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Antimicrobial drug discovery: greater steps ahead

Summary

Infectious diseases account for a substantial proportion of deaths worldwide. Continuing progress in the treatment of many infections is threatened by the growing resistance of pathogens to antimicrobial drugs. For example, in the European Union (EU) it is estimated that 25,000 people die annually of sepsis caused by resistant bacteria. The epidemiology of resistance is complex but the problem is compounded by recent lack of success in developing novel antibiotic classes.

In this Statement, EASAC builds on a long-standing interest in the opportunities and challenges associated with tackling infectious diseases to re-examine the current situation, to consider how to search for new scientific directions for antimicrobial innovation and to remove impediments in translating research advances to drug development. In March 2014, EASAC, together with its member academies in Germany and the Netherlands, organised a meeting in Hannover to explore new paths in antibiotic research. Among key topics elucidated and exemplified were the following:

- How can we learn from previous examples of success, and lack of success, in antibiotic research and development?
- What are the functions of antibiotics in their natural environments?
- What are the opportunities for novel approaches to tackling pathogens, for example based on virulence modulation or immune stimulation?
- How might pathogen-specific pathways be influenced?
- Can host cell targets be found that inhibit intracellular bacterial infection?
- Are there new sources of antimicrobial compounds and delivery systems that can capitalise on emerging technologies?

There was consensus among the participants at the meeting on the urgency to develop critical mass to support and generate good new science, to dismantle the bureaucratic obstacles to using the outputs from that science and to ensure that innovation can be sustained in the longer term.

EASAC recommendations focus on the following areas.

Support for basic research to include the social sciences as well as biosciences and allied disciplines, to understand antimicrobial resistance and provide the resource to underpin diverse scientific approaches to combatting pathogens. Increased investment in fundamental research must be accompanied by other action to ensure the field is attractive to young investigators and draws on appropriate

multidisciplinarity; this requires attention to the present procedures by which research is evaluated and rewarded.

Platforms for compound identification, lead optimisation and characterisation to capitalise on the new scientific opportunities coming within range. These discovery platforms include using transcriptomics to define and differentiate modes of action; investigating new natural product sources; deciphering the rules for chemical compound penetration into cells; utilising prodrugs, other delivery systems and combinatorial chemistry approaches; standardising mechanisms for activating silent genes; culturing the hitherto unculturable micro-organisms; and identifying off-target effects.

Resolving the bottlenecks for pre-clinical and early clinical development activities by developing collective EU resource. This requires clarification of the science and technology competencies that are limiting current efforts to reach proof-of-principle stage, in particular relating to animal models, medicinal chemistry, drug metabolism and toxicology, and capturing new sources of funding to provide structured, coordinated resource in support of academic investigators. It is also necessary to sustain clinical skills and infrastructure for infectious disease research and to facilitate faster recruitment of patients into clinical trials.

Optimising current EU partnerships by building on strategies including the Innovative Medicines Initiative and the Joint Programme Initiative on Antimicrobial Resistance, to ensure sufficient EU funding, concentration on the best research, tools and therapeutic assets, and flexibility to pursue new research directions.

Rethinking regulatory frameworks to introduce simpler data requirements where appropriate, to increase the use of conditional licensing followed by comprehensive monitoring of patients, and to take account of the expected availability of new diagnostic tests. Although regulation should be facilitated to focus on the priority clinical indications, it is also necessary to enable preparedness for the unexpected, (re-) emerging threats.

Raising public and political awareness of the threats from antimicrobial resistance and of the steps that must be taken to counter the challenges. There is much to be done to engage with the public and decision-makers to preserve the efficacy of the antibiotics already available, but also to stimulate sustained support for research and innovation. This necessitates better appreciation of (1) the importance of animals in research, (2) the improbability of generating medicines with zero side effects and (3) the need to reduce bureaucracy while providing greater public resources to accelerate innovation.

1 Introduction and previous work by the academies

Infectious diseases account for a substantial proportion of deaths worldwide. Continuing progress in the treatment of many infections is now threatened by the increasing numbers and widening distribution of pathogens resistant to antimicrobial (antibacterial, antiparasitic and antifungal) drugs. There are many reasons for the crisis, and the misuse and overuse of the available antibiotics in human medicine, veterinary medicine and agriculture has favoured the selection and emergence of resistant micro-organisms. The problem is severely compounded by the lack of progress in antibiotic innovation during the past 25 years.

Many of the national academies of science and medicine have a long history of interest in antimicrobial resistance, in analysing the issues and proposing solutions, and have begun to work together (Table 1) to convey stronger messages.

Box 1 draws on the previous EASAC analysis to demonstrate the importance of coordinated action across a broad front. It is not the purpose of the present Statement to repeat this comprehensive analysis but rather to consider how to search for new scientific directions to provide ideas for innovation and how to remove the obstacles in translating research advances to drug development.

Our Statement is based on discussion at a meeting in Hannover in March 2014, organised by EASAC together with two member academies, the German National Academy of Sciences Leopoldina and KNAW, the Royal Netherlands Academy of Arts and Sciences. This meeting brought together scientists from across Europe and from diverse fields to explore new paths in antibiotic research in uninhibited discussion.

Among key topics addressed were the following. What are the functions of antibiotics in natural environments? What are the opportunities for alternative approaches to innovation, for example based on virulence modulation or immune stimulation? How might pathogen-specific pathways be targeted? Can host cell targets be found to inhibit intracellular bacterial infection? Are there new delivery systems that can capitalise on developments in emerging technologies? In the following sections we describe the nature of the problem and the current concerns about innovation status (section 2) and review examples of the advancing science (section 3). Our conclusions and recommendations (section 4) on the imperative to stimulate and use research must be regarded as part of the broader strategy worldwide to respond to the growing threat (Box 1).

