Tackling AMR – A Cross Council Initiative
Theme 1: Understanding resistant bacteria

AMR Theme 1 Collaboration Grant Awards (announced in 2015) x2

The focus of the call was to drive forward innovative high quality multi-disciplinary collaborative research to address the broad challenges presented in AMR initiative - theme 1.

AMR Theme 1 Innovation Grant Awards (announced in 2014 & 2015) x11

This call was for small, novel, high risk proposals to address the broad challenges presented in AMR initiative - theme 1. The focus of this call was on research that is potentially transformative, stimulating creative thinking across disciplines.

Links:
http://www.mrc.ac.uk/research/initiatives/antimicrobial-resistance/tackling-amr-a-cross-council-initiative/

http://www.mrc.ac.uk/funding/browse/tackling-amr-theme-1-understanding-resistant-bacteria-in-context-of-the-host/

Theme 1 Large Collaboration Grant

Grant Holder	Institution	Title of Award
Professor Christopher Dowson	University of Warwick	Mechanistic understanding of cell wall biosynthesis to combat antimicrobial resistance

Co-Investigators

University of Sheffield:

Professor Simon J Foster
Dr Stephane Mesnage
Professor Jamie Hobbs
Dr Simon Jones

University of Warwick:

Dr David Ian Roper
Dr Adrian John Lloyd
Professor Matthew Turner

Newcastle University:

Professor Waldemar Vollmer
Professor Richard Lewish

University of Southampton:

Dr Syma Khalid

University of Oxford:

Professor Christopher Schofield

Summary

The discovery of the antibiotic penicillin opened the door to the treatment of a wide range of infections. It works by stopping bacteria from making the polymer in the cell wall (peptidoglycan, PG) that holds them together. This is assembled by specialised proteins (called penicillin-binding-proteins or PBPs, which are present in all bacteria) that either have the ability to stitch together both the sugar backbone and the peptides (these are known as bi-functional enzymes), or either just the sugar back bone or just the peptide-crosslinks (mono-functional enzymes). We know little about how the polymerization and cross linking activities are controlled or co-ordinated, or how they truly interact with their natural substrates. Furthermore, the construction of peptide cross-links by PBPs is famously the target inhibited by penicillin which stops cell wall construction and kills the bacterium.

Penicillin has been an excellent antibiotic, not least because it targets multiple PBPs simultaneously within a bacterium and resistance rarely develops by altering the PBP target (with the notable exception of bacteria that can acquire altered PBP genes from other species that are poor targets for the antibiotic). Unfortunately, many bacteria have acquired resistance to penicillin by other mechanisms. Primarily this has been due to the acquisition of enzymes that degrade the antibiotic (beta-lactamases), or reduce penetration (influx) of antibiotic into the bacterium or increasing the rate of efflux out of the bacterium. We urgently need to fight back and the strategy of exploring PBPs to make better versions of current antibiotics that are more active, can evade beta-lactamases or resistance due to changing influx or efflux. Global pharmaceutical companies have a real interest in progressing such developments, however, they need better mechanistic insight into how PBPs work. We attend to address these fundamental gaps in our understanding.

Why can we succeed where others have failed?

1. Progress in achieving this mechanistic insight has been hampered by past inability to routinely synthesise the key chemical components or precursors that make this polymer. From past MRC and BBSRC funding we can now make key chemical components at Warwick, and have developed an exceptional track record of providing reagents to study peptidoglycan biosynthesis to academia worldwide.

2. Having studied how to synthesise all of the chemical precursors used by different PBPs we have developed completely new continuous assays that will now help us to understand how PBPs polymerise precursors or how they crosslink these. We have one assay to finalise, which would bring together our ability to study polymerization and crosslinking in one reaction. Alongside a continuous crosslinking assay, these new technologies represent a 70 year long breakthrough and world first.

