings new hope in the potential of prospecting untapped sources of antibiotics in the development of new compounds.

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