Tackling AMR – A Cross Council Initiative
Theme 3: Understanding the Real World Interactions

Call 1: Antimicrobial Resistance in the Real World

AMR Theme 3 Research Grant Awards (announced in 2016) x4

AMR Theme 3 Pump Priming Grant Awards (announced in 2016) x9

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Theme 3 Research Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Dov Stekel	University of Nottingham	EVAL-FARMS: Evaluating the Threat of Antimicrobial Resistance in Agricultural Manures and Slurries

Co-Investigators

University of Nottingham:

Dr Jonathan Hobman
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Summary

Antibiotics are used extensively to fight bacterial infections and have saved millions of lives. However, the bacteria are becoming resistant to antibiotics and some antibiotics have stopped working. We refer to this as antimicrobial resistance - AMR. We don't just use antibiotics for people; similar amounts are given to farm animals. More than 900 million farm animals are reared every year in the UK and antibiotic treatments are vital for their welfare, for farms as businesses, and for us to enjoy affordable food. However, farms may be contributing to the development of AMR. The aim of this project is to improve our understanding of how farm practice, especially the way in which manure is handled, could lead to AMR in animal and human pathogens. This understanding will help farmers and vets find new ways to reduce AMR, without harming their animals or their businesses.

For research purposes, Nottingham University maintains a typical high performance dairy farm - its 200 cows produce a lot of milk and a lot of manure. The waste is stored in a 3 million litre slurry tank, any excess goes into a 7 million litre lagoon. This slurry is applied to fields as organic fertilizer. Cow manure contains many harmless bacteria but some, e.g. E. coli O157, can cause severe infection in people. When cows get sick they are treated with antibiotics. Udder infections are treated by injection of antibiotics into the udder. Since this milk contains antibiotics, it cannot be sold but is discarded into the slurry. Foot infections are treated with an antibacterial footbath, which is also emptied into the slurry tank.

As a result, slurry tanks contain a mixture of bacteria, antibiotics and other antimicrobials that are stored for many months. The bacteria that survive in the presence of antibiotics are more likely to have antibiotic resistance. This resistance is encoded in their genes so passed to the next generation. Worse still, the genes can be passed on to other bacteria in the slurry.

Before we wrote this proposal, we investigated our own farm's slurry tank to see if this might be happening. We tested 160 E. coli strains from the slurry; most carried antibiotic resistance. We also found antibiotics in the tank - including some that bacteria were resistant to. Our mathematical modellers showed that reducing spread of resistance genes in the tank might be more effective in preventing resistance than cutting the use of antibiotics. Conversations with the farm vets revealed that they knew about AMR and had changed some of their antibiotic prescriptions. But these analyses leave us with more questions than answers.

In this project, we want to find out if current farming methods are contributing to the development of harmful antibiotic-resistant bacteria in slurry, bacteria that may then be encountered by humans and animals. To do this, we need to integrate scientific and cultural approaches:

- What bacteria are in the slurry? How many are harmful? What resistance genes do they carry? How do these genes spread?
- How long do antibiotics remain in the tank? Do they degrade?
- What happens to the bacteria and antibiotics after they are spread on fields?
- How do farmers, vets and scientists interpret evidence about AMR? What are their hidden assumptions? Can we improve collaborative decision making on AMR risk management?
- Can we reduce resistance by avoiding mixing together bacteria and antimicrobials in slurry?
- Can we predict the risk of emergence of and exposure to resistant pathogens? Can we predict which interventions are likely to be most effective to reduce AMR, taking into account both human and scientific factors?

Through this research, we will learn what can realistically be done to reduce this risk; not just on this farm, but UK wide. We will work with farmers, vets and policy makers to ensure that our results will make a difference to reducing the risk of harmful AMR bacteria arising in agriculture.

Theme 3 Research Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Andrew Singer	NERC Centre for Ecology and Hydrology	NEC05839 Chicken or the Egg: Is AMR in the Environment Driven by Dissemination of Antibiotics or Antibiotic Resistance Genes?