2 What needs to be done better?

Antimicrobial resistance: a new diplomatic campaign

In the United Kingdom, currently 7% of deaths are caused by infectious disease; before the introduction of antibiotics it was 43%. Sally Davies, the UK Chief Medical Officer, described her efforts[1] to raise visibility of the threat of antimicrobial resistance, already a deadly reality in the EU where, for example, 25,000 die annually of sepsis caused by resistant bacteria. The epidemiology is complex, involving antibiotic use in food and companion animals and aquaculture as well as in human medicine. If the growing threat, coupled with the present antibiotic discovery void were to return society to the pre-antibiotic era, then the broad expectations of modern medicine could no longer be satisfied. Urgent action is needed on stewardship (conserving current antibiotics), improving surveillance and hygiene, and increasing commitment to research and development, all actions that need to be underpinned by greater appreciation of the economics

Table 1 Previous collective academy activities relevant to antimicrobial resistance

Academy body	Year of publication	Title of publication
EASAC	2007	Tackling antibacterial resistance in Europe
EASAC	2011	European public health and innovation policy for infectious disease: the view from EASAC
German National Academy of Sciences Leopoldina and Academy of Sciences Hamburg	2013	Antibiotics research: problems and prospects
G8 science academies with other science academies	2013	Drug resistance in infectious agents – a global threat to humanity
InterAcademy Panel (IAP) and InterAcademy Medical Panel (IAMP)	2013	Antimicrobial resistance – a call for action

[1] Annual Report of the Chief Medical Officer, Volume 2, 2013 "Infections and the rise of antimicrobial resistance", http://media.dh.gov.uk/network/357/files/2013/03/CMO-Annual-Report-Volume-2-20111.pdf.

Box 1 Recommendations for the EU from EASAC (2007, 2011)

Reducing spread of resistance

Heightening awareness: accurate and timely communication to policy-makers, health professionals and the public.

Implementing infection and hygiene control measures in hospitals and communities.

Improving and standardising coordinated surveillance of infection and resistance in hospitals and the community.

Supporting prudent antibiotic use for human and animal healthcare, based on evidence and education.

One Health: to integrate strategies for control of use of antibiotics in human healthcare, veterinary medicine and agriculture.

Sustained commitment to supporting innovation to generate new therapeutic approaches

Strengthening the science base and investing in fundamental, translational and clinical research, including the social sciences.

Developing novel, rapid diagnostics and vaccines.

Improving public–private partnership in research and development, across biological and chemical disciplines.

Providing new incentives for smaller and larger companies to invest in antibiotic innovation.

Simplifying the regulatory framework.

Global integration

Increasing EU involvement at the global level for surveillance, research, innovation and strategy development.

Supporting capacity building in lower and middle income countries worldwide.

involved: the costs of inaction and new mechanisms to incentivise innovation.

Action is needed at all levels: local, regional and global. The Executive Board meeting of the World Health Organization in January 2014 took an important step towards developing the World Health Organization Global Action Plan on antimicrobial resistance. The academies have an important continuing responsibility to inform global initiatives, particularly to emphasise that:

- Achieving advances in the fundamental science in this area is complex and difficult, but essential.
- Long-term solutions are needed to sustain innovation that is equally applicable to the lower- and middle-income countries.

The antimicrobial crisis: where did things go wrong?

In reviewing the history of antimicrobial drug discovery, Jos van der Meer, President of EASAC, provided further analysis of the selection pressure for resistance induced by indiscriminate use of antimicrobials in humans, animals and plants. The concomitant problem of drug persistence in the environment necessitates combining insights from research in ecology as well as the molecular biosciences and social sciences, to clarify epidemiology and search for solutions. Mechanistically, resistance develops in various ways (dependent on pathogen mutation or acquisition of foreign DNA): the antibiotic may no longer reach the target, it is inactivated or broken down, it no longer affects the target (for that has altered) or it is pumped out of the cell.

The present drug discovery void can be attributed to various causes: assumptions that natural products are exhausted (unlikely, but the easy gains may have been realised), an unwise focus on drugs tailored to single targets (rather than multiple targets), an over-rating of what could be achieved by genomics, a declining industry interest because of perceived low economic returns (by comparison with those obtainable in other therapeutic areas, including chronic diseases) and, generally, a paucity of new scientific ideas.

What has not worked so far?

Heike Brötz-Oesterhelt (Institut fur Pharma Biol & Biotechnologie, Heinrich Heine University, Düsseldorf, Germany) further highlighted some of the current weaknesses: in regulating use of antibiotics, preventing the spread of resistance, maintaining a pipeline of truly novel classes and retaining pharmaceutical company

interest in antibiotic discovery. Discovering and developing a good antibiotic are demanding goals:

- Bacteria have short generation times, strong adaptive capabilities, intrinsic defence mechanisms and can develop resistance rapidly.
- Quick, effective and thorough killing is required, without side effects.
- Generally, a high dose is needed compared with other therapeutic indications and this requires good pharmacokinetic profile and tissue penetration.
- A novel drug, if kept in reserve by health service providers, may have low economic value.

Lessons learned from past successes and failures include the following:

- *The target*: must be validated for all relevant pathogens and demonstrable *in vivo*; if it is a single gene target, it needs to be protected from developing resistance, in combination therapy; complex mechanisms seem to be more successful in attaining bactericidal activity.
- *The assay format*: whole cell pathway assays are found to have better success rates; focused approaches are superior to random screening.
- *The compound leads*: most compound libraries of pharmaceutical companies contain relatively few antibiotic-like structures (most of which have complicated chemical structures) and antibiotics do not follow generalised lead optimisation rules (customarily based on physiochemical characteristics; Lipinski rule of 5[2]); it is not yet possible to design cell uptake characteristics into a novel antibiotic entity.

Most commercial antibiotics have their origin in natural products but the starting point in nature is not, of course, optimised for safety or tolerability, stability or pharmacokinetic profile. Natural products should still be regarded as a source both of chemical leads and new modes of action. As discussed in detail in subsequent presentations, new approaches to sustainable innovation must incorporate new target areas, focused screening strategies, a willingness to use drug combinations or narrow spectrum drugs when appropriate, as well as attention to alternative ways to control infection, based on virulence inhibition or immunological stimulation.

3 Reviewing the evidence on novel approaches

What is the function of antibiotics and antibiotic resistance genes in nature?