3. Super high resolution imaging is now available so that we can see how PBPs work inside bacteria and in the test tube, how they interact with each other and other proteins or lipids within bacterial cells. We can also study PBP structure at ultra-high resolution to understand how PBPs interact at the molecular level with their natural substrates and different well known antibiotics. We also have access to new chemical approaches, which along with our assays and structural biology will help direct us to new ways to stop these enzymes.

4. Finally, we have brought together international academic experts from across the UK with skills in microbiology, chemistry and physics to work in synchrony and closely with many industry experts and a wider scientific advisory panel. This concentration of effort across a wide skill base with new technology will help ensure rapid progress and results with broad application that will be valuable for future programs of antibiotic discovery and development.

Theme 1 Large Collaboration Grant

Grant Holder	Institution	Title of Award
Dr Mark Holmes	University of Cambridge	Determination of the dynamics of antimicrobial resistance genes in the human and animal gut microbiome.

Co-Investigators

University of Cambridge:

Professor Duncan Maskell
Dr Alexander Tucker
Dr Andrew Grant
Dr Olivier Restif
Professor James Woods

University of Edinburgh:

Professor Mark Stevens

Summary

The aim of the proposed research is to define the nature and frequency of transfer of antimicrobial resistance (AMR) genes between pathogenic and commensal bacteria within their hosts under varying selection pressures.

The overarching hypothesis to be tested is that the unculturable microbiome provides a reservoir of AMR genes that may both receive and donate AMR genes, in particular when the microbiome is under antimicrobial selective pressure.

The specific objectives are:

1. To establish the accuracy and reproducibility of two novel metagenome sequencing methodologies that enable the individual genomic backgrounds of bacteria containing AMR genes to be identified from within faecal microbiome samples.

2. To use these novel genomic techniques in experimental conditions (in mice and pigs) to investigate horizontal AMR gene transfer from E. coli expressing Extended Spectrum Beta-Lactamase (ESBL) and Salmonella enterica serovar Typhimurium with plasmid-mediated fluoroquinolone resistance within the gut microbiome both following the therapeutic use of antimicrobials that lead to bacterial cure, and following the use of antimicrobials to which these strains are resistant.

3. To perform longitudinal studies of the gut microbiome in farmed pigs receiving antibiotics for treatment of clinical disease to test the external validity of observations made from experimental animal infections.

4. To develop mathematical models that capture the flux of AMR genes in bacterial populations at the individual host and population levels to identify key parameters in the build-up or transmission of resistance.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Dr Alex O'Neill	University of Leeds	'Silent' antibiotic resistance genes: an overlooked issue of considerable importance in antibacterial chemotherapy?

Summary

Antibiotics enable the treatment and cure of life-threatening bacterial infections, and represent one of the great successes of modern medicine. Unfortunately, the utility of these agents is being progressively eroded as bacteria evolve to resist their effects, and antibiotic resistance is now considered one of the three greatest threats to human health.

A key aspect of dealing with antibiotic resistance effectively in medical practice is strategic intelligence. Being in possession of up to date information about the proportion of bacterial strains in a given location that are resistant to particular antibiotics allows doctors to decide which would be the best antibiotics to use routinely to treat bacterial infection, and to avoid those which are probably not going to work because resistance is so commonplace. In the case of a life-threatening bacterial infection, knowing precisely which antibiotics the specific bacterium present in the patient is resistant or susceptible to enables the doctor to select the best antibiotic treatment to cure the patient.

This project is focussed on investigating a phenomenon that may be seriously undermining our strategic intelligence regarding antibiotic resistance. Recent work in the applicant's laboratory has established that some bacteria that are sensitive to antibiotics nonetheless carry genes that are normally associated with antibiotic resistance, but that these genes have become switched off ('silenced'). This phenomenon, which we have termed 'silencing of antibiotic resistance by mutation' (SARM) is of considerable concern, as bacteria with SARM would appear susceptible to an antibiotic when tested, but could then very quickly and easily become resistant to the antibiotic during treatment in a patient. Currently, we do not know how widespread SARM is amongst bacteria that cause disease, nor do we understand properly how SARM occurs. The present proposal aims to investigate both of these issues in the so-called 'superbug', Staphylococcus aureus.