Co-Investigators

CEH:

Dr MJ Bowes
Dr Francois Edwards

Rothamsted Research:

Mr Andrew Mead

University of Warwick:

Professor EMH Wellington

University of Exeter:

Dr WH Gaze

Summary

Antimicrobial resistance (AMR) in the environment is driven by antibiotics released in the urine of humans and animals into sewage and ultimately the receiving rivers. AMR is also released from within the gut bacteria that are shed in faeces of both humans and animals. In both cases, antibiotics and AMR-containing gut bacteria are released into the environment through sewage. Despite the continued release of both antibiotics and antibiotic-resistant bacteria into our rivers, we still don't know the relative role that they play in explaining the amount of antibiotic resistance that we see in our environment. This is a critically important knowledge gap as it prevents industry and policy makers from determining where to spend our time and resources so as to lower this 'environmental reservoir of antimicrobial resistance'.

Sewage contains thousands of chemicals, many of which are at concentrations sufficient to inhibit or kill bacteria. Microbes defend themselves from these chemicals with a range of strategies, all of which have genes that are broadly classified as 'resistance genes'. Hence, sewage is an excellent place to find bacteria rich in resistance genes. Many of these genes are known to be mobile, which allows for the genes to be shared, thereby increasing its abundance within the environment. This mobility of genes is key to why it is so difficult to know what is driving AMR in the environment-a bit like 'which came first, the chicken or the egg.' Are the concentrations of antibiotics present in sewage sufficiently high to select for resistance genes in the environment or are the genes for resistance simply spreading from the gut-derived bacteria into the native environmental microorganisms? The keys to answering this question lie in the following two questions: 1) Do genes released from sewage move into and persist in the natural microbial community without continued exposure to critical threshold concentrations of antibiotics; and 2) Are the critical threshold concentrations in the environment sufficiently high to maintain gut-derived AMR genes in the natural microbial community or select for them all on their own?

In the proposed research we aim to answer these two key questions using four innovative experimental systems: 1) a small laboratory microfluidic system for the precise control and manipulation of microbial biofilms; 2) an in situ river mesocosm and 3) ex situ macrocosm which can also control and manipulate microbial biofilms under controlled conditions with the addition of antibiotics and/or antibiotic resistance genes; and finally 4) the use of the freshwater shrimp, Gammarus pulex, as an indicator species of environments where the reservoir of antibiotic resistance is elevated. In the case of the Gammarus, we will study the microorganisms that live within this shrimp and determine if these microbes acquire similar antibiotic resistance traits as those found in identically-exposed biofilms. Modern molecular techniques (i.e, metagenomes, plasmid metagenomes, qPCR, meta-transcriptomes), will be used to quantify treatment effects within biofilms and Gammarus. The data from these studies will be used to parameterise a mathematical/statistical model that will be designed for use by regulators, industry and academia to better predict and understand the risks posed by AMR in the environment.

Theme 3 Research Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Matthew Avison	University of Bristol	Acquisition and Selection of Antibiotic Resistance in Companion and Farmed Animals and Implications for Transmission to Humans

Co-Investigators

North Bristol NHS Trust:

Professor Alasdair MacGowan

University of Bristol:

Professor Alastair Hay
Professor David Barrett
Dr Kristen Reyher
Dr Tristan Cogan
Dr Severine Tasker
Dr Katherine Turner
Dr Margaret May

Royal Veterinary College:

Dr Rachel Casey

Summary

Without antimicrobial drugs, the risk of bacterial infection would render many common medical procedures too dangerous to contemplate because of the risk of infections caused by "opportunistic bacteria". They can live on the patient's skin, or in their intestines, and infection occurs when bacteria get into parts of the body that are normally sterile. A perfect example is urinary tract infection (UTI) caused by faecal bacteria. E. coli is particularly abundant in human faeces so is perfectly placed to cause opportunistic infections. It is one of the most common causes of healthcare pneumonia, surgical site infection, bloodstream infection and UTI in the UK. In order to prevent against and treat opportunistic infections, patients are given antimicrobials.

Almost all antimicrobials are "antibiotics", which means they are derived from natural chemicals produced by microbes found in the environment. Natural antibiotics have been present in the environment for millions of years, and so bacteria living in their presence have had time to evolve mechanisms that can resist their actions, encoded by "resistance genes". Opportunistic bacteria like E. coli can randomly acquire these pre-evolved resistance genes and in a single step, they become insusceptible to a particular antimicrobial. If that insusceptible E. coli colonises a person and then causes an opportunistic infection, the infection will not be treatable with that particular antimicrobial. We refer to this as "antimicrobial resistance" (AMR); however AMR bacteria don't just resist clinical antimicrobial therapy, they beat it.

