The science of antimicrobial resistance

How bacteria evade treatment

The discovery of antibiotics in 1928 was a major breakthrough in medical history, rendering many deadly diseases easily treatable for the first time. Today, nearly 100 years later, medical experts are painting dystopian scenarios of a post-antibiotic era as antimicrobial resistance (AMR) is claiming more than 700,000 casualties globally each year and estimated to dramatically increase to up to 10 million by 2050[1]. Looking back in time, Alexander Fleming already urged for caution in appropriately dosing antibiotics, when awarded the Nobel Prize for his discovery in 1945, as "exposing microbes to non-lethal quantities of the drug make them resistant." Despite his warning, AMR development by bacterial evolution and natural selection is fuelled by uninformed prescription. Furthermore, AMR is aggravated by the prophylactic use of antibiotics in agriculture and animal husbandry making AMR the major healthcare threat of the 21st century.

Antimicrobial resistance (AMR) is a term that covers resistance by both bacteria and fungi to existing drugs. In this article, we focus on bacterial resistance and discuss why these pathogens continue to resist treatment by current antibiotics. We suggest measures that can be taken to address this problem, including the role of antibiotic stewardship in maintaining the effectiveness of current drugs. Additionally, we explain how diagnostics, including one developed by our company, can aid the development of new drugs and guide patient treatment.

AMR is encoded in the DNA

What makes bacteria resistant? As there are numerous mechanisms of action for different antibiotics, there are various mechanisms of resistance. AMR can be conferred through preventing the antibiotic to reach its target (e.g. reduced permeability, increased efflux), changes made to antibiotic targets (e.g. by mutation) or direct modification or degradation of antibiotics (e.g. β-lactamases). Such mechanisms of resistance are encoded in the bacterial DNA and bacteria may be intrinsically resistant to certain antibiotics or acquire AMR. The simplest example of intrinsic resistance in an individual species results from the absence of a susceptible target of a specific antibiotic. The biocide triclosan for example, has broad efficacy against Gram-positive bacteria and many Gram-negative bacteria, but it is unable to inhibit growth of members of the Gram-negative genus *Pseudomonas*. Although this was initially thought to be due to active efflux, it has more recently been shown that it is instead due to the pathogen's mutations in the genetic code[2].

However, the reason that AMR is constantly evolving lies in the ability of bacteria to modify their genetic code under the selective pressure of antibiotics. This was the mechanism that Alexander Fleming had in mind. While humans tend to believe that evolution is something that happens slowly, the dramatic speed of this process was recently illustrated in a cinematic approach by scientists from Harvard in the much-cited "MEGA plate experiment"[3].

Typical examples of such acquired mutations are modifications of the drug's target, e.g. mutations in the genes coding for bacterial gyrases, enzymes required for DNA replication during bacterial cell division, which render fluoroquinolone antibiotics ineffective. Fluoroquinolones, inhibiting bacterial gyrases are a daunting example of bacterial evolution. During their introduction in 1987, the development of resistance was deemed unlikely since at least two mutations of the drug's target would be required to generate significant resistance. However, mutants of the target bacterial gyrase genes and efflux of fluoroquinolones from the cell have increasingly been encountered globally. Additionally, fluoroquinolone-modifying enzymes have made their appearance as well as a peptide (Qnr) that protects the cellular target, gyrase[4]. This demonstrates that the development of AMR is only a matter of time.

Not only are microbial pathogens able to continuously adapt to changing environmental conditions, develop mutations and evolve, they also have the ability to acquire resistance from one another. Intra- as well as interspecies gene transfer contributes significantly to the spread of AMR. In fact, many resistance genes may already be encoded in some genomes and they may also have developed as natural ecological phenomena even before human activity, demonstrated by micro-organisms found at pristine sites, including isolated caves and permafrost[5]. Such resistance genes are then passed on vertically through transmission of DNA from one generation to the next. Alternatively, they can be transferred horizontally, i.e. the transfer of genetic material from outside a clonal pathogen lineage by mobile DNA elements such as plasmids. A prominent example in this context are β-lactamase enzymes.