To establish how common SARM is, a large collection of 1500 S. aureus isolates recovered from patients around the world will be tested. Each strain will undergo DNA sequencing of its genome to establish its complete genetic make-up, which will allow for the identification of genes that are known to be associated with antibiotic resistance. In addition, the susceptibility of each isolate to a wide range of commonly used antibiotics will be established to determine if the bacterium displays resistance to all the drugs that it has the genetic potential to display resistance to. Strains that carry antibiotic resistance genes, but do not exhibit resistance to the corresponding antibiotics, represent potential SARM strains. These strains will be studied in detail to establish how easily they can switch their 'silenced' resistance genes back on, and to understand the mechanism(s) by which SARM works.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Professor Martin Buck	Imperial College London	Role of RNA repair in the tolerance of bacteria to antibiotics.

Co-Investigators

Imperial College London:

Professor Michael Stumpf

Summary

The killing of bacteria by antibiotics is preceded by a period of adaptation and tolerance to the antibiotic(s). Similarly sub lethal amounts of antibiotics cause adaptations in the bacterial cells allowing them to better survive in the presence of antibiotics. Identifying the mechanisms behind antibiotic tolerance will afford us with new ways to manage the administration of antibiotics for controlling infections, and also inform new strategies for enhancing the action of antibiotics. We have discovered certain antibiotics cause the induction of a repair system in bacterial cells. The system repairs particular molecules in the cell called RNA, which perform key functions for cell growth and maintenance. By working out how the cells use the RNA repair system to help them survive antibiotic challenges we will be able to plan how to use different specific combinations of antibiotics to kill the bacteria, and also embark on a program of work to consider inhibiting the repair system itself. We anticipate that we can potentiate the killing actions of existing antibiotics through careful establishment of appropriate combinatorial treatments of bacterial infections.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Dr David Roper	University of Warwick	Multi-Targetting of tRNA synthetases: A paradigm shift in combating AMR

Co-Investigators

University of Warwick:

Professor Christopher Dowson
Dr Adrian Lloyd

Summary

Antimicrobial resistance to existing antibiotics threats future healthcare at multiple levels and has been acknowledge as a worldwide issue with an impact as important as climate change. However, a number of factors has lead to a steady decline in the discovery and development of antimicrobials in the pharmaceutical industry despite the clear clinical need. The remaining pharmaceutical company antibacterial research and development in this area has focused generally in the past on either attempts to discovery new targets for antibiotics or new compounds with limited activity profiles based on existing targets. Generally this approach has met with very limited success which combined with other factors, means we have a decreasing number of drugs to treat bacterial infection leading to a crisis on healthcare.

It is clear that bacteria are adept at the selection of resistance to drugs for single gene targets (i.e. their protein products) and that the successful antimicrobial chemotherapy of the past targets activities where there are multiple essential activities e.g. protein synthesis at the ribosome, the proteins which are responsible for DNA supercoiling in the cell and mechanisms by which cross-linking of the bacterial cell wall is achieved. We have identified another area of bacterial metabolism which has these similar properties and if correctly targeted may provide an avenue for next generation antimicrobial discovery.