Animals also carry an abundance of E. coli in their intestines and are frequently treated with antimicrobials. This can select for the acquisition of AMR E. coli which can then be passed on to another animals, directly, or via contamination of the environment with faeces. Theoretically, the AMR E. coli could also be passed on to people, and there is much debate about whether such "zoonotic transmission" happens to any significant degree. This is an important debate because it has led to calls from some to dramatically reduce the amount of antimicrobials that are given to animals with the view that it will reduce the level of AMR in animals, and so the possibility of zoonotic transmission to people. But the potential impact on welfare and food production means this should only be done if there is evidence that it will work.

In this project we will identify what drives acquisition of AMR in animals using E. coli as the exemplar bacterium and dairy cows and dogs as exemplar farmed and companion animals. We will test whether AMR bacteria encountered by an animal as it interacts with the environment influence the AMR profile in its faeces, and/or whether early life antimicrobial use plays a part in selection of AMR bacteria in animals. We will also test whether reducing antimicrobial use in dairy cows actually does reduce AMR in the near-farm environment that is contaminated with their faeces. We will test whether exercising in these contaminated near-farm environments influences the abundance of AMR bacteria in dogs, and whether there is any evidence of direct acquisition of AMR E. coli by dogs from near-farm environments, which might be brought into the home.

Finally, we will investigate whether AMR abundance in human UTI E. coli reduces as antimicrobial drug prescribing reduces in primary care; whether living close to a farm affects AMR abundance in UTI E. coli; whether there is direct evidence for E. coli carried by dogs or found in near-farm environments contaminated by cattle faeces also causing UTIs in humans.

These interlaced studies will provide much needed data about the management changes that might reduce AMR in animals and in humans, and are designed to address the fundamental question of whether zoonotic transmission is particularly significant as a driver of AMR in people relative to antimicrobial drug use by doctors.

Theme 3 Research Grant		
Grant Holder	**Institution**	**Title of Award**
Professor Derrick Crook	University of Oxford	The environmental REsistome: confluence of Human and Animal Biota in antibiotic resistance spread (REHAB)

Co-Investigators

University of Oxford:

Dr Nicole Stoesser
Professor Timothy Peto
Professor Ann Walker
Dr Daniel Wilson
Dr Nicola De Maio
Dr Anna Sheppard

Mount Sinai School of Medicine:

Dr Robert Sebra

NERC Centre for Ecology and Hydrology

Professor MJ Bailey
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Summary

OVERALL STUDY AIM

We do not fully understand how important types (species) of bacteria and packages of genetic material (genes) coding for antibiotic resistance move between humans, animals and the environment, or where, how and why antibiotic resistance emerges. This study aims to look in detail at the genetic level at bacteria in farm animals, human/animal sewage, sewage treatment works and rivers, to work out the complex network of transmission of important antibiotic-resistant bacteria and antibiotic resistance genes. We will use this information to work out how best to slow down the spread of antibiotic resistance between humans, livestock and the environment.

STUDY BACKGROUND AND AIMS IN MORE DETAIL

Infections are one of the most common causes of ill-health in human and animal medicine, and are caused by a range of different micro-organisms, including viruses and bacteria. Amongst bacteria, there are some species, or types, of bacteria, which can live harmlessly in human and animal intestines, sewage, and rivers, but can also cause disease in humans and animals if they get into the wrong body space, such as the bloodstream or urine. Examples of these bacteria include E. coli, and other similar organisms, which belong to a family of bacteria called "Enterobacteriaceae".

It has generally been possible to treat infections caused by bacteria using several classes of medicines, known as antibiotics. Different antibiotics kill bacteria in different ways: for example, they can switch off critical chemical processes that the bacteria need to survive, or they can break down the outer shell of the bacteria. In response to the use of antibiotics, bacteria have changed over time, finding ways to alter their structure so that antibiotics no longer have a target to act on, or by producing substances that break down the antibiotic before it has a chance to kill the bacteria. These changes to the bacteria's genetic code, so that they are no longer killed by an antibiotic, create antibiotic resistance. Bacteria can also acquire packages of genes that cause antibiotic resistance from other surrounding bacteria. This is known as horizontal gene transfer. Through these mechanisms, members of the Enterobacteriaceae family of bacteria have developed antibiotic resistance to a number of different antibiotics over a short period of time. In some cases we are no longer able to treat these infections with the antibiotics we have available.

