A new paradigm for antibiotic resistance research

*By drawing from the insights of EvoTAR Coordinator **Dr Rob Willems** in the project's final newsletter, we highlight how important innovation is to progressing knowledge on the ways in which antibiotic resistance operates*

The decision to fund the Evolution and Transfer of Antibiotic Resistance (EvoTAR) project came about in an unusual way. Can you share with us the background of the support for such a study?

I have been coordinator of EvoTAR for the past four years, but the story of EvoTAR started much earlier. More than a year before the start of EvoTAR, a group of talented and ambitious scientists came together to form a consortium. We were successful in developing an innovative research strategy that covered many relevant aspects of the evolution and transfer of antibiotic resistance and were very thankful that the European Commission decided to support our proposal. I appreciate how the different groups that were involved in EvoTAR have worked together. Several collaborations between groups from different European countries have been fostered in EvoTAR and I sincerely expect that these will continue after EvoTAR has ended.

From your perspective, what do you consider the highlights of the EvoTAR collaboration to be?

It is difficult to say what I enjoyed most during my time as coordinator of EvoTAR. The recent International Conference on the Evolution and Transfer of Antibiotic Resistance 2015 (ICETAR2015), which was organised under the auspices of the EvoTAR consortium, was definitely a highlight. I was very pleased with the excellent presentations by EvoTAR scientists at ICETAR2015 and was happy to welcome a large number of scientists from outside the EvoTAR consortium to the conference.

In what ways do you consider EvoTAR is taking an innovative approach to antibiotic resistance research?

Clearly, EvoTAR's mission in approaching antibiotic resistance from an ecological and evolutionary framework has struck a chord with many scientists. In addition, this approach has led to some completely original approaches, developed by the SMEs BioMar and DaVolterra, to counter the emergence and spread of antibiotic resistance. We have managed to uncover many new and relevant aspects of the emergence and spread

of antibiotic resistance. Importantly, we have also trained a new generation of young scientists in the field of antibiotic resistance research. I expect that, in the future, research will continue to build on the foundations that are laid down by the EvoTAR consortium, thereby contributing to the fight against the rising tide of antibiotic resistance.

A fighting chance

The challenges facing the world in managing the growing resistance to antibiotics have been well documented. By adopting a novel approach to this major threat, the EvoTAR project is using an ecological and evolutionary framework to improve understanding about how antibiotic resistance spreads and changes

Late last year, the World Health Organization (WHO) held the first ever World Antibiotic Awareness Week to increase awareness of global antibiotic resistance and to improve the way antibiotics are prescribed and used. This brought world attention to the growing concern amongst the healthcare industry of what is considered to be the most urgent drug resistance trend today: the way in which, over time, the world's ability to treat infectious diseases is being compromised. WHO estimates that in the EU, drug-resistant bacteria 'cause 25,000 deaths and cost more than US$1.5 billion every year in healthcare expenses and productivity losses', and the number of deaths is accelerating significantly for less developed countries.

As antibiotics for some of the common infectious bacterial diseases become less and less effective, the only way to address this issue is through developing new medicines and changing behaviours in regard to prescribing and using antibiotics. Everyone must play their part, including doctors, the general public, policy makers, medical researchers and the agricultural sectors. Scientists, in particular, have an important role through their work investigating new antibiotics, vaccines and diagnostics. This is where the EU-funded Evolution and Transfer of Antibiotic Resistance (EvoTAR) project is helping to

provide some answers. With a consortium of 17 partners, including 15 institutions and two private companies from seven EU countries, the EvoTAR team has been able to draw on a highly experienced pool of scientists who have in-depth knowledge and understanding of antibiotic resistance research. The group of scientific experts in antibiotic resistance, microbial genomics and mathematical modelling is hoping to garner some new insights into the human reservoir of antibiotic resistance genes.

HOW BACTERIA INTERACT INSIDE THE HUMAN BODY

Relatively little is known about the 'resistome', or the pool of genes that humans have to antibiotic resistance. By using high-throughput sequencing methods the researchers are aiming to characterise the resistome. They are also keen to learn more about the ways in which resistant and non-resistant bacteria interact in the human body. The ultimate goal for this research is to identify and develop novel intervention strategies. To achieve this, the project has been broken down into eight work packages covering five key focus areas: Dynamics and Evolution; Reservoirs; Transfer; Modelling; and Novel Interventions.