Since the introduction of β-lactam antibiotics, β-lactamases have become well-studied enzymes, amounting to almost 2,800 known unique proteins in 2018. Extended-spectrum β-lactamases (ESBLs) and carbapenemases confer resistance by hydrolysing β-lactam antibiotics, including penicillin, carbapenems, and cephalosporins. The horizontal inter- and intraspecies conjugation is spreading resistance in pathogens of highest concern, for example between *Enterobacteriaceae, Pseudomonas*, and *Acinetobacter*, even in microenvironments such as hospital sinks[6].

The need for antibiotic stewardship

Generally, the use of antibiotics increases the selection pressure for resistant strains, making antibiotic consumption a primary driver of antibiotic resistance[7]. Therefore, surveillance programmes to monitor antibiotic consumption have been established such as the Global Action Plan on AMR endorsed by the member states of the World Health Organization. The data shows that the antibiotic consumption rate in low-and middle-income countries has been converging to (and in some countries surpassing) levels typically observed in high income countries. For example,

between 2000 and 2015, antibiotic consumption increased from 3.2 to 6.5 billion (103%) defined daily doses (DDD) in India, from 2.3 to 4.2 billion DDDs (79%) in China, and from 0.8 to 1.3 billion DDDs (65%) in Pakistan.

Projections for global antibiotic consumption by 2030, assuming that there are no changes in policy, are up to 200% higher than the 42 billion DDDs estimated for 2015[8]. Acting on these alarming trends, antimicrobial stewardship programmes, such as those recommended by the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR), are increasingly being implemented in order to limit the use of antibiotics and slow the spread of resistance.

A key challenge for antibiotic stewardship programmes is the current diagnostic practice that still largely relies on culturing pathogens in a patient sample for pathogen identification and then exposing such cultures to antibiotics to determine their susceptibility or resistance. This process takes 48 to 72 hours - too long for clinicians to wait to take a decision on prescribing an antibiotic. Hence, the vast majority of antibiotic prescriptions are done empirically with the majority of patients being over-treated. This needlessly accelerates resistance while at the same time putting those who need treatment at risk. Consequently, novel rapid diagnostic methods delivering results in hours instead of days are increasingly important in the fight against spreading AMR. Such methods are either phenotypic, i.e. still relying on culture but trying to reduce culture time through miniaturisation to the microscopic level, or genotypic, i.e. based on the detection of bacterial DNA in the patient sample. With PCR (polymerase chain reaction) as the current molecular diagnostic workhorse technology, the latter are very fast and robust.

While it is comparatively easy to design PCR assays that sensitively and specifically detect pathogen DNA in a sample, AMR detection relies on assays specifically looking for genetic alterations conferring resistance. PCR panels however, due to their size limitation can only cover some of the more prevalent genetic markers of AMR, they are by far not exhaustive, leaving a significant diagnostic gap. Nevertheless, such panels are very useful in flagging patients that may need specific attention due to resistance to one or more commonly used drugs. Numerous panels including AMR markers have already been approved by the regulatory authorities.

The role of next-generation sequencing and AI

Recent advances and lower costs of DNA sequencing, mainly through the advent of next-generation sequencing (NGS), have not only made this technology indispensable in AMR research, but it is now also widely accepted for infection control purposes and AMR surveillance. Genome-wide association studies using NGS have also been applied for the systematic discovery of novel genetic AMR markers thereby closing the diagnostic gap that current molecular technologies leave. Beyond research applications, NGS has set out to transform public health surveillance and outbreak investigations by providing highly reproducible and accurate pathogen identification, antimicrobial resistance profiling, transmission tracking and biological risk assessment[11].

A prominent example is a study that tracked the *mcr-1* gene, now present across the globe and conferring resistance to a last resort antibiotic, namely Colistin. The outbreak was tracked back to a single event around 2005 when it moved from bacteria in pigs at a Chinese farm into pathogens that affect humans[12]. As a result, the European Centre for Disease Prevention and Control (ECDC) is strongly advocating the use of NGS to better track and trace AMR in the fight against antibiotic resistance[13].