Studying antibiotic resistance and horizontal gene transfer in bacteria found in humans, animals and the environment is difficult because we cannot directly see how bacteria and their genetic material move between them. However, new "Next Generation Sequencing" (NGS) technologies allow scientists to look in great detail at the genetic code of large numbers of bacteria. Comparing this information across bacteria which have been living in the different parts of the environment (e.g. sewage treatment works, rivers) and in human and animal sewage allows us to see how bacteria have evolved to become resistant to antibiotics, and how resistance genes have been shared between them.

This study will use NGS technologies to look at the genetic code of large numbers of Enterobacteriaceae bacteria found in humans, animals (pigs, sheep and poultry), sewage (pre-, during and post-treatment), and rivers. These different groups/areas will be sampled in different seasons of one calendar year to determine how antibiotic resistance genes move around between these locations and over time, and what factors might influence this movement. We will also be investigating whether various chemicals and nutrients in the water may be affecting how quickly horizontal gene transfer occurs. Understanding this is essential to work out how we might intervene more effectively to slow the spread of antibiotic resistance genes and bacteria, and keep our antibiotic medicines useful.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Professor Charles Keevil	University of Southampton	Occurrence and horizontal gene transfer of carbapenemase and ESBL genes in soil microbiomes

Co-Investigators

University of Southampton:

Dr Marc Dumont

Summary

This project aims to understand the potential of environmental reservoirs and transmission of AMR in members of autochthonous soil microbiomes and between transient allochthonous human and animal pathogens entering the environment, for example from faecal agricultural wastes or domesticated or wild animal and bird faecal ingress. It also seeks to understand if horizontal gene transfer occurs successfully between potential pathogens in the various soil microbiomes and complex matrices encompassing clay, loam and sandy soils. The work will utilize modern molecular biology and genomics to screen microbiome populations and defined third generation ESBL and fourth generation carbapenemase E. coli and Klebsiella pneumoniae donor strains, with an E. coli sensitive strain as the recipient.

The University of Southampton team comprises an established scientific group with expertise in survival of faecal pathogens in soil and water, and HGT of ESBL and carbapenemase resistance genes between different species, in collaboration with an Early Career Researcher with expertise in soil microbiology and processes, and stable isotope probing (SIP) to understand community viability, metabolic turnover and gene acquisition. Previous work for UKWIR and Defra/FSA has developed grassed soil microcosms of clay, loam and sandy soils and demonstrated survival of zoonotic pathogens therein for several weeks following faecal waste irrigation. These pathogens were subsequently released from the complex soil matrices using a novel, gentle pulsification procedure followed by membrane filtration and quantitative resuscitation on appropriate selective agar media.

This proposal will help identify the specific environmental drivers of the HGT and beta lactamase selection processes, including implications for both anthropogenic (animal husbandry, human wastewater disposal) and non-anthropogenic (wild animal and bird faecal ingress) drivers. The work will also include identifying the implications for pathogens of clinical and/or veterinary importance such Klebsiella pneumonia and E. coli. The research will be able to help inform AMR policy and management strategies, and will form the basis of a subsequent large research proposal to address these policies and strategies more fully.

The approaches and methodologies to be developed in this proposal could be translated to other environments, antibacterials or other bacterial communities of interest to the AMR funding bodies.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Charles Knapp	University of Strathclyde	Quantifying Spatial AMR Patterns across Urban and Rural Landscapes

Co-Investigators

Newcastle University

Professor DW Graham
Dr MP Cooke

Summary

Antimicrobial resistance is increasing in nature and threatens the effectiveness of our drug therapies and infection control. However, it remains difficult to distinguish what originates from human activities or what is natural. Therefore, we must extend the scale and depth monitoring efforts to better understand what is driving the increases in resistance traits.

This project will use two collections of previously characterised soils to compare and contrast distributions of AR genes under widely varying conditions, ranging from urban, agriculture, legacy mining, and pristine rural environments. The project will utilise DNA extractions and new genetic technology to quantify over 230 AR genes in the samples. Soil inventories provide us well-characterised soils and the wealth of information that describes both the soils and the impacts at source locations.