Protein synthesis, itself a golden area for past antimicrobial discovery as described above, is reliant upon the delivery of single amino acids to the ribosome which are activated by chemical ligation to transfer (t)RNA molecules. Each amino acid has its own synthetase but some of these enzymes lack the ability to select the correct amino acid initially and are reliant on other systems to produce the correct amino acid-tRNA liganded product. It is this selection procedure, which we can circumvent with potential drugs and thus produce a new line of antimicrobial chemotherapy this is less likely to be overcome by resistance through mutation since these potential drugs would require mutations in multiple genes. Whilst there is a great deal of existing biochemical data to draw upon in this field we lack the three-dimensional structures of some which are required for the next stage of this discovery process. The goal of this project will be to produce these data.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Dr Helen Mariott	University of Sheffield	Re-engaging antimicrobial killing by macrophages to combat antimicrobial resistance

Co-Investigators

University of Sheffield:

Professor David Dockrell
Dr Martin Bewley
Dr Ashley Cadby
Dr Simon Jones
Professor Jamie Hobbs

Summary

Serious bacterial infections arise because of defects in the ways that our body's immune system protects against these infections. We have established that effective clearance of bacteria in body tissues requires a type of immune cell called the macrophage to eat bacteria and when the macrophages ability to kill bacteria becomes exhausted to commit a form of cell suicide called apoptosis. This cell death helps clear the remaining viable bacteria and importantly does so by a process that is less dependent on inflammation and therefore is less likely to result in damage to organs in the body. We have defined the key molecules that regulate this process in macrophages and shown that this mechanism of bacterial killing is not working properly in several groups of patients who are at risk of serious bacterial infection. We wish to use these findings to develop a totally new approach to treating bacterial infection.

Our approach will take several types of medicine that are used by doctors to treat other types of human disease and test their ability to enhance macrophage cell death and bacterial killing. We have selected these medicines because they re-engage some of the processes that cause macrophage cell death and which may not be working properly in patients at risk of serious bacterial infection. We will modify some of these medicines to enhance their uptake by macrophages so that the treatment will be specific for macrophages and less likely to have effects on other cells in the body. We will then screen these medicines for their ability to increase macrophage cell death and to increase bacterial killing in these macrophages, using cells we have genetically engineered to be more susceptible to bacterial infection. We will explore in more depth how the most promising medicines achieve macrophage cell suicide and kill bacteria. We will do this to confirm they are acting on the specific process we believe are important. We will also test their ability to kill antibiotic resistant bacteria. We will then test whether the most promising medicines we have identified from this initial screen also enhance macrophage cell suicide in mice and whether this increases bacterial clearance, reduces inflammation or reduces death when these mice have bacterial infection in the lungs.

Our overall goal is to develop a new treatment for bacterial infection that relies on modulating the body's own response to infection. This approach will reduce our reliance on antibiotics, decreasing the development of antibiotic resistance and develop a new treatment that will act on bacteria that have become resistant to the antibiotics we currently use.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Dr Paul Elkington	University of Southampton	Investigating multi-drug resistant tuberculosis in the 3-dimensional bioelectrospray cell culture model

Co-Investigators

Imperial College London:

Professor Francis Drobniewski

Summary

Tuberculosis is a bacterial disease that used to cause one third of all deaths in this country and is now is becoming progressively more difficult to treat. Indeed, some strains are now completely resistant to all antibiotics previously used to cure tuberculosis infection. One of the difficulties in identifying new antibiotic treatments is the lack of good systems to study how antibiotics work when the bacteria are inside human cells. We have developed a new way of studying tuberculosis and human blood cells by making tiny droplets that are impregnated with both cells and tuberculosis bugs. We will use this system to study how normal tuberculosis and drug-resistant tuberculosis are killed by antibiotics when the bacteria are infected into human cells. We have experiments showing that antibiotics that we know work in patients with tuberculosis also work in our new system but do not work in standard infection systems, and so this approach provides a new way of identifying antibiotics that will not be found by more traditional approaches.

Once we have developed the model further, we will investigate standard and newly discovered antibiotics in the droplets. We will then add a second level to the model, starving the cells of oxygen and nutrients to reflect conditions in patients with tuberculosis more accurately. We will study how this stress changes the behaviour of both the bacteria and the human cells. We can then integrate this system with modern fluidic systems to screen a large array of new treatments. Furthermore, once the system has been developed, we will be able to use this to investigate other bacterial infections which are also becoming more resistant to antibiotics. Therefore, this is a system of broad potential that can be used to address one of the major medical challenges of the modern era.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Professor Xiaodong Zhang	Imperial College London	An inhibited state of bacterial RNA polymerase as a framework for antibiotic design.

