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Discovery research: the scientific challenge of finding new antibiotics

David M. Livermore* on behalf of the British Society for Antimicrobial Chemotherapy Working Party on The Urgent Need: Regenerating Antibacterial Drug Discovery and Development†

Antibiotic Resistance Monitoring & Reference Laboratory, HPA Microbiology Services - Colindale, 61 Colindale Avenue, London NW9 5EQ, UK

*Tel +44-20-8327-7223; Fax +44-20-8327-6264; E-mail: david.livermore@hpa.org.uk
†Members are listed in the Acknowledgements.

The dwindling supply of new antibiotics largely reflects regulatory and commercial challenges, but also a failure of discovery. In the 1990s the pharmaceutical industry abandoned its classical ways of seeking antibiotics and instead adopted a strategy that combined genomics with high-throughput screening of existing compound libraries. Too much emphasis was placed on identifying targets and molecules that bound to them, and too little emphasis was placed on the ability of these molecules to permeate bacteria, evade efflux and avoid mutational resistance; moreover, the compound libraries were systematically biased against antibiotics. The sorry result is that no antibiotic found by this strategy has yet entered clinical use and many major pharmaceutical companies have abandoned antibiotic discovery. Although a raft of start-up companies—variously financed by venture capital, charity or public money—are now finding new antibiotic compounds (some of them very promising *in vitro* or in early trials), their development through Phase III depends on financial commitments from large pharmaceutical companies, where the discouraging regulatory environment and the poor likely return on investment remain paramount issues.

Keywords: genomics, high-throughput screening, antibiotic discovery, antimicrobial discovery, antibacterial discovery

How we reached the present situation

For half a century, from 1940 to 1990, the repeated and successful response to emerging resistance was to discover new antibacterials. In recent years this strategy has failed, with resistance accumulating faster than new antibiotics have been developed. The absolute number of new antibiotics licensed has declined as well, with a particular shortage of new agents against Gram-negative pathogens. Several factors have led to this failure. Most importantly, it reflects a move by 'big pharma' away from antibiotics, which have proved increasingly difficult to license and which, as short-course treatments, are less lucrative than drugs for chronic conditions.[1] In addition, the failure reflects the *particular* challenges of antibiotic discovery. Antibiotics must attack multiple target species that change over time, by developing resistance, and must do this in multiple body compartments. Even the developer of a new β-lactamase inhibitor (targeting bacterial enzymes rather than bacteria themselves) must guess whether KPC or metallo-carbapenemases will be the greater problem 5 years from now, as these need quite different inhibitors.[2] This flexibility is not demanded in other therapeutic areas: a drug for hypertension, diabetes or Alzheimer's disease must bind one constant target at one body site, and although anti-cancer drugs, like antibiotics, are prone to resistance, this is not transmissible among cancers or patients. Antibiotics must also be remarkably non-toxic, for their daily dosages are higher than for other pharmaceuticals, being measured in grams rather than milligrams.[3]

As if these challenges were not enough, the pharmaceutical industry radically rethought its method of seeking antibiotics in the early 1990s, adopting a strategy that appeared sophisticated when compared with what went before, but which ultimately proved less successful. Nearly all the antibiotics used today belong to classes discovered before 1970. They are products of a 'golden age' of discovery, lasting from 1945 to 1965, which sought naturally produced antibiotics from soil streptomycetes and fungi. This process hit the law of diminishing returns by the 1960s, with the same classes (particularly tetracyclines) being repeatedly rediscovered and few new ones emerging.[4] Since 1970 the only indisputably new antibiotic classes to reach the market are the oxazolidinones (discovered 1978, launched 2000) and lipopeptides (discovered 1986, launched 2003), though carbapenems (discovered 1975, launched 1985) arguably have sufficient differences to earlier β-lactams to warrant being counted as well.

Most advances since 1970 have come via improvements—some very substantial—*within* antibiotic classes, yielding analogues with increased potency and a greater ability to evade existing resistance, as with fluoroquinolones versus nalidixic acid, amikacin versus kanamycin, and glycylcyclines versus tetracycline. Gradually this approach, too, became harder, with the emergence of mechanisms giving class resistance, such as multiple topoisomerase mutations, compromising all fluoroquinolones;[5] metallo- and KPC

β-lactamases, compromising nearly all β-lactams;[6] 16S rRNA methylases, compromising nearly all aminoglycosides[7]; and up-regulation of resistance, nodulation and division (RND) efflux pumps,[8] compromising multiple drug classes.

The disappointment of genomics

A sense that the limits of existing antibiotic classes had been reached, along with advances in molecular biology, led to the adoption of genomics-based antibiotic discovery in the 1990s. The approach was to sequence the genomes of multiple pathogens, to identify essential conserved genes encoding targets that lacked counterparts in mammalian cells, and then run high-throughput screens of existing compound libraries to identify 'druggable' molecules that bound to these targets. Natural product screening was quietly abandoned, partly because it had ceased to identify new leads, partly because it was expensive and time consuming and partly because it fitted poorly with the changing logistics of high-throughput screening.