José Martínez (Departamento de Biotecnología Microbiana, Centro Nacional de Biotecnología, Spain) explored the role of non-clinical environments in the development of resistance. In natural environments, at low concentrations, antibiotics serve as signalling molecules (between bacteria and other organisms) as well as having concentration-dependent defence roles. Antibiotic resistance genes are acquired from the natural environment and these have various functions in nature. For example, the bacterial multi-drug efflux pump, a major determinant of antibiotic resistance, is highly conserved and has important natural roles in the efflux of heavy metals, plant-derived antibacterials and other toxins. To these natural functions is added antibiotic resistance—not because of evolution but because of changes in the environment. There is evidence that resistance develops even at non-inhibitory concentrations of antibiotics, as found in environmental pollution.

The speed and extent of the environmental dissemination of resistance depends significantly on ecological connectivity (more likely where there is selection pressure, for example in aquaculture and sewage systems, particularly near to hospitals) and on the associated fitness costs that can impede fixation of a given resistance determinant. However, with regard to the latter, the net burden on bacterial metabolism (fitness and transmissibility) depends on the gene acquired so that resistance determinants may be allowed where the fitness cost is lower or compensated more easily. The impact of human activities on natural and managed environments and hence on antimicrobial resistance, hitherto often underestimated, and the concept of resistance genes as pollutants, are described in detail elsewhere[3].

Antibiotic biosynthesis and microbial interaction

Further insight was provided by Christian Hertweck (Leibniz-Institute for Natural Product Research and

[2] Lipinski CA (2004) Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies 1, 337–341.
[3] Martínez JL (2012) *Bottlenecks in the transferability of antibiotic resistance from natural ecosystems to human bacterial pathogens*. Frontiers in Microbiology, doi: 10.3389/fmicb.2011.00265; Martínez JL (2012) *Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials*. Frontiers in Microbiology, doi: 10.3389/fmicb.2012.00001.

Infection Biology, Germany), who reinforced the points made by previous speakers in observing that 'biologically validated' natural products have greater success in lead generation than synthetic sources. However, as natural products are not designed for human therapy, significant effort may be required in lead optimisation to address side effects and improve bioavailability. Moreover, in improving the starting material, there is also need to improve on the traditional approaches used in discovery, based on chemical screening and bioactivity-guided isolation of active substances. For example, better approaches to mass screening must also tackle those micro-organisms that have proved difficult to culture, a challenge that was discussed in more detail by subsequent speakers.

Case studies presented at the meeting illustrated some of the possible approaches to designing and assessing leads derived from natural product sources:

- *Chemo-biosynthesis*: to complement chemical derivatisation of natural products, derivatives of antibiotics can be generated by merging biosynthesis and chemical synthesis, for example by adding altered building blocks to a mutant, also referred to as mutasynthesis. In this way, the scope of structural modifications is greatly enhanced (exemplified by doramectin).

- *Pathway engineering by synthetic biology*: for example based on erythromycin (from *Saccharopolyspora erythraea*), where recent advance in understanding of the genetics and biochemistry of biosynthesis has been used to construct a modular assembly line to generate a library of diverse molecules for selection. Although reconstruction of pathways *in vivo* ('mix and match' with heterologous gene cluster expression) is now possible, the exact knowledge of the enzymatic functions substantially increases chances of success.

- *Genome mining*: many putative biosynthesis genes are silent under laboratory conditions such that micro-organisms may be neglected and valuable antibiotics overlooked. Gene analysis can be used to develop predictive models, and cryptic natural product biosynthesis can be triggered by changing culture conditions, using stress factors or co-culture with signal molecules. Proof-of-principle was provided by experimental activation of a cryptic gene cluster in *Burkholderia thailandensis*. In addition, research to activate secondary metabolism in *Clostridium cellulolyticum* by culturing with additional soil samples has produced the first antibiotic from an anaerobic bacterium, closthioamide, found to be highly active against methicillin-resistant *Staphylococcus aureus* (MRSA).

Biological systems can continue to serve as a source of new ideas. For example, the pathobiology of mushroom soft rot, caused by *Janthinobacterium*, is not yet understood in detail but experimental work on the infection process to search for active compounds, together with development of suitable culture systems, led to the isolation of the lipopeptide jagaricin. In proof-of-principle studies, this has been found active against human fungal pathogens.

In discussion, various points were raised that provided a basis for continuing debate during the meeting. Can systems be standardised for culturing all micro-organisms or for activating all cryptic gene clusters? What obstacles are encountered in scaling up screening and synthesis for industry purposes? Are industry requirements unrealistic, in expecting from academia in-depth characterisation and demonstration of animal model and clinical activity? What are the pathogen clinical priorities and how should these urgent needs inform and direct the search for natural product leads? Who should be responsible for prioritisation? In addition, although there is good reason to focus on priority pathogens, what more should be done to prepare for the unexpected, based on a better understanding of generalisable pathways?

Tackling pathogenicity pathways of extracellular bacteria

The aim in antibiotic therapy is to counter pathogenic bacteria, not kill all bacteria: what selective targeting might achieve this anti-virulence strategy? Jürgen Heesemann (Max von Pettenkofer Institute, University of Munich, Germany) cited examples from the increasing understanding of the mechanisms of host–pathogen interaction to enable new specific approaches. Besides adhesins and toxins, pathogen-specific secretion systems and metabolic pathways are attractive targets.

A case study of the extracellular lifestyle of *Yersinia*, focusing on pathogenicity factors, has characterised the subversion strategies used to create an immune suppressive environment (inhibiting phagocytosis and T- and B-cell activation). The diverse secretion proteins (types 1–7, including enterotoxins, adhesins, cytotoxins, modulins, haemolysin and anti-host effectors) are generally present in pathogenic

bacteria but not in the harmless microbiota and may, therefore, serve as good targets. Other case studies to inhibit pathogenicity presented experimental evidence on *Clostridium difficile*, rationally designing inhibition of autocatalytic cleavage of the virulence factor TcdB, and on *Staphylococcus aureus*, targeting prothrombin activities with the commercially available anti-coagulant drug argatroban to establish proof-of-principle. Additional approaches were described that involved inhibition of proliferation by affecting the host-microbe competition for iron (and other nutrients).