Each of these focus areas is tasked with specific research. The Dynamics and Evolution area includes the key jobs of metagenomics sequencing of the

human microbiome during and after administration of antibiotics, population dynamics of resistant and susceptible enterococci and *Enterobacteriaceae* during and after administration of antibiotics, and experimental adaptive evolution of resistance genes. The Reservoirs area is looking at functional metagenomics of antibiotic resistance genes in the human microbiome, detection and quantification of antibiotic resistance genes in human and non-human reservoirs, and genomic characterisation of antibiotic resistant bacteria from different reservoirs.

The Transfer area is working on the determination of factors affecting the bacterial host range of resistance plasmids, and the contribution of environmental conditions to the efficiency of transfer of antibiotic resistance genes, as well as analysis of fitness costs incurred by resistance mutations and resistance plasmid carriage and genetic adaptation of resistance plasmids to novel bacterial hosts. The Modelling area is studying the development of mathematical models that describe the probability and rate of resistance development, taking into account several levels of modular trait interactions. They are also tasked with the development of generic and predictive models which will lead to a detailed description of the

within-host dynamics and between-host dynamics of antibiotic resistance modules and spread of antibiotic resistance at the population level.

The Novel Interventions group is working on assessment of the efficacy of compounds that inhibit emergence and spread of antibiotic resistance, and also the identification of novel targets for therapeutic interventions. At the commencement of EvoTAR, a Scientific Advisory Board was set up to provide technical guidance to the different work packages throughout the entire project. Project Coordinator Dr Rob Willems believes that through all this extensive investigative work the EvoTAR team has added new knowledge about the evolution and spread of antibiotic resistance to ultimately develop novel interventions: 'I can confidently say that EvoTAR made many important contributions to our collective understanding of the emergence and spread of antibiotic resistance genes and antibiotic resistant bacteria.'

CLINICAL TRIALS
Through this work two important groups of antibiotic resistant bacteria have been studied, being *Enterobacteriaceae* and enterococci, which include important antibiotic resistant 'hospital bugs'. The team's findings show that heavy metals found in both polluted external environments and used to treat animals can lead to antibiotic resistant disease. In addition, as a part of the research looking at *Salmonella* and *Klebsiella*, the team identified how different mutations foster resistance to antimicrobial peptides. The researchers note in the project's final report that this includes the antibiotic colistin, 'which is generally considered to be an antibiotic "of last resort" for the treatment of infections caused by multi-drug resistant Gram-negative bacteria'.

The investigators generating models and data to show how fast and in what ways antibiotic resistance emerges and transmits in natural settings saw 'significant difference in the dynamics of resistance to methicillin and tetracycline', which they consider could be associated with the different historical patterns of antibiotic use

in the healthcare sector and the livestock industry. The EvoTAR project has been able to undertake a randomised clinical study of healthy humans looking at how an enteric-coated activated charcoal-based product (DAV132) can combat the emergence and spread of antibiotic resistance. They showed that DAV132 is successful at selective absorption of drug compounds without impacting on antibiotics. A similar product is now being developed to identify potential for use in veterinary settings by one of the project partners.

A NEW PATHWAY
Sharing of this new knowledge is now an important focus. During the project information was communicated via a project website and Twitter account, conference presentations, institute visits, articles and publications in industry journals. In order to encourage new research collaborations and disseminate their results, the EvoTAR consortium organised the International Conference on Evolution and Transfer of Antibiotic Resistance 2015. This was attended by 130 scientists from 20 different countries, where they were exposed to oral presentations and posters about the work EvoTAR had completed over the past four years, as well as outside speakers talking about antibiotic resistance in general.

The results from the entire EVOTAR research programme will enable new intervention approaches for the clinical application which are targeted towards reducing the emergence and spread of antibiotic resistance genes in human pathogens. These will be used by healthcare specialists and policy makers in the health industry. Researchers in the wider field will also be able to use the findings to continue discovering and developing additional intervention approaches. From Willems' perspective, their efforts will support ongoing studies into antibiotic resistance: 'I expect that, in the future, research will continue to build on the foundations that are laid down by the EvoTAR consortium, thereby contributing to the fight against the rising tide of antibiotic resistance.'