In the event, genomics-based discovery proved notable for its disappointments despite huge early enthusiasm and investment by, among others, SmithKline Beecham, Glaxo, Merck, Pfizer and Wyeth. Targets were found, but the compound libraries yielded around 5-fold fewer hits than for other therapeutic areas,[3] and fewer still converted to development leads. From 1995 to 2002, SmithKline Beecham [now part of GlaxoSmithKline (GSK)] identified 300 potential targets and ran 67 high-throughput screens, each of 260000–530000 compounds. Sixteen screens led to 'hits'—meaning compounds that bound selectively to a target giving a reproducible positive signal in the assays—and five of these translated into 'lead' compounds.[3] Of the five corresponding targets, two (FabI[9] and Mrs) were not universally essential or conserved, meaning that they could not form the targets of broad-spectrum antibiotics, and it proved impossible to incorporate 'drug-like properties' into molecules that bound two others. The final target identified was peptide deformylase, for which GSK now has a molecule (GSK 1322322) in Phase II trials, although this did not come from high-throughput screening. This performance appears typical of other companies that followed the genomics strategy. Thus, 20 years after its advent, no antibiotic developed by this approach has reached the market.[3]

The problem partly lay with the compound libraries.[3,4] Though impressively large, these were biased towards molecules meeting Lipinski's 'rule of five', a chemical algorithm used to predict whether a drug is likely to be absorbed orally and have acceptable tissue distribution based on its molecular mass, hydrogen bonding potential and lipophilicity.[10] The rule's applicability to antibiotics, particularly parenteral ones, is questionable and many existing antibiotics do not conform to its stipulations.[3]

Another problem is that finding a compound that binds to a conserved target does not equate to finding one with antibiotic activity. Drugs that bind may fail to penetrate the bacteria or may be removed by efflux.[3,4] What is more, drugs with single targets are especially vulnerable to mutational resistance, as should have been realized from experience with rifampicin, streptomycin and fosfomycin. Some former industry researchers now stress that the ideal antibiotics should bind multiple targets, as with β-lactams, quinolones and aminoglycosides.[4]

The failure of genomic strategies to address the growing and often-changing demands of regulators and the likely poor return on investment compared with other therapeutic areas has led many companies to abandon antibiotic discovery, with this exodus exacerbated by corporate mergers. In 1980 more than 20 large, profitable, companies had antibiotic discovery programmes whereas now only GSK, Astellas, AstraZeneca, Novartis, Merck and Cubist remain, though others (Forest, for instance) are developing antibiotics discovered by others. Where Lederle, Pharmacia, Parke-Davis, Upjohn, Wyeth and Pfizer once all had antibiotic discovery teams, these are all now part of Pfizer, which recently announced the closure of its antibacterial research in the USA, saying that it will relocate to Shanghai, though the scale and timescale remain unclear. Lesser moves are all too frequent: Merck shifted antibiotic research from New Jersey to Montreal in 2008 and back again in 2010; Novartis is transferring its efforts from Boston to California; and AstraZeneca bought Novexel for US$400 million in 2009, then closed Novexel's Paris laboratories, making the staff redundant. It is hard to see how such disruptions encourage the retention of scientific expertise.

Revitalizing discovery

The BSAC Working Party heard much on these problems, but also some positive developments. Companies have learned from the disappointments of genomics and have shifted to other strategies, some of them highly innovative. One approach is to seek new classes of molecules that bind to established targets, as with GSK's non-quinolone topoisomerase inhibitors[11] and with various non-β-lactam inhibitors of β-lactamases, including NXL104 (Novexel/AstraZeneca),[12] MK-7655 (Merck) and ME1071 (Meiji). NXL104 and MK-7655 are related molecules that inhibit class A and C enzymes,[2] including extended-spectrum β-lactamases (ESBLs) and KPC types, whereas ME1071, a dicarboxylic acid, inhibits metallo-β-lactamases.[13] Other inhibitors are under investigation, including those that interfere with multidrug RND pumps (Mpex/GSK), thereby restoring quinolone activity against *Pseudomonas aeruginosa*, a species in which most resistance is efflux mediated.[14]

Advances also continue within classes. Tetraphase's innovative chemistry is yielding a huge array of tetracycline analogues, potentially providing a breakthrough as significant as when Beecham first derived 6-aminopenicillanic acid from benzylpenicillin, opening the route to arrays of semi-synthetic penicillins.[15] The lead Tetraphase compound, TP434, now entering Phase II, evades acquired efflux pumps and TetM-mediated resistance, with MICs ~4-fold lower than those of tigecycline.[16] Its pharmacokinetics also are more straightforward than those of tigecycline. Other companies (e.g. Northern Antibiotics in Finland and Novozymes in Denmark) seek to modify polymyxins, minimizing their toxicity and maximizing potency.[17]