Co-Investigators

Imperial College London:

Professor Martin Buck

Summary

Successfully managing bacterial infections in humans and in animals has been possible through the use of antibiotics, agents that kill the bacteria and or stop them from growing. Resistance to these compounds is now wide spread, in part because bacteria mutate to evade the actions of these agents and in part because for all known natural antibiotics, a resistance mechanism is genetically encoded in the organism which makes the anti-bacterial compound. These intrinsic resistance mechanisms are portable and can transfer between bacteria to help spread drug resistance.

Recently we have unravelled, in great detail, how one natural protein inhibitor works to prevent an essential bacterial machinery from functioning. This machine accesses and converts the genetic information stored in DNA in a process called transcription which eventually allows proteins to be made. Proteins carry out the majority of cellular activities. Therefore, without the proper function of this essential machine, the bacteria can't survive. Interestingly this machine is relatively conserved among bacteria but very different from humans and animals, so it is a good target for broad spectrum antibiotics to work. We plan to use our newly acquired in-depth knowledge to design new non-native compounds that will inhibit and/or kill bacteria. To do so we will use methods that allow us to look at how these molecules are organized in 3-dimension to understand the exact detailed interactions at the molecular and atomic levels, and use this information to design small inhibitors of this essential machinery.

We have a wide range of methods to study in mechanistic details this essential machinery, called the bacterial multi-subunit RNA polymerase, and so can work out how best to inhibit it based on our knowledge of a novel naturally occurring factor which normally inhibits the machine. This approach has not been previously taken, rather naturally occurring small molecules have been sought which inhibit the RNA polymerase machine.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Professor Matthew Gibson	University of Warwick	Targeting cell wall glycans: an untapped approach for therapeutics and diagnostics to combat antimicrobial resistance?

Co-Investigators

University of Warwick:

Dr Elizabeth Fullam

Summary

Tuberculosis (TB) is one of the leading causes of death worldwide from a single infectious agent. In 2013, 1.5 million people died from TB and 9 million people became infected, with an estimated 2 billion latent global infections. TB is caused by the bacterium Mycobacterium tuberculosis (Mtb). Treatment of TB requires a lengthy, complicated drug regimen, which means that many patients fail to comply with the recommended course of treatment. Poor compliance, in turn, has led to the evolution of multi-drug resistant, extensively drug-resistant and totally drug-resistant TB (MDR-TB, XDR-TB and TDR-TB). MDR- and XDR-TB strains are difficult and costly to treat. In some instances XDR and TDR TB, few, if any, therapeutic agents remain. In conjunction with HIV infection, this deadly infection now presents us with a global time bomb that could devastate societies across the globe. The problem of TB is not confined to developing countries and in the last decade cases of TB have doubled in the UK with London now known as the TB capital of Western Europe. Mycobacterium bovis is a growing threat to livestock and can spread to humans causing TB

There have been no new drugs to treat TB for > 40 years and there is thus a clear and pressing need for better, innovative, methods to treat and diagnose TB in the hope that we can find new weapons in the fight against this fearful pathogen.

Mtb is unique from most bacteria that cause infection in that it has a distinctive, unusual cell wall. The cell wall has an unusually high fat and sugar content that provides a barrier to drugs, such as penicillin, and protects Mtb from the immune defence system.

The majority of current antibiotic drugs are reliant on targeting a single biological process inside the bacteria; this approach, however, has lead to the resistance that is now widespread. As these targets are inside the bacteria, the drugs must be able to cross the cell wall to function, which in the case of TB, is hugely challenging.

The aim of this innovative project is to challenge the current beliefs that exist in the field of anti-microbial research. Most drug searches focus on screening libraries of small molecules that fulfill a limited range of criteria (known as Lipinski's rule of 5) and focus on cell-well permeation potential.

