



K O N I N K L I J K E N E D E R L A N D S E
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ANTIBIOTICS – NOW!

Journalist report by the joint Academy-University of Groningen symposium 'Antibiotics – Now!', on finding solutions for antibiotic resistance, 2-3 May 2016, Groningen

door René Fransen

Could infectious diseases that are now considered harmless become a plague in the near future? Thanks to the discovery of penicillin almost a century ago, many dangerous infections can be cured with just a few pills. Antibiotics are also vital in preventing complications during complex surgical procedures. But antibiotic resistance is on the rise, and the pipeline for new compounds is running dry. During a joint Academy-University of Groningen symposium on 2-3 May 2016, approximately 160 specialists in drug discovery and development, clinical use and policymaking gathered in Groningen to discuss the way forward.

Excess and access

The Dutch Minister of Health, Welfare and Sport Edith Schippers kicked off the conference with a recorded opening address. She referred to the changes brought about by Ian Fleming's discovery of penicillin and added that medicine is now at a tipping point. Government, science and industry have to work together to fight antibiotic resistance. 'I count on you,' she said.

The keynote speaker was Dame Sally Davies, Chief Medical Officer for England, who has put antibiotics on the agenda of the UK Government and internationally. She stressed that antibiotic resistance is a global problem, saying: 'There are rivers in India which have a higher concentration of ciprofloxacin than the blood values you would expect to find in a patient.'

Davies outlined the global initiatives in which she is involved, most notably the WHO Global Action Plan for antimicrobial resistance, where she chaired the strategic and technical advisory group (STAG) advising the WHO. She has also cooperated with many stakeholders and politicians to get the issue on the agenda for the next UN General Assembly. She acknowledged the help of Edith Schippers and her Swedish counterpart in getting this done. 'And it will be discussed in the week when heads of state are present at the UN,' she said

Davies stressed that excess use of antibiotics is a problem, but access is also a concern: 'At present,' she said, 'more people die from lack of access to antibiotics than of resistant bacteria.' But projections show that by 2050, the world might face 10 million deaths from untreatable infections. That is why she continues to travel the globe to raise awareness of the problem and encourage innovation.



Pipeline

Innovation is exactly what the rest of the conference was about. The organisers had opted for an interdisciplinary approach, with talks on policy, the search for new drug candidates, current antibiotic development, and alternative approaches to fighting infections. Clinicians, biologists, chemists and physicists presented their views on the problem of antibiotics resistance.

One important issue is the inadequate drug pipeline. The last time a new class of antibiotics was discovered was 1987. Development costs have increased and the current economic model, based on 'selling pills', cannot cover the investment. Antibiotics are typically used for short-term treatments, and new antibiotics are used as sparingly as possible to prevent resistance development.

Indeed, whereas 'Big Pharma' normally takes new drugs from discovery to the clinical phases, small and medium-sized enterprises are now taking on this – expensive – role, explained Ursula Theuretzbacher, Principal of the Center for Anti-Infective Agents (CEFAIA), Austria. Other economic models are required to encourage antibiotic research and development that take the unique situation of antibiotics into account. Several speakers talked about de-linking revenues from volume drug sales, for example by offering a payment for bringing a new drug to the market that is effective against the most resistant bacteria. Finding a new economic model is a bit like 'finding a solution for global warming,' Theuretzbacher confessed. Indeed, one response to her talk was that any system not based on market forces would not be acceptable to the US.

Along with the inadequate pipeline, Theuretzbacher issued another warning: with Big Pharma not investing in antibiotics research and with so many years of neglect in this area, small drug discovery companies are having a hard time finding employees experienced in drug discovery and development.

New leads

Be that as it may, there were quite a few presentations by scientists who are doing just that. Traditionally, fungi (as in the case of penicillin) or bacteria have been sources of new antimicrobial compounds. In the 1990s, however, another paradigm emerged: find a drug target, purify it and test a huge library of up to a million compounds against the target. This seemed like a valuable approach, but the results were disappointing, explained Heike Brötz-Oesterhelt (Düsseldorf University).

The compounds contained in the libraries were often small ones, a far cry from complex natural antibiotics that are 'optimised by nature'. One drawback of natural drug candidates, however, is that they are not optimised for use in humans. 'These leads get you up the Matterhorn in part, but there is still quite a climb remaining,' said Brötz-Oesterhelt. Lead compounds may turn out to be insoluble, unstable or not optimisable for human application. In the end, the overall drug-like profile of a new antibacterial compound is what counts most.

Several talks discussed mining nature for these 'optimised' natural antibiotics. For example, many bacteria carry gene clusters that are silent under standard cultivation circumstances in the lab. They can be found using bio-informatics to search bacterial genomes. Another approach is to take the ecology of bacteria into account. In some cases, adding a soil extract to soil bacteria triggers the production of new secondary metabolites. Florian Kloss (Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute) described this approach in anaerobic bacteria – a much-overlooked source, as they are difficult to cultivate and generally unknown in antibiotics production.