The view that the compound libraries were too narrow has encouraged a search in unconventional classes. The most interesting developments here are boron-based Leu-tRNA synthetase inhibitors discovered by Anacor and being developed by GSK.[18] These are active at 0.12–2 mg/L against staphylococci, enterococci, Enterobacteriaceae, *Acinetobacter* spp. and *P. aeruginosa*; the lead compound (variously numbered AN-3365, GSK2251052 or GSK'052) has successfully completed Phase I trials.

There is even a small renaissance in natural product screening, with companies such as Galapagos (also in collaboration with GSK) working in the area, along with several academic groups. Two strategies are being used to outflank the previous barriers. One is to screen different organism groups, not just soil streptomycetes. There is interest, for example, in molecules produced by plants,[19] deep-sea bacteria,[20] and by *Actinomycetes* that colonize ants' nests, protecting them from fungi.[21] The other approach recognizes that many existing antibiotics are growth-phase-dependent regulatory products and seeks to identify further non-expressed regulatory gene clusters in streptomycetes, manipulating their expression (in the streptomycetes themselves or after cloning) and characterizing the products to identify any with antibiotic activity.[22–24]

Conclusions

Despite the disappointments of genomics and the exodus of big pharma, antibiotic innovation does continue, and discovery failures are the lesser problem compared with the downstream issues of licensing and poor return on investment. Nevertheless—and exactly because of these latter barriers—discovery is starved of funding. Many of the discoveries now being made depend upon venture capital, charity or public money, not on long-term investment by profitable companies making objective commercial decisions about the prospects of future income.

Venture capital is a major funding source, but (i) is more comfortable with progressing known compounds through Phases I and II than with *de novo* discovery; (ii) often favours short-term returns, selling the company as soon as possible; and (iii) has become more difficult to source since the 2008 financial crisis. Among charities, the Wellcome Trust is investing ~£91 million annually to sponsor 'innovative drug development', with around half of this going (though not hypothecated) towards antibacterials and anticancer agents.[25] Readers may be surprised to learn that GSK's antibiotic discovery efforts—though not the more expensive Phase II/III trials—partly depend on this charitable source: GSK received a £4 million award from the Wellcome Trust to accelerate development of anti-Gram-negative agents, with the company making a matching contribution and with the Trust receiving a return on any compound commercialized. Achaogen's development of aminoglycosides that evade modifying enzymes[26] is financed by the US Biodefense Program, and the US National Institutes of Health are seeking to enable academic researchers to undertake high-throughput screens of compounds against the targets they have identified.[27]

These initiatives are welcome, but discovery is only the first, and least expensive, part of development, begging the question: 'Will big pharma pick up these discoveries and take them through the expense of Phase III trials?' Unless this happens—or a way is found to reduce the cost of these trials—new discoveries, however impressive, will not be translated into drugs that cure infections due to resistant pathogens.

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Members of the Working Party

The BSAC Working Party comprised Professor Richard Wise (Chair), Dr Richard Bax (Transcrip Partners LLP, UK), Dr Frances Burke (Eli Lilly, UK), Professor Ian Chopra (University of Leeds), Dr Lloyd Czaplewski (Biota Europe Ltd, UK), Professor Roger Finch (Molecular Medical Sciences, University of Nottingham and Nottingham University Hospitals NHS Trust, Nottingham, UK), Dr David Livermore (Director, Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, London UK), Professor Laura J. V. Piddock (President, BSAC; University of Birmingham, UK) and Tony White (BSAC Council member).

Transparency declarations

D. L. has received conference, speaking and research support from numerous pharmaceutical companies. He holds shares in AstraZeneca, Merck, Pfizer, Dechra, and GSK, and, as executor, manages further holdings in GSK and Eco Animal Health. He is an employee of the UK HPA and is a UK taxpayer. R. B. is currently a senior partner at Transcrip partners LLP and works with several large and small pharmaceutical companies in the area of antibiotic development. He is also a non-executive director of Helperby Therapeutics Ltd. R. F. has provided consultative advice to Destiny Pharma, GSK, Menarini Recherche and Novartis. F. B. is an employee of Eli Lilly and Company Ltd. I. C. is a member of the Scientific Advisory Board of Destiny Pharma Ltd and has recently received research funding from Cubist, Destiny, Galapagos, Leo, Pfizer, Novartis and Novacta. T. W. is an independent consultant, a retired employee and shareholder of GSK, and in the past 5 years has received financial remuneration for consultancy or presentations from GSK and Chiron/Novartis. The remaining members of the Working Party have none to declare.

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