In this study the work will be carried out at the University of Warwick. The aim is to identify whether the unique cell wall of mycobacteria can itself prove to be a new target for therapies and/or diagnostics - essentially turning its own cell wall against itself. The objective of this work is to identify specific molecules that are able to bind to the unique cell wall sugars in the cell wall of mycobacteria and then to understand how this affects and disrupts essential cellular processes. This elegantly bypasses the need for drugs to reach an intracellular target - a major obstacle for anti-tubercular drug efficacy - and will exploit the unique nature of Mtb's cell wall to develop weapons against itself. The specific cell-wall binding process will also lead to potential diagnostic agents, increasing the value of this work.

Potential applications and benefits resulting from this work are in the development of a new set of tools that can be used for diagnostics, and in identifying new drug targets for TB treatment. This work will stimulate untapped avenues and lead to paradigm shift in thinking to tackle the huge challenge of AMR.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Professor Gad Frankel	Imperial College London	Exploiting commensal-pathogen competition to treat mucosal infection

Co-Investigators

Perkin Elmer Inc:

Dr Kevin Francis

Summary

Recently, there has been a dramatic rise in the number of bacteria that cause disease acquiring resistance to antibiotics that are commonly used to treat them. These bacteria are known as antimicrobial resistant (AMR) and carry gene/s that mediate insensitivity to, degradation of, or expulsion of, these drugs. In the not too distant future, we are facing a situation where once trivial, easily treatable, infections could potentially prove fatal. Currently, E. coli, a bacterium that constitutes an important part of the normal gut flora, is one of the most frequently isolated AMR hospital acquired infections in Europe and the UK and is a major cause of blood poisoning, diarrhoea and recurrent urinary tract infections. Misuse and overuse of antibiotics (e.g. during chemotherapy or in intensive care units) is at the heart of the AMR problem. However, little research has been performed to date to elucidate the in vivo consequences of AMR in relevant animal models.

For the last 20 years my lab has been modelling human intestinal infections with pathogenic E. coli in mice, using the mouse pathogen Citrobacter rodentium. C. rodentium infection is an excellent bacterial colonisation model as it does not cause severe disease in mice. Recently, we found that when treatment of mice infected with C. rodentium that is resistant to the antibiotic kanamycin (Kan), with high level of Kan leads to a phenomenon we termed antibiotic induced bacterial persistence (AIBP). C. rodentium in the AIBP state persists within the gastrointestinal tract for many days and develops a new relationship with the host, which is different from a typical pathogen-host interaction. In particular, virulence genes are turned off in C. rodentium in the AIBP state and the pathogen is non-infectious. In contrast, treating infections with Kan resistant C. rodentium with low levels of Kan leads to delay clearance of the pathogen, which coincided with growth of commensal gut bacteria, which could potentially outcompete or kill C. rodentium.

In this study we will characterise C. rodentium in the AIBP state and its relationship with commensal bacteria. Although we appreciate that the mouse intestinal microbiota is not identical to that in humans, this project will reveal common transferable principles underpinning bacterial competition and adaptation in the intestine during antibiotic treatment, the consequences of using the wrong antibiotics to treat AR infections and lead to the identification of novel new natural products expressed by the microbiome that impact on pathogen-host interactions. These principles are likely to be applicable to other enteric pathogens that have to compete with the gut microbiota during colonisation.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Dr Lynn Dover	Northumbria University	Post-translational lipidation of proteins with mycolates in Rhodococcus equi: a novel drug target in the mycolata?