In discussion, it was observed that the conventional industry view is sceptical about anti-virulence approaches because of the dogma that, to be effective, a compound should be bactericidal. However, recent work has shown that targeting key virulence factors can effectively clear pathogens from relevant models *in vivo*, including lung and gastro-intestinal infections. An infection is not in a naive host and the immune system may clear an infection if the pathogen is disabled. It will also be important to test the assumption that anti-virulence strategies will generate much weaker selection for resistance, because this assumption is controversial[4]. The combination of using an anti-virulence agent with an antibiotic to achieve synergy is also attractive. Discussion further suggested that using anti-virulence approaches might first be tested in farm animals where antibiotic use is likely to be increasingly restricted.

Intracellular pathogen–host cell interdependence: exploration of a new therapeutic concept

Thomas Meyer (Max Planck Institute for Infection Biology, Germany) described how intracellular pathogens are broadly dependent on host cell determinants. In addition, host cell reaction can cause tissue destruction and there is risk of damaged host cells remaining after therapy. Case studies were presented in pursuit of the objective of blocking host susceptibility by understanding and transiently blocking host cell functions. For example, the study of influenza A virus has identified host cell targets as essential determinants of virus replication but not essential for mammalian host function; these targets are being employed to generate novel host-directed anti-influenza drugs[5].

Another case study described the bacterium *Chlamydia trachomatis,* associated with significant clinical problems including trachoma and urogenital tract infection. Antibiotic treatment has often failed because the infections were refractory, inaccessible to treatment or resistant. Gene function analysis showed that host nucleotide metabolism plays an important role in *Chlamydia* infection. Experimental study of the available purine biosynthesis inhibitors such as acivin (a GMP synthase inhibitor) and mycophenolic acid (an IMPDH inhibitor) facilitated fast assessment of proof-of-principle, to furnish the confidence to use the leads as starting points to embark on a novel drug research and development programme (recognising that it is difficult to elicit industry interest in old drugs for new indications).

Chlamydia also provides a useful model with which to explore the risk of remnants of infected cells. An observation of unexpected coincidence between anti-chlamydial and anti-cancer drug targets stimulated consideration of whether *Chlamydia* might be involved as a cofactor in carcinogenesis and, if so, whether nucleotide metabolism is implicated. Experimental investigation is beginning to clarify the link between infection of the host cell and DNA damage/imprinting. Thus, the ultimate target should be the whole infected cell rather than only the pathogen. It might also be assumed that host-directed treatment avoids the emergence of pathogen resistance; however, this hypothesis was again challenged in discussion, and requires evidence.

Microbial cell biology, finding targets

Jeff Errington (Centre for Bacterial Cell Biology, Newcastle University, United Kingdom) returned to the characterisation of the ideal target: essential function, conserved across a broad spectrum of bacteria, absent from humans (or sufficiently different to allow specificity) and druggable. These characteristics were well understood by the pharmaceutical industry, but still there is a discovery void and some of the reasons for this had been addressed by previous speakers: initial hits were often weak, optimisation by medicinal chemistry was often inadequate and expensive, many inhibitors found *in vitro* were inactive in cells, and compounds

[4] Nonetheless, it is possible that selection for resistance could be reduced or even reversed using appropriate combinations of target and treatment environment: see Allen RC et al. (2014) *Targeting virulence: can we make evolution-proof drugs?* Nature Reviews Microbiology **12**, 300–308.

[5] Further information on the ANTIFLU Framework Programme 7 project is at http://www.antiflu-project.eu.

acting on single targets often suffered from resistance issues.

The problem of resistance was exemplified by the company Prolysis in work on inhibitors of bacterial cell division in *Bacillus subtilis.* Cell-division proteins are essential, broadly conserved and druggable. Prolysis developed two distinct classes of cell division inhibitor: one proved intractable to medicinal chemistry, the other (benzamides) were easier to manipulate but resistance emerged by mutation in the binding pocket. Although benzamides in combination therapy might still be conceived, this finding substantiates the previous conclusion that the more successful antibiotics have multiple lethal targets or non-protein-based targets that are not susceptible to resistance. Other work, by the company Demuris in searching for novel antibiotics from natural products in a diverse collection of actinomycete bacteria including those from extreme environments, using cell-based assays, has identified novel chemistry and been successful in activating dormant gene clusters. The challenge here is how to prioritise multiple leads from primary screening and systematically eliminate known activities (de-replicate) in order to focus on new mechanisms.

New technologies to develop antimicrobials from natural products

Effective therapy may be undermined not only by the development of antimicrobial resistance but also by subpopulations of dormant persisters[6], protected from antibiotic action. Kim Lewis (Department of Biology, Northeastern University, USA) described four new (revived) opportunities for drug discovery and selection[7]:

- Addressing the cell penetration barrier of Gram-negative bacteria by developing physicochemical rules of penetration (analogous to Lipinski's rule of 5, characterising drug-likeness). This requires constructing a large database of chemical structures that would then be a very useful resource for medicinal chemistry to optimise penetrability in tailored libraries and, thereby, revive the genomics-based high throughput platforms, so far found wanting.

- Resuscitating the prodrug platform to deliver a benign agent converted specifically to reactive drug within the cell. The history of antimicrobial drug research and development testifies to many effective prodrugs, for example prontosil, isoniazide, metronidazole and valaciclovir, but the prodrug platform has, undeservedly, become less popular and merits revival. There are opportunities both to revisit existing libraries for prodrug candidates and to design products based on new understanding about mechanisms of action intracellularly, as a basis for improved selectivity rather than relying on the previous prodrug paradigm of extended pharmacokinetics.

- Reviving the Waksman platform (based on the work of Selman Waksman, whose laboratory isolated many antibiotics in the mid-20th century, including streptomycin): developing techniques for those bacteria that do not easily grow in culture, for example by co-culturing with other bacteria producing essential growth factors.

- Resuscitating old compounds (repurposing), in particular to eliminate persisters in dormant cells. Proof-of-principle was demonstrated with a protease dysregulator to achieve sterilisation of the culture and efficacy in a mouse model.

