Antibiotics: where does the future lie?

As we mark the anniversary of the discovery of penicillin, what are the prospects for new drugs to fight infectious diseases?

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By common consent, Alexander Fleming's discovery of penicillin made by the mould that contaminated one of his culture dishes 80 years ago was one of the most important medical landmarks of the 20th century. Its development into a wonder drug by Howard Florey and Ernst Chain at Oxford in the early 1940s revolutionised the treatment of bacterial infections caused by staphylococci and streptococci and saved countless lives. Numerically, though, the major producers of antibiotics are a group of soil microbes that shot to fame from relative obscurity after the 1943 discovery of streptomycin by Selman Waksman's group at Rutgers University in New Jersey. Streptomycin was the first effective treatment for tuberculosis. It is made by *Streptomyces griseus*, the type species of a large genus within the actinomycetes, later shown to be true bacteria rather than being, as earlier supposed based on their fungus-like growth, a group intermediate between fungi and bacteria (or even actual fungi).

Penicillin and streptomycin ushered in the antibiotic era that transformed the management of infectious disease. Their discovery was followed by the finding of many further antibacterial drugs. One was another fungal product, cephalosporin, discovered at Oxford by Edward Abraham, another member of Florey's department. It falls into the same chemical class as penicillin, and chemical derivatives of these two molecules over subsequent decades account for about half the current market in antibiotics. The rest is made up of many actinomycete products, mostly discovered in the 1950s and 1960s in a period named retrospectively as the 'Golden Age' of antibiotic discovery. They include antibacterials such as the tetracyclines, erythromycin, kanamycin and vancomycin. The antifungal agents candicidin and amphotericin were also found, as well as anticancer drugs like doxorubicin and bleomycin.

The Golden Age of antibiotic discovery was followed by decades in which far fewer useful natural products were discovered (Figure 1), although the antiparasitic compound avermectin was a big success for the treatment of worm and warble fly infestations of livestock, and with a human application to prevent river blindness, caused by a microscopic worm, in Sub-Saharan Africa. It was joined by important immunosuppressant drugs for controlling organ transplant rejection such as cyclosporin from a fungus and tacrolimus from an actinomycete.

Needs for new antibiotics

With so many successes, why could we possibly need new antibiotics? The most urgent need stems from the rise of acquired antibiotic resistance. Almost as soon as antibiotics were introduced into medicine the bacteria fought back. They have evolved over countless millions of years to survive the insults of their environments, and they could readily combat the threat posed by clinically used antibiotics. The huge numbers that bacterial populations achieve in a small space and in a short time help them mutate to survive, though the main source of medically important resistance is not fresh mutations but genes conferring drug resistance transferred into them by mating with non-pathogenic relatives. The ultimate source of resistance to many antibiotics is almost certainly the antibiotic-producing organisms themselves, since they need to have genes for protection against suicide by their own antibiotics.

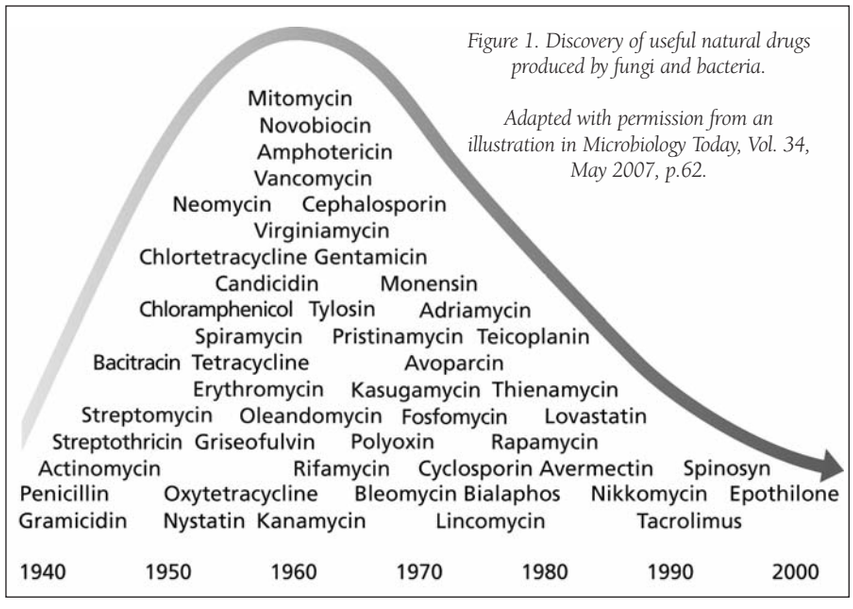

Figure 1. Discovery of useful natural drugs produced by fungi and bacteria.