With costs coming down and turn-around times getting shorter and shorter, NGS is also increasingly attractive for rapidly and precisely diagnosing infections and AMR using native patient samples and analysing the entire DNA in a sample at base pair resolution, creating a universal molecular test for infectious diseases. Taking a step towards this and building on recent advances in machine learning and artificial intelligence, academic scientists and newly emerging start-ups are trying to develop diagnostic tests that can accurately predict drug resistance or susceptibility based on DNA sequencing data.

Predicting drug responses

In contrast to current molecular diagnostics, the wealth of information gained from NGS data makes it possible to predict drug responses as accurate as minimum inhibitory concentrations (MICs), the lowest concentration of antibiotics that can inhibit the growth of a pathogen[14]. MICs are the output of the still widely applied culture-based antibiotic testing methods that usually take 48 hours or even weeks. However, MIC predictions based on DNA sequencing data obtained from NGS technology require artificial intelligence and deep machine learning to produce a result. These methods require comprehensive databases to provide the training datasets for the machine learning algorithms.

Lower costs for microbial sequencing have made it possible to create huge databases with genome and associated antimicrobial susceptibility data for thousands of clinical pathogens. Yet despite this, several hurdles remain before NGS can inform antibiotic therapy. Characterising and cataloguing genetic AMR mechanisms and biomarkers is key to improved molecular diagnostics as well as informing the development of new drugs. While the amount of NGS data generated and pathogenic strains sequenced is skyrocketing, NGS data itself is useless for training predictive artificial intelligence models unless combined with high quality antimicrobial susceptibility data.

Unfortunately, existing databases are often lacking this information and/or biased towards certain, multi-drug resistant pathogens. This is where expert-curated genetic databases of newly emerging start-ups such as ARESdb by Ares Genetics come into play. To sustainably maintain ARESdb, currently accumulating approximately 40,000 pathogens with quantitative antimicrobial susceptibility data for more than 100 antibiotics, the company is setting up a sustainable public-private partnership (PPP) model connecting globally leading diagnostic and pharmaceutical industry players with public and academic institutions. Because even though ARESdb enables molecular detection of AMR for more than 150 pathogen/drug combinations with up to 98% accuracy in its current version, it needs to be continuously updated to sustainably keep up with bacterial evolution.

With increasing economic pressure on the antibiotics

market, there is a dire need for more cost-effective approaches for bringing new treatments to the market. These approaches may include repurposing old drugs in novel combinations, or increasing clinical trial success rates using genetic markers allowing for targeted narrow-spectrum treatment[15]. Furthermore, drug candidates may be screened pre-clinically using genomic data to identify those with the most beneficial properties, e.g. the least likelihood of encountering resistance according to the resistome (the collection of all the antibiotic resistance genes and their precursors) in general or for a specific indication. This screening can be performed *in-silico* using databases such as ARESdb which link genotypic to phenotypic resistance data[16]. Similarly, genomic databases can also inform drug repurposing, aiming at using existing therapies or drugs that have stalled in development to treat conditions for which they were not originally intended. Given the long development time, high cost and low success rates of new therapies that seek market approval, drug repositioning is an attractive option. It is efficient and potentially less costly because the candidate drugs may already have established safety profiles from Phase I clinical trials. This is data that can be used in new studies and in the registration dossier[17].

During clinical trials of antibiotics, companion diagnostics may help identify patients with a specific microbial aetiology of their illness. For example, a clinical trial of a novel antimicrobial agent with activity against both methicillin-resistant and sensitive *Staphylococus aureus* (MRSA and MSSA, respectively) in skin and skin structure infections may benefit from a rapid test to screen wound specimens from potential patients to identify those specifically with MRSA infections[18].

Finally, extrapolated data on resistance mechanisms, genetic basis of resistance, occurrence of cross-resistance and susceptibility data can significantly support the approval of such drugs by regulatory authorities. After regulatory approval, a more widespread use of molecular diagnostics would have the potential to turn the current empirical antibiotic treatment regimens into informed treatments, further enabling the use of narrow-spectrum antibiotics and limiting the spreading of AMR.