Co-Investigators

Northumbria University:

Dr Iain Sutcliffe

Summary

Novel antimicrobials are desperately needed as both antibiotic resistance increases and new pathogens emerge. The development of novel antimicrobials will be greatly facilitated by the characterisation of as yet unexploited pathways that contribute to the interactions between pathogenic bacteria and their hosts. We propose to study one such pathway, which we expect will prove to be an overlooked but crucial aspect of the interaction between mycolic acid containing bacteria and their human or animal hosts. The mycolic acid containing bacteria are an important group of bacteria which have a common feature of a waxy cell envelope based on characteristic lipids, the mycolic acids. Many very significant pathogens belong to this group of bacteria, including the causative agents of tuberculosis, leprosy and diphtheria in humans and of bovine farcy, bovine tuberculosis, rhodococcal bronchopneumonia in foals and caseous lymphadenitis in sheep and goats. The biosynthesis of the unusual cell envelopes of these bacteria is already established as valid drug target (e.g. in tuberculosis treatments). We hypothesise that there is a pathway in these bacteria by which proteins are localised to the mycolic acid cell envelope by modification of the proteins with mycolic acids, which will provide a lipid anchor holding such proteins onto the waxy cell surface layer. This type of protein modification is thus likely to influence the virulence of mycolic acid containing bacteria as cell envelope proteins are crucial to the interactions between pathogen bacteria and their hosts. We therefore propose to verify that this type of lipid modification is indeed widespread in mycolic acid containing bacteria and to establish the mechanism by which it occurs. We will examine a representative range of mycolic acid containing bacteria to demonstrate that mycolic acid modified proteins are present, using selective extraction and chemical characterisation methods. We will also examine the sites at which the proteins are modified as this should give insights into the underlying mechanism and also allow sequence based prediction of which proteins are likely to be similarly modified. As a model system in which to study the mycolic acid modification pathway in detail, we will use the important equine pathogen Rhodococcus equi (a global cause of rhodococcal bronchopneumonia in foals) as we have extensive experience of methods for studying the cell envelope biology of this bacterium. We have already identified a candidate enzyme that is likely to be the catalyst for mycolic acid modification of proteins. Genetic modifications of the gene encoding this enzyme will allow us to create mutant strains of R. equi that we predict will be attenuated in their ability to cause disease, which we will verify using novel tissue culture method for assessing bacterial virulence in vitro. This will provide evidence for the importance of this pathway that should be relevant to other mycolic acid containing pathogens. Finally, we will characterise biochemically the protein mycolic acid modification machinery in R. equi and thus gain mechanistic insights that will allow us to determine whether this pathway is a suitable target for the development of novel antimicrobial therapies. As part of this work we will also devise assays suitable for adaptation in high throughput screens that can be used for the discovery of novel antimicrobials targeting this pathway. In summary, we expect this project to identify a new pathway influencing host-pathogen interactions in an important group of bacteria and to demonstrate that this pathway represents a target suitable for the development of novel antimicrobials.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Dr William Gaze	University of Exeter	Selection for AMR in complex microbial communities at sub-therapeutic antibiotic concentrations

Co-Investigators

University of Exeter:

Professor Angus Buckling

Summary

Antimicrobial resistance (AMR) is an increasing problem in human and animal pathogens, and has been highlighted as a serious threat to public health by the Chief Medical Officer. There is increasing evidence that antibiotic usage in agriculture may be contributing to the emergence of AMR in the clinic and the government's 5 year AMR strategy highlights improved knowledge and understanding of AMR as a key priority. This project will investigate the evolution of AMR in complex microbial communities using laboratory evolution experiments. For the first time, selection for AMR in a clinically important opportunistic pathogen, E. coli, will be studied in the presence and absence of a complex community of bacteria. The community will be from pig faeces, and will be incubated in anaerobic fermenters that have previously been used as simple gut models.

Traditional approaches to studying AMR in bacteria often consider only one species in isolation. This approach can be useful in studying mutation based adaptation to antibiotics; however it does not consider the impact of horizontal gene transfer where resistance genes are acquired from the microbial community. Single species experiments also fail to consider competition between different species of bacteria that have differing intrinsic resistance to antibiotics.