Another approach is to co-cultivate several microbial species. After all, no micro-organism grows alone in nature. The interaction stimulates the production of secondary metabolites. Adding plant extracts and specific exudates to *Streptomyces* species, for example, activated biosynthetic pathways for antibiotics. This approach led Gilles van Wezel (Leiden University) to discover several interesting lead compounds.

Another out-of-the-box method for finding new secondary metabolites involves using the microbial richness present in organisms like insects and marine sponges. Insects have their own microbiomes and form an almost untapped source, explained Rainer Fischer (Fraunhofer Institute for Molecular Biology and Applied Ecology). Anna Vagstad (ETH Zürich) described them as 'nature's incubators': 'Insects and sponges develop their own drugs,' she said. Her talk focused on the natural products of a marine sponge (*Theonella swinhoei*) and the biosynthetic pathway for polytheonamides produced by an endosymbiont bacteria.

However, some new drug candidates are based on old systems. Arnold Driessen (University of Groningen) showed how even good old *Penicillium chrysogenum* produces some as yet untapped secondary metabolites. Also, by silencing secondary metabolite pathways, the fungus becomes an ideal 'plug and play' production platform for new antibiotics.

Conference host Oscar Kuipers explained his work on lantibiotics, highly modified peptides. They were introduced as food preservatives decades ago and their role as antibiotics seemed marginal at best, but Kuipers took a synthetic biology approach that allowed him to design and produce new lantibiotics. Some compounds show promising activity against gram-negative bacteria, in particular when acting synergistically with small eukaryotic peptides. Gram negatives are a very difficult class to defeat, as they have two membranes with different properties that must be penetrated to kill them.

Nisin, a well-known lantibiotic that is active against gram-positive bacteria, was also one of the topics covered by Nathaniel Martin (Utrecht University), who discussed the bacterial peptidoglycan layer as a potential target. Nisin binds to lipid II, a peptidoglycan precursor molecule. Like Kuipers, Martin discussed ways to modify nisin to increase its effectiveness and potential for therapeutic use.

Martin also discussed undecaprenyl phosphate (C55-P), which carries lipid II across the bacterial membrane, as a target. His group recently discovered that the natural compound Laspartomycin C acts on C55-P via a mode of action unlike any antibiotic in clinical use. According to Martin, even though the low-hanging fruit is gone, there are still plenty of apples a little higher up the tree.

Alternatives

But fighting antibiotic resistance isn't just about finding new drugs. Ben Feringa, professor of organic chemistry at the University of Groningen and designer of both the world's first light-driven molecular motor and the first molecular 4-wheel drive molecular car, showed how light-operated switches can control the activity of drugs. Switches can for example be used to make sure drugs are no longer active after excretion. They are also useful in drug delivery, to open pores in a vesicle on the exact spot of an infection. Furthermore, auto-deactivation, dual activation or other switching options all have a high level of spatial and temporal control and are triggered by a perfectly harmless stimulus.

Another approach for targeted drug delivery was discussed by Jan van Hest (Radboud University Nijmegen). He synthesised polymeric nanovesicles that can be made to target different compartments. These vesicles can cross the blood-brain barrier, and he described ways to get the vesicles to target specific cells by attaching peptides, something that may also help them to penetrate the cell membrane.



Synthetic chemistry is an intrinsic part of drug development. Adri Minnaard (University of Groningen) gave a demonstration showing, first of all, how he identified and synthesised compounds produced by *Mycobacterium tuberculosis* to avoid being killed by macrophages. Two *M. tuberculosis* gene products arrest lysosome maturation in these immune cells and could prove to be interesting targets for antibiotics. But Minnaard also showed how to block the phosphorylation of a vital hydroxyl group on the aminoglycoside antibiotic kanamycin by changing its orientation. This may make it possible to overcome the bacterial resistance to kanamycin that results from such phosphorylation.

But the resistance genes are not the only reason why antibiotics fail. Bob Hancock (University of British Columbia, Vancouver) listed a number of alternative causes: sepsis (which causes cytokine-driven immune suppression), biofilms (which protect bacteria from antibiotics), or a reduced patient immune system due, for example, to chemotherapy all require more than just antibiotics. Additional medication, for instance to stimulate the immune system or attack biofilms, is needed to overcome these challenges.

Traditionally, clinicians have been hesitant to adopt multi-drug treatments for infection, but in addition to Hancock, several other speakers stressed the benefits of doing so. One was Floris Rutjes (Radboud University Nijmegen), who showed how synthetic pantothenamides can block a key microbial metabolic pathway *in vitro*, but are rendered useless *in vivo* by enzymes present in the blood. However, blocking these pantotheinases of the vanin family with a selective vanin-inhibitor makes the antibiotics effective again.