Conclusion

The technology exists to identify and track the resistance mechanisms of bacteria, even as these pathogens change in response to pressures from the environment. A combination of stewardship of current drugs, surveillance of new pathogens and the targeted treatment of patients with infections offers a way forward.

References:

1. O'Neill, J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations the Review on Antimicrobial Resistance. (2016). doi:10.1016/j.jpha.2015.11.005

2. Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O. & Piddock, L. J. V. Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology* **13**, 42–51 (2015).

3. Pesheva, E. A cinematic approach to drug resistance – Harvard Gazette. (2016). Available at: https://news.harvard.edu/gazette/story/2016/09/a-cinematic-approach-to-drug-resistance/. (Accessed: 9th May 2019)

4. Davies, J. & Davies, D. Origins and Evolution of Antibiotic Resistance. *Microbiol. Mol. Biol. Rev.* **74**, 417–433 (2010).

5. Bush, K. Past and Present Perspectives on β-Lactamases. Antimicrob. Agents Chemother. **62**, e01076-18 (2018).

6. Lerminiaux, N. A. & Cameron, A. D. S. Horizontal transfer of antibiotic resistance genes in clinical environments. *Can. J. Microbiol.* **65**, 34–44 (2018).

7. Malhotra-Kumar, S., Lammens, C., Coenen, S., Van Herck, K. & Goossens, H. Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. *Lancet* **369**, 482–490 (2007).

8. Klein, E. Y. *et al.* Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E3463–E3470 (2018).

9. Jaillard, M. *et al.* A fast and agnostic method for bacterial genome-wide association studies: Bridging the gap between k-mers and genetic events. *PLoS Genet.* **14**, e1007758 (2018).

10. Suzuki, M., Shibayama, K. & Yahara, K. A genome-wide association study identifies a horizontally transferred bacterial surface adhesin gene associated with antimicrobial resistant strains. **Sci. Rep**. **6**, 37811 (2016).

11. Mellmann, A. et al. High Interlaboratory Reproducibility and Accuracy of Next-Generation-Sequencing-Based Bacterial Genotyping in a Ring Trial. *J. Clin. Microbiol.* **55**, 908–913 (2017).

12. Wang, R. *et al.* The global distribution and spread of the mobilized colistin resistance gene mcr-1. *Nat. Commun.* **9**, 1179 (2018).

13. European Centre for Disease Prevention and Control. Expert opinion on whole genome sequencing for public health surveillance. *ECDC* (2016). doi:10.2900/12442

14. Monk, J. M. Predicting Antimicrobial Resistance and Associated Genomic Features from Whole-Genome Sequencing. *J. Clin. Microbiol.* **57**, e01610-18 (2019).

15. Wong, C. H., Siah, K. W. & Lo, A. W. Estimation of clinical trial success rates and related parameters. *Biostatistics* **20**, 273–286 (2019).

16. Coll, F. *et al.* Genome-wide analysis of multi- and extensively drug-resistant Mycobacterium tuberculosis. *Nat. Genet.* **50**, 307–316 (2018).

17. Pritchard, J.-L. E., O'Mara, T. A. & Glubb, D. M. Enhancing the Promise of Drug Repositioning through Genetics. *Front. Pharmacol.* **8**, 896 (2017).

18. Caliendo, A. M. *et al.* Better Tests, Better Care: Improved Diagnostics for Infectious Diseases. *Clin. Infect. Dis.* **57**, S139–S170 (2013).

This article was written by Andreas Posch, managing director and chief executive of Ares Genetics GmbH; Achim Plum, managing director, and Richard Zahrl, life science business associate, also of Ares Genetics GmbH. Dr Posch joined Ares Genetics from Siemens where he was responsible for bioinformatics R&D at Siemens Healthcare. Dr Plum, who is also chief business officer of Curetis Group, previously worked at Siemens and the medical diagnostics company Epigenomics AG. Dr Zahrl joined Ares Genetics from the Austrian Centre of Industrial Biotechnology at the University of Natural Resources and Life Sciences in Vienna.