Competition experiments in the presence and absence of a complex microbial community will help us understand how evolution of AMR occurs. We will expose experimental microbial communities to different concentrations of antibiotic representing the sub-therapeutic concentrations found in the animal and human gut during oral antibiotic therapy. This will give insights into the way complex interactions that occur within microbial populations affect evolution of AMR, relative to selection for AMR in single species experiments. Exposure to low concentrations of antibiotics was traditionally thought to be unimportant in selection for AMR. However recent data suggests that selection occurs at much lower concentrations than previously thought, and preliminary data in Gaze's lab suggests that selection for AMR gram-negative opportunistic pathogens can be greater at lower sub-therapeutic concentrations than at higher concentrations closer to those used to treat infections.

We will also investigate the effects of selection by a single antibiotic on relative abundance and diversity of all known resistance genes. This will provide data on indirect co-selection for AMR genes due to genetic linkages in the genomes of bacteria in the complex community and on mobile genetic elements capable of transferring multiple genes between bacteria. Cell sorting and next generation sequencing techniques will identify all known AMR genes transferred to a genetically tagged E. coli under antibiotic selection. Conversely, we will also investigate AMR gene transfer from a tagged E. coli to all other bacteria within the complex community.

Theme 1 Innovation Grant

Grant Holder	Institution	Title of Award
Dr Suzanne Hingley-Wilson	University of Surrey	Macrophage-induced drug tolerant persisters in tuberculosis

Co-Investigators

University of Surrey:

Professor Johnjoe McFadden

Summary

More than 1.8 million people die worldwide each year from Tuberculosis (TB), making it the biggest cause of mortality due to a single bacterial infection. This equates to the passengers of more than 10 full Boeing 747's succumbing to the disease every single day. This is despite the availability of antibiotics against the causative agent of the disease Mycobacterium tuberculosis (Mtb). Antibiotic treatment for any disease is a lengthy process; however, in TB, eradication of the infecting bacilli takes 6-9 months and requires at least 3 highly toxic drugs. This is primarily due to the presence of a small group or sub-population of bacteria that can resist and survive antibiotic treatment. Termed drug-tolerant persisters, these bacilli were first discovered in 1942 and yet we are still a long way from understanding these elusive bacteria. One of the reasons for this is that they are present at such low numbers (approximately 1 persistent bacilli in 10, 000) that they are difficult to study.

Preliminary results from our laboratory have shown that interacting with the very cell that we, the host, deploy to kill invading pathogens can induce much higher numbers of these persister bacilli. Upon infection with Mtb or many other invading pathogens, our immune system deploys white blood cells to kill the invader, one of which is called the macrophage. From our results it appears that Mtb uses our own macrophages as a Trojan Horse and when inside this host cell changes into a virtually untreatable drug-tolerant persister cell. We have called these macrophage-induced persisters or MIPs. The trigger(s) for the formation of drug-tolerant persisters is currently unknown but could represent an ideal target for future drug development

Our aims

1) To identify the bacterial trigger genes required for the formation of MIPs. Discovering the bacterial genes vital to the formation of drug-tolerant persisters would add to our understanding of how Mtb is able to evade killing and could represent future drug targets.

2) To determine the macrophage conditions responsible for MIP formation. Persister cells are notoriously difficult to treat due to their rarity in a population. By identifying what factors or conditions the bacterium is sensing to induce the formation of MIPs, we will be able to generate enough persisters to enable further study. We also intend to develop a novel drug testing platform so we can identify drugs capable of killing these MIPs, i.e. persisters formed whilst within our own bodies.

Potential applications and benefits

Targeting drug-tolerant persisters is key to combatting TB. Understanding the nature of this sub-population of bacteria will enable us to develop new treatment strategies that either attack the bacteria themselves or push the host immune response in the right direction. This could improve treatment tolerance among patients, and improve treatment success rates. The current threat of drug resistance combined with the HIV epidemic makes the development of new TB treatment strategies of the upmost importance.