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The most famous resistant pathogen is MRSA – methicillin-resistant *Staphylococcus aureus* – responsible for much hospital-acquired infection. Others include vancomycin-resistant *Enterococcus* after abdominal surgery, and multi-drug-resistant *Mycobacterium tuberculosis*. *Clostridium difficile* is also a much talked about threat, ironically in patients whose antibiotic treatment has cleared their gut of beneficial bacteria that keep *'C. dif.'* at bay in healthy people. Another growing problem is resistant respiratory pathogens, especially in immuno-compromised patients. There is no doubt that antibiotic over-use and misuse have greatly exacerbated the problem of acquired resistance, but at some level it is an inevitable consequence even of sensible antibiotic use itself.

New antibiotics through genetic engineering

How are we going to find new treatments if the supply of naturally produced antibiotics has been exhausted? One answer was been to go back to the roots of the pharmaceutical industry before the antibiotic era and use synthetic chemistry to make new drugs. This endeavour, aided by modern developments in robotic synthesis – combinatorial chemistry – was heavily backed by the big drug companies in the 1990s as they closed their natural product discovery efforts. The companies also backed the idea of sequencing the genomes of the pathogens in order to discover genes encoding proteins that are found in them but not in humans and which could therefore provide potential new targets for compounds that should not damage human cells. Unfortunately the approach has had negligible success, mainly because the synthetic compounds tend not to make good drugs and the new targets were not as susceptible as those already refined in nature during the process of natural selection.

A more promising approach is to harness the enzymes that microbes have evolved to make complex molecules that interact specifically with cellular targets, but in new ways. This is the field of combinatorial biosynthesis of 'unnatural natural products', so called because the compounds are made by microbes, and so are 'natural', but not by those found in the wild, hence 'unnatural'. It builds on knowledge about the genetics of the actinomycetes that has developed over the decades since the mid-1950s, reaching an advanced enough stage for the rational genetic manipulation of antibiotic biosynthesis around 1990.

The first example of making a hybrid antibiotic in this way capitalised on the natural colours of the antibiotics: some of the genes for a blue antibiotic were introduced into a strain making a brown compound, whereupon a purple hybrid was produced. This demonstration of hybrid antibiotic production was academic, but as knowledge accumulated about how the complex biochemical pathways that make natural products are 'programmed', the field has burgeoned.

Many such compounds consist of a skeleton made from a long carbon chain, decorated and folded in characteristic ways, and are made on protein templates in which a linear arrangement of enzyme sites forms an 'assembly line'. The final structure of the product depends on the number, properties and arrangement of these sites, the equivalent of workstations along the assembly line, which are determined directly by the DNA sequence of the genes encoding the proteins and so can be read just by DNA sequencing, now that many of the rules have been worked out. Since the changes introduced at each workstation along the protein assembly line are nearly all independent of one another, the number of possible combinations is enormous. The trick is to alter the programming in predictable ways, guided by prior knowledge of structure-activity relationships of the compounds. In this way it is possible to do complex 'chemistry by genetics'. It is still relatively early days, but some of the first products are in clinical trials.

Back to nature?

Meanwhile, the idea that nature's bounty was exhausted during the Golden Age has been challenged. The sequencing of several complete *Streptomyces* genomes since the turn of the Millennium has revealed that these organisms must be capable of producing much greater numbers of interesting natural products than are found by traditional screening procedures. Expression of the genes is tightly regulated, presumably because many of them encode products that are useful to the organisms under special conditions that they encounter only sporadically. Isolated examples of waking up these sleeping genes in the laboratory are now being published. We need sustained investment in the fundamental microbiology required to understand the natural roles of the compounds, and their regulation, in order to find general, and therefore commercially attractive, methods to reveal the full potential of the organisms to make useful compounds.

Commercial caveats

The development of antibacterial agents suffers from problems not encountered in launching other classes of drugs. Anti-cancer medicines, for example, always have side effects, often serious, but they make it to the clinic because their benefits are deemed to outweigh their drawbacks. Not so with antibiotics. They must be devoid of side effects, in all members of the population, and this is an almost impossible ideal: hardly any of the antibiotics that have saved millions of lives since the 1940s would have been launched in the current regulatory climate. So companies are reluctant to spend hundreds of millions of pounds on clinical trials that are likely to fail on the grounds of, perhaps rather small, drawbacks. Added to this, a successful antibiotic might be taken for only a week and the patient recovers. Moreover, the expectation is that antibiotics will be inexpensive compared with anti-cancer drugs, even of marginal benefit, or treatments for chronic conditions that large numbers of the population will take for years on end. If the pharmaceutical industry is to be persuaded back to the task of combating the danger of medicine entering a scary 'post-antibiotic' era, some of these commercial caveats must be urgently addressed.