Before the advent of antibiotics, bacteria-killing viruses called bacteriophages were seen as an interesting treatment option. And in both Poland and Russia, phage therapy has never ceased to be an object of study. The clinical results were never very impressive, explained Henri Verbrugh (Erasmus MC, Rotterdam), but in the food industry, phages turned out to be very good at cleaning carcasses and surfaces. A number of clinical trials for therapeutic use are ongoing. Having a better understanding of how phages interact with bacteria is paramount.

One question that should indeed be explored is what phages contribute to the spread of antibiotic resistance, explained Maite Muniesa (University of Barcelona). The 'head' of a phage, called the capsid, usually packs the DNA of the virus. But packaging is not always selective, and bits of bacterial DNA can get into a phage, to be mobilised and introduced into another bacterium upon infection. Since phages don't always lyse their victim, this can lead to the emergence of new resistant bacteria. Because phages are so abundant (they are the most abundant entities on Earth, with an estimated 10^{31} virions), they may play an important role in the spread of antibiotic resistance genes.

Of course, drugs are not the only tools to combat infectious disease. Monitoring outbreaks is important for containment. But whereas a century or so ago disease spread neatly in concentric circles around the source, our increased mobility means that infections like SARS or MERS can jump to a different continent in the blink of an eye.

Physicist Dirk Brockmann (Humboldt University of Berlin) reviewed the challenges in modelling disease outbreak and presented a very neat solution that got quite a buzz during the coffee breaks. In the global air transport network, New York's JFK airport, for example, is in a practical sense closer to Frankfurt, Germany, than to a remote town in the State of New York. By redefining the notion of distance in this way, it turns out that diseases still follow the 'traditional' wave-like patterns according to the concept of effective distance. Identifying transport nodes helps to predict how a disease will spread. Brockman also showed data from an experiment that logged all the movements and face-to-face interactions of 1000 Danish students over three years using cell phone data. This underlined how important individual social interactions are: 'Averages don't determine disease spread, but individual interactions,' he said. Such information is vital to determine the effect of vaccination campaigns.



Clinic

Finally, all this work should have an impact on clinical use of antibiotics. Willem van Schaik (UMC Utrecht) showed how ICU patients differ from the general population in their gut microbiome. ICU patients carried increased levels of the opportunistic pathogen *Enterococcus* and suffered from a decrease in butyrate-producing species, which are both unhealthy changes. These are caused, at least in part, by selective digestive tract decontamination (SDD), a standard procedure in most Dutch ICUs that involves administering multiple antibiotics simultaneously to eradicate opportunistic pathogens from the patient's microbiome. Clinicians need to find a balance between preventing SDD-induced infections in ICU patients and the possible emergence of resistance.

Indeed, some resistance to colistin, which is used in SDD but is also seen a 'last resort' antibiotic, was found in a *Klebsiella* outbreak in one Dutch ICU. Both Van Schaik and Christina Vandenbroucke-Grauls (VU University Medical Center, Amsterdam) blamed circulating *Klebsiella* that had already acquired resistance to some antibiotics used in SDD and that easily evolve resistance to colistin. This underlines the importance of detailed and real-time monitoring of the gut microbiome, and its resistance genes, during ICU hospitalisation. Vandenbroucke-Grauls had a sobering message: sooner or later resistance always arises. Resistance genes were even detected in 32,000-year-old permafrost soils. Resistance genes, or proto-resistance genes, are everywhere: 'Nature always strikes back,' she said.

So what is to be done? The last speaker, Bhanu Sinha (UMC Groningen), described an integrated view of the entire chain of antibiotic development summarising much of what had been said. New – targeted – drugs are important, and new economic models are needed to stimulate their development. Drug screening should go beyond the minimum inhibitory concentration (MIC), as this measures stasis rather than killing. Inappropriate killing actually drives the development of resistance. During development, constant feedback must be sought on issues of pharmacokinetics, pharmacodynamics and toxicity, to ensure maximum clinical effectiveness.

And of course, sustainable clinical use is important to delay the evolution of resistance. Rapid diagnostics are essential. Better imaging techniques can help clinicians to increase diagnostic certainty and to monitor therapy. Debulking infected tissues could improve the results of treatment. Hospitals should also be vigilant about transmission between wards and healthcare institutions. New treatment protocols and guidance (Antimicrobial Stewardship) have already reduced antibiotic use at UMC Groningen and also made it more cost-effective.

In his final summing up, the chairman of the last session, Jos van der Meer (Radboud University Medical Center), reiterated that staying ahead of antibiotics resistance will require combining many approaches from many different disciplines. But, as one of the speakers quoted from *Alice in Wonderland*, it will take all the running we can do to keep in the same place.

Preferred citation: KNAW (2016). *Antibiotics – Now! Journalist report by the joint Academy-University of Groningen symposium 'Antibiotics – Now!', on finding solutions for antibiotic resistance, 2-3 May 2016, Groningen (Author: René Franssen)*. Amsterdam, KNAW.