Pathway analysis: microbes are not the same: a systems approach to understanding immunology

Mihai Netea (Radboud University Medical Centre Nijmegen, The Netherlands) returned to a point made previously: do not forget the host. However, approaches to strengthen the host in sepsis using adjuvant immunotherapy have proved unsuccessful. New evidence, which helps to explain these past failures, indicates that sepsis is characterised by an early hyperimmune response followed by hypoimmune status (at the time when many patients reach hospital). Furthermore, the type of immune response elicited may vary for Gram-positive and Gram-negative bacteria and for fungal and bacterial sepsis: that is, the initial immune response is not as non-specific as had originally been assumed. Transcriptomics now enables identification of the specific signature of the pathway in different infections. Thus, analysis of differential gene expression pathways might be used to identify whether sepsis is bacterial or fungal, but more validation is necessary to develop a predictive test or diagnostic.

[6] Bacterial persisters are multi-drug-tolerant phenotypic variants, associated with relapsing infections. For discussion of the main physiological features that define persistence and the implications for antibiotic treatment regimens, see Balaban NQ et al. (2013) *A problem of persistence: still more questions than answers*. Nature Reviews Microbiology **11**, 587–591.

[7] Further details are in Lewis K (2012) *Rediscover the lost art of drug discovery.* Nature **485**, 439–440; Lewis K (2013) *Platforms for drug discovery.* Nature Reviews Drug Discovery **12**, 371–387.

In attempting to rebalance the immune system, proof-of-principle has been sought using the adjuvant immunotherapy recombinant interferon-gamma in *Candida* infection. In early work, a strong improvement in critical markers of the immune response was observed but studies to evaluate clinical endpoints still need to be completed. Thus, it may be possible to use immune-based therapy in combination with antibiotics to improve outcome, based on deciphering the nature of the host response and personalising treatment according to both pathogen and phase of infection.

Can nanotechnology contribute?

Bertus Beaumont (Technical University, Delft, The Netherlands) reviewed some of the literature on nanotechnology applications. These include, for example, use of nanosilver as an antimicrobial agent; gold nanoparticles[8] functionalised with antimicrobials to improve pharmacokinetics and targeting; and modular polycarbohydrates, self-assembling nanoparticles inducing selective lysis of microbial membranes. Nanotechnology can also be employed as a tool in the search for new antimicrobials, for example using atomic force microscope cantilevers to detect low concentrations of bacteria and screen their response to antibiotics[9]. Nanofluidics might also be used as a technology to facilitate the various platforms described by previous speakers, for example for high-throughput applications in combinatorial chemistry and screening of antibiotic-producing species and compounds.

More speculatively, would it be possible to engineer bacteriophages to overcome their weaknesses as therapeutics (stability and DNA transfer)—to make programmable machines by modular construction, to be non-toxic and biodegradable with pre-emptive strategies to counter resistance developing? Admittedly ambitious, a start on an engineered bacteriophage tail could be made by a better understanding of key mechanisms at the nanoscale and the application of engineering principles (now robustly established in many synthetic-biology laboratories). In discussion, while some doubted the value of bacteriophages as the starting point for engineering (partly because of their very limited host range), there was recognition of the theoretical potential of nanotechnology and synthetic biology to engineer and target new antimicrobial entities that could adapt to circumstances.

Knowledge-based discovery of modern anti-infectives

Another alternative approach to filling the discovery void was presented by Rainer Fischer (Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany) based on biotechnology studies of insects, chosen because of their evolutionary success and biodiversity, representing an incredible compound library. Various case studies were reviewed, including the following:

- Rat-tailed maggots of the drone fly that can produce enzymes degrading biofilms.
- Identification of insect metallo-protease inhibitor (IMPI) in the moth *Galleria mellonella*; tailoring IMPI variants to inhibit other proteases, for example pseudolysin, a key secreted virulence factor in *Pseudomonas aeruginosa*.
- Transcriptomics-based identification of antimicrobial peptides in secretions of the maggot of the common fly *Lucilia sericata*.
- Studies on the immunobiology and invasion biology of the harlequin ladybird *Harmonia oxyridis* identified a lead compound, harmonine, with broad spectrum antimicrobial activity, including multi-stage anti-malaria activity. The characterisation and regulation of multiple antimicrobial peptides in *Harmonia* has been described in detail[10].

Capitalising on insects as a source of novel therapeutics provides the underpinning for an integrated knowledge-based approach whereby academia can be a central part of discovery and development. Integrating this drug discovery hub for library construction, screening and lead selection (supported by public funding and private sector collaboration) has required some important lessons to be learned: validating standard operating procedures to collect and compare data from multiple sources; establishing access to new sources (soil sample collections, international collaborative projects to use biodiversity in global

[8] A recent study incorporated gold nanoparticles to stabilise liposomes for sustained topic antimicrobial delivery in a *Staphylococcus aureus* mouse model: Gao W et al. (2014) *Hydrogel containing nanoparticles-stabilised liposomes for topical antimicrobial delivery*. ACS Nano **8**, 2900–2907.

[9] Longo G et al. (2013) *Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors*. Nature Nanotechnology **8**, 522–526.

[10] Vilcinskas A et al. (2013) *Expansion of the antimicrobial peptide repertoire in the invasive ladybird* Harmonia oxyridis. Proceedings of the Royal Society B, doi: 10.1098/rspb.2012.2113.

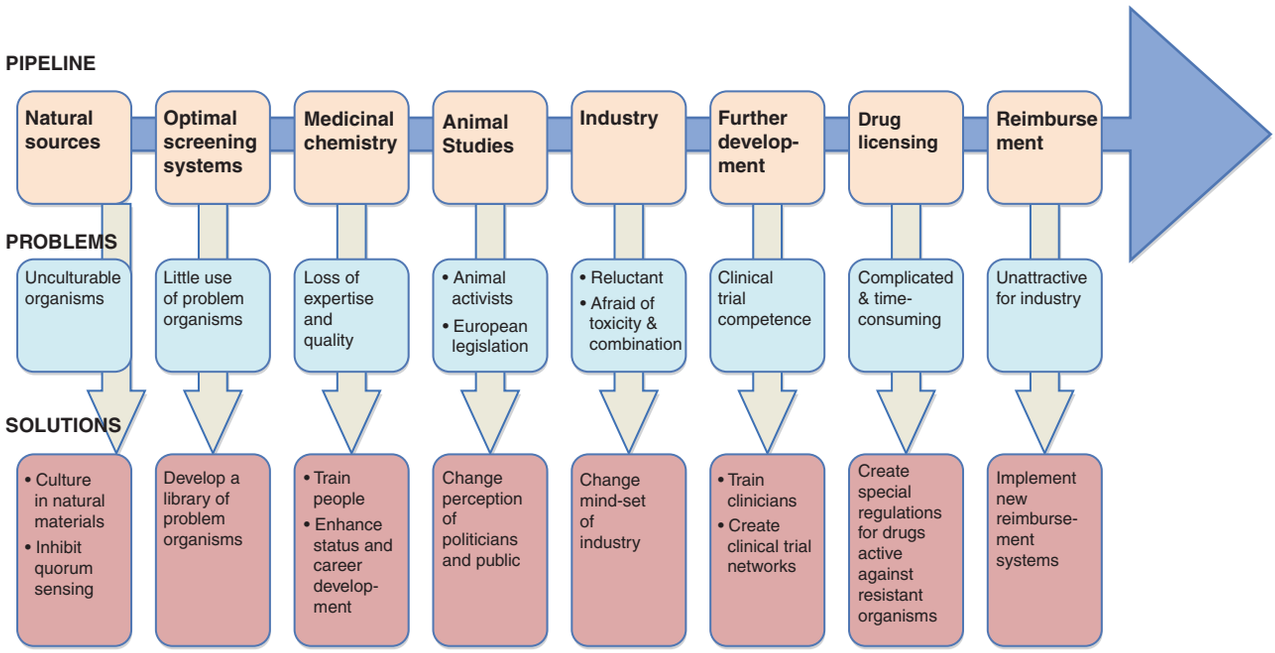

Figure 1 Problems and solutions in antimicrobial drug development.

hot spots, public–private partnership with Sanofi); generation of novel host-based screening models; and the development of production platforms to optimise protein expression. Infrastructure requirements can be demanding, for example for good manufacturing practice facilities, but are achievable in academia.

4 Conclusions and recommendations

Much needs to be done to enhance antibiotic innovation: to define and validate better targets, to ensure high-quality clinical research facilities, to streamline regulation and to tackle the market problems so that companies are attracted back into the therapeutic area[11].

There was consensus among the participants at the meeting on the urgency:

- to develop the critical mass to support and generate good new science;
- to remove the bureaucratic obstacles to using the outputs from that science;
- to ensure that innovation can be sustained in the longer term.

Satisfying these needs depends on raising the political visibility of the threat of antimicrobial resistance and tackling the challenges for research and innovation across a broad front, as described in the following recommendations (and summarised in Figure 1, shown for simplicity as a linear sequence but in reality subject to feedback and iteration).

(i) Support for basic research

Developing novel approaches to countering infection and understanding antimicrobial resistance is intellectually exciting—as exemplified by presentations at the meeting. Basic research merits more support by the European Commission and Member State funding agencies. This should include the study of virulence factors, signalling molecules in recognition and communication, and the dimensions of host–pathogen interaction. It should also include those research activities associated with identification of new antibiotics, namely the study of new mechanisms of action, target validation and exploration of new lead structures.

There is also room to do more in social sciences research, to understand and then to influence the determinants of human behaviour associated with the spread of resistance, and to support interdisciplinary connections across all the sciences including, for example, environmental and ecological research.

Increasing investment in fundamental research should result in the field becoming more attractive to young

[11] There is some evidence that a few large pharmaceutical companies are re-investing in the area (including Sanofi, with the Fraunhofer Institute in 2011, after spinning out antibiotic research in 2007) although many industry experts remain concerned that the commercial incentives do not yet outweigh the challenges of antibiotic development: Anon. (2014) *An antibiotic comeback*. Nature Reviews Drug Discovery **13**, 165. Although it is too soon to be sure, there may be some amelioration of the unfavourable market economics emerging in consequence of the 'Generating Antibiotics Incentives Now' 2012 provision of the US Food and Drug Administration Safety and Innovation Act.

investigators. However, there is concern that the best scientists are deterred because journals publishing research in this area may have a low Impact factor. Increasing attention to this purported measure of quality by research funding bodies and others, taken together with the complications of recognising individual contributions in large, perhaps multidisciplinary teams, represents an increasing problem for the robust evaluation of high-quality science. It is timely to examine more broadly how the impact of research should be assessed and rewarded: these matters will be considered in a project of the InterAcademy Partnership that will explore the issues for science assessment practices and the consequences of the current evaluation regimes.

At the same time, there must be commitment to revive the training of scientists in the relatively neglected disciplines, for example medicinal chemistry, and to reverse the migration of skilled staff in pharmaceutical companies from the EU.

(ii) Platforms for compound identification, lead optimisation and characterisation

Funding agencies must also be helped to understand the value of supporting broader platforms to facilitate lead discovery and validation, as well as the importance of continuing to invest in-depth within a broad range of research topics. As detailed in the presentations, new scientific opportunities are coming into range, and discovery platform priorities include investigating new natural product sources (for example insects and microbes from extreme environments), standardising mechanisms for activating silent genes, culturing the hitherto non-culturable, deciphering the rules of cell penetration, capitalising on prodrugs and new delivery systems, identifying off-target effects, transcriptomics to define and differentiate modes of action, and the development of combinatorial approaches.

(iii) Addressing the bottlenecks for preclinical and early clinical development activities

Currently there is a major problem in the EU in the lack of expertise and resources available for academia to progress interesting agents into animal models to reach proof-of-principle stage and attract industry attention. In the USA, the National Institutes of Health accepts compounds from academia for free testing in animal models subject to satisfying certain criteria[12]. The recent announcement from the National Institutes of Health[13] of a new US initiative, 'Accelerating Drug Discovery', to tackle the disconnect that exists between the preliminary identification of biological targets and the production of viable effective treatments, bringing together government, non-profit and industry stakeholders, may also serve as a model for further discussion in the EU for collective work, focused on antibiotic innovation with a central role for academia.

Various other competences that may be necessary to reach proof-of-principle are also scarce in academia in the EU, in particular medicinal chemistry to optimise lead generation, but also skill sets for evaluation of drug metabolism and toxicity. It is vitally important to increase public funding available for preclinical activities in the EU, associated with the achievement of agreed milestones on the path to proof-of-principle. Resources might be structured in various ways: a single, centralised EU institution is one option, but there are other possibilities, for distributed, contracted-out expert resources and (virtual) networking. Whichever system is chosen, it must ensure interdisciplinarity and teamwork, and embody oversight by an expert advisory board to assess the value of proposed entrants to preclinical development according to agreed criteria. Considerations about intellectual property would also need to be addressed.

It is urgent to consider new funding sources for these preclinical development functions—possibly the European Investment Bank would be interested. In some cases, it will also be desirable to seek new funding for early clinical development. For example, a not-for-profit consortium could draw income from Member States, international organisations and pharmaceutical companies, among others, with financial oversight by an executive board and income reinvested from subsequent sale of assets to industry. In the present meeting, time did not allow detailed discussion of incentives and new business models to encourage industry partnership and innovation on antibiotics, but the issues have been highlighted in previous academy work (Table 1) and suggestions by other bodies have been published recently[14]. It is also

[12] The US National Institute of Allergy and Infectious Diseases (NIAID) is leading a comprehensive Antibacterial Resistance Program that offers an extensive range of preclinical and clinical services. NIAID recently created a leadership group to develop, design, implement and manage clinical studies to focus on antimicrobial resistance.

[13] The US initiative will sponsor three to five pilot projects in three disease areas: Alzheimer's disease, type 2 diabetes and autoimmune disorders including rheumatoid arthritis and lupus. Further details are at http://www.nih.gov/science/amp and in the editorial *Accelerating drug discovery* in the Lancet (2014) **383**, 575.

[14] For example, the Chatham House Working Group paper on new business models for sustainable antibiotics, February 2014, http://www.chathamhouse.org/publications.

important to note that clinical research depends not only on funding but also on the availability of excellent clinical researchers. There may be opportunities in the EU, by analogy with the US National Institute of Allergy and Infectious Diseases initiative, to develop consortia of clinicians, to sustain skill development in infectious disease research and to facilitate faster recruitment to trials.

It is also highly desirable that EU publicly funded development activities serve as a basis for international collaboration, perhaps particularly with the US National Institutes of Health, to ensure strategic complementarity and to make the most of limited infrastructure, for example pathogen-handling facilities.

(iv) Optimising EU partnerships for research and strategy in the collective endeavour

Current scientific partnerships between academia and the pharmaceutical industry, exemplified by the Innovative Medicines Initiative (IMI)[15] are highly welcome. However, in continuing to improve the performance of IMI to deliver the objective of additional value, it is essential that pharmaceutical company partners are encouraged to contribute their best assets (compounds and ideas) to the pre-competitive projects and that IMI has the flexibility to explore new findings emerging during the projects. Moreover, it is crucial that the concentration of attention on IMI does not act inadvertently to constrain other funds to academia to generate and develop novel ideas.

The Joint Programme Initiative on Antimicrobial Resistance[16], providing a mechanism for Member States to agree research needs and with the objectives to enable greater impact and avoid duplication, is also welcome. However, in the view of participants at the meeting, it is very much underfunded for development of new drugs, and the available funds are too much diluted.

In efforts to build new strategic partnerships at the EU level, there is the opportunity to learn from and emulate the initiative of the German National Academy of Sciences Leopoldina following its joint 2013 report, in bringing together researchers, funders, regulators and others to tackle the investment and translational research issues. As one next step, EASAC and its member academies will consider what can be done to engage with other stakeholders in the EU and to extend the debate globally.

(v) Rethinking the regulatory frameworks

Faster antibiotic innovation also requires attention to regulatory frameworks. It would help if different regulatory authorities could agree on requirements for registration (with flexibility to consider different types of clinical evidence). Among the suggestions made by participants at the meeting were simpler regulatory requirements for narrow spectrum agents (with approval based on smaller clinical trials), for combination of established therapies, and where the need is critical, for example in tuberculosis. There must be renewed commitment to conditional licensing where early marketing on the basis of core clinical trial data is followed by comprehensive monitoring for collection of post-marketing data in routine use. It is also likely that registration requirements for new antibiotics will be increasingly influenced by, and coincident with, the development of rapid, simple diagnostics to detect resistance and direct therapy.

A case can be made for facilitating regulation to focus on the priority indications: multidrug-resistant Gram-negative bacteria, MRSA and other multidrug-resistant Gram-positive bacteria, and drug-resistant tuberculosis. However, it is also essential to ensure flexibility in regulatory frameworks to enable preparedness for unexpected (re-)emerging threats.

(vi) Raising public awareness

The challenges are scientific, technical, regulatory and economic. They cannot be tackled without increasing the visibility of the issues with society. Better public engagement is centrally important: to describe the global threats and new pressures (for example, ageing populations); to educate on how to preserve the efficacy of the antibiotics currently available (including reduction in their use outside human health); to encourage support for research and innovation and, in particular, the appreciation that this necessitates the use of animals in research; and that we cannot succeed without reducing bureaucracy or revisiting societal expectations of zero side effects. EASAC's member academies have an important continuing responsibility to raise public awareness.

[15] The IMI project series New Drugs for Bad Bugs (ND4BB) recently launched its third public–private partnership 'European Gram-negative antibacterial Engine' to tackle the discovery void. ND4BB will also start a project currently entitled 'Driving re-investment in R&D and responsible use of antibiotics to focus on new business models and economic strategies': see further details in Rex JH (2014) *ND4BB: addressing the antimicrobial resistance crisis*'. Nature Reviews Microbiology, doi:10.1038/nrmicro3245.

[16] The Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) Strategic Research Agenda was published in January 2014: http://www.jpiamr.eu/activities/strategicresearchagenda.

Appendix 1 Workshop Participants, 6–8 March 2014, Hannover, Germany

Professor Dan Andersson, Uppsala University, Sweden

Dr Bertus Beaumont, Technical University Delft, The Netherlands

Professor Axel Brakhage, Friedrich Schiller University Jena, Germany

Dr Eefjan Breukink, University of Utrecht, The Netherlands

Dr Heike Brötz-Oesterhelt, Heinrich Heine University Düsseldorf, Germany

Professor Dame Sally Davies DBE, Chief Medical Officer, Department of Health and National Health Service, United Kingdom

Professor Nynke Dekker, Delft University of Technology, The Netherlands

Dr Christiane Diehl, EASAC Executive Director, German National Academy of Sciences Leopoldina, Halle (S.), Germany

Professor Alan Dobson, University College Cork, Ireland

Professor Arnold Driessen, University of Groningen, The Netherlands

Professor Mikael Elofsson, University of Umea, Sweden

Professor Jeff Errington, Newcastle University, United Kingdom

Dr Robin Fears, EASAC Biosciences Programme Secretary, United Kingdom

Professor Ben Feringa, University of Groningen, The Netherlands

Professor Rainer Fischer, Fraunhofer Institute for Molecular Biology and Applied Ecology, Aachen, Germany

Ms Anna-Maria Gramatté, German National Academy of Sciences Leopoldina, EASAC, Halle (S.), Germany

Dr Oliver Grewe, VolkswagenStiftung, Hannover, Germany

Professor Jörg Hacker, President, German National Academy of Sciences Leopoldina, Halle (S.), Germany

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Dr Henrike Hartmann, VolkswagenStiftung, Hannover, Germany

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Professor Mariana Pinho, Universidade Nova de Lisboa, Portugal

Dr Andrew Roe, University of Glasgow, United Kingdom

Professor Hans-Georg Sahl, University Hospital Bonn, Germany

Professor Philippe Sansonetti, Institut Pasteur, Paris, France

Dr Tanja Schneider, University Hospital Bonn, Germany

Professor Volker ter Meulen, Co-Chair InterAcademy Partnership, German National Academy of Sciences Leopoldina, Halle (S.), Germany

Professor Jos van der Meer, EASAC President, Nijmegen Medical Centre, The Netherlands

Professor Gilles van Wezel, Leiden University, The Netherlands

Professor Christina Vandenbroucke-Grauls, VU University Medical Center Amsterdam, The Netherlands

Professor Ada Yonath, Weizmann Institute of Science, Rehovot, Israel

Appendix 2 Acknowledgements

This Statement of the Hannover meeting was prepared by EASAC drawing on the presentations and discussions, and other recent literature. We thank all participants and presenters.

Appendix 3 Meeting agenda

EASAC brainstorm:
'Antimicrobial drug discovery, greater steps ahead'

6th-8th March 2014
V E N U E: Schloss Herrenhausen | Herrenhäuser Straße 5 | 30419 Hannover

Thursday, March 6th	17:00	**Welcome** | *Chair: Jörg Hacker* **Welcome** | *Henrike Hartmann, Volkswagen Foundation*
	17:15	I N T R O D U C T I O N **Why are we here?** | *Dame Sally Davies* **The antimicrobial crisis: where did things go wrong?** | *Jos van der Meer* **What hasn't worked so far?** | *Heike Brötz-Oesterhelt*
	18:00	**First plenary discussion** | *Chair: Christina Vandenbroucke-Grauls*
Friday, March 7th	S E S S I O N 1 | *Chair: Hans-Georg Sahl*	
	09:00	**What is the function of antibiotics and antibiotic resistance genes in nature?** | *José L Martínez*
	09:45	**The biosynthesis and biological interactions of antibiotics** | *Christian Hertweck*
	11:00	**The pathogenetic concepts** a. **Life as an extracellular pathogen** | *Jürgen Heesemann* b. **Intracellular pathogen-host cell interdependence – exploration of a new therapeutic concept** | *Thomas F. Meyer*
	12:00	**Discussion in multidisciplinary groups**
	S E S S I O N 2 | *Chairs: Jos van der Meer / Volker ter Meulen*	
	13:30	**Microbial cell biology, finding targets** | *Jeff Errington*
	14:15	**New technologies to develop antimicrobials from natural products** | *Kim Lewis*
	15:00	**Pathway analysis: microbes are not equal** | *Mihai Netea*
	16:00	**Can nanotechnology contribute?** | *Bertus Beaumont*
	16:45	**Working groups (multidisciplinary)**
	18:15	**Reports from the working groups and plenary discussion**
Saturday, March 8th	S E S S I O N 3 & C O N C L U S I O N | *Chairs: Jos van der Meer / Volker ter Meulen*	
	09:00	**Knowledge based discovery of modern anti-infectives** | Rainer Fischer
	09:30	**Did we accomplish something?**
	9:45	**Working groups**
	11:30	**Reports from the working groups and plenary discussion**

The meeting was made possible with support from The Royal Netherlands Academy of Arts and Sciences (KNAW), the German National Academy of Sciences Leopoldina, and the VolkswagenStiftung.

EASAC

EASAC – the European Academies' Science Advisory Council – is formed by the national science academies of the EU Member States to enable them to collaborate with each other in providing advice to European policy-makers. It thus provides a means for the collective voice of European science to be heard. EASAC was founded in 2001 at the Royal Swedish Academy of Sciences.

Its mission reflects the view of academies that science is central to many aspects of modern life and that an appreciation of the scientific dimension is a pre-requisite to wise policy-making. This view already underpins the work of many academies at national level. With the growing importance of the European Union as an arena for policy, academies recognise that the scope of their advisory functions needs to extend beyond the national to cover also the European level. Here it is often the case that a trans-European grouping can be more effective than a body from a single country. The academies of Europe have therefore formed EASAC so that they can speak with a common voice with the goal of building science into policy at EU level.

Through EASAC, the academies work together to provide independent, expert, evidence-based advice about the scientific aspects of public policy to those who make or influence policy within the European institutions. Drawing on the memberships and networks of the academies, EASAC accesses the best of European science in carrying out its work. Its views are vigorously independent of commercial or political bias, and it is open and transparent in its processes. EASAC aims to deliver advice that is comprehensible, relevant and timely.

EASAC covers all scientific and technical disciplines, and its experts are drawn from all the countries of the European Union. It is funded by the member academies and by contracts with interested bodies. The expert members of EASAC's working groups give their time free of charge. EASAC has no commercial or business sponsors.

EASAC's activities include substantive studies of the scientific aspects of policy issues, reviews and advice about specific policy documents, workshops aimed at identifying current scientific thinking about major policy issues or at briefing policy-makers, and short, timely statements on topical subjects.

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