The project will generate an astonishing 120,000 AR-related data points (400 locations x 300 genes), each with extended background information on environmental conditions-creating among the largest geographic representation of AR gene distribution across landscapes ever created; sufficiently detailed to make cross-cutting observations of landscape effects on acquired vs innate AR levels. With advanced multi-parametric statistics, we will relate specific environmental conditions and factors with observed AR genes levels in soils to identify risk factors associated resistance development and impacts on human and agricultural health.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Emily Rousham	Loughborough University	Spatial and temporal dynamics of AMR transmission from the outdoor environment to humans in urban and rural Bangladesh

Co-Investigators

Loughborough University:

Dr Paul Wood
Mr Michael Smith

Summary

Antibiotic resistant bacteria can be found in freshwater, soil, wastewater and among livestock. It is not yet known, however, how easily these resistant bacteria in the outdoor environment can be transmitted to humans. Research is needed on the human health risk from antimicrobial resistance (AMR) in the natural environment, and research is required in locations with high levels of AMR in the environment and where humans are at high risk of environmental exposure. Bangladesh is one such location where many factors favour the transmission of antibiotic resistance such as the widespread bacterial contamination of soil and drinking water; high human population densities; inadequate sanitation and poor treatment of wastewater alongside regular floods and natural disasters. Furthermore, inexpensive antibiotics are readily available from over-the-counter suppliers leading to widespread use in humans and animals.

This study will examine whether there is a health risk to humans from being exposed to AMR in the outdoor environment in Bangladesh. The novelty of the study is that it will measure the quantity of resistant bacteria in the outdoor environment (freshwater, soil, wastewater) and relate this to the presence of resistant bacteria in livestock (poultry) and humans at the same time and in the same locations. This will also be one of the first studies to measure AMR in humans with high and low exposure to contaminated environments.

The study will take place in three locations where transmission is likely to be high because of human interaction with animal and environmental reservoirs of AMR contamination. These locations are urban markets selling live poultry in Dhaka city, commercial poultry farms and rural villages where poultry and humans share living and sleeping areas.

We will measure the quantity of resistant bacteria in the gut (faeces) of humans who live or work closely with poultry (such as live poultry sellers and slaughterers, commercial poultry farm workers and village women who have poultry living in the household). These rates will be compared to humans who share similar environments but have little or no exposure to poultry. We predict that the resistant bacteria from the gut of chickens will be present in poultry faeces and that this is a potential route of AMR transmission to humans through close contact. We also predict that there will be more AMR bacteria in poultry that are given antibiotics regularly in poultry feed or as medication and this will increase the risk of AMR bacteria in humans.

A further aim is to study how resistant bacteria in the environment change over time by comparing the quantity of resistant genes in water, soil and waste during the dry season and wet season in Bangladesh. Understanding the seasonal changes of AMR in the outdoor environment will enable us to target interventions at times when the risks of transmission of AMR are highest.

Finally, we will examine the cultural and social practices in animal husbandry and poultry keeping in relation to the use of antibiotics as medicine and in animal feed. We will observe the practices of farm workers, slaughterers and market sellers to assess which aspects of food production, selling and disposal of poultry waste contaminate the environment, and what human activities (hand washing, use of gloves, handling of poultry) increase or decrease risk of exposure to AMR. We will use this information to identify ways of reducing the spread of AMR among animals and humans.

This study will provide new insight into whether AMR in the environment is a serious threat to human health. As new forms of resistant genes and bacteria are spreading rapidly in south Asia, there is an urgent need to establish how AMR can be transmitted through water, soil, waste and livestock, and identify what can be done to reduce this transmission at a global level.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Igor Morozov	Coventry University	Identification of novel double-stranded RNA elements in developing antibiotic resistance in the agricultural environment

Co-Investigators

University of Liverpool:

Dr Daniel Rigden

Coventry University:

Dr Jess Rollason
Dr Lauren Acton

Summary

Many types of antibiotics (AB) which are used in humans (e.g. chloramphenicol and its derivatives) are also used in farms (e.g. thiamphenicol, a methyl-sulfonyl derivative of chloramphenicol) either to treat or prevent disease. They have a similar antibacterial spectrum and may significantly increase a possibility that clinical pathogens will develop cross-resistance to drugs used in human medicine. The intestinal microbiota is the epicentre but underexplored source for antibiotic resistance (AR) emergence in response to the selective pressure of AB. The vast majority of bacteria cannot be cultured in laboratory conditions and this limits our knowledge of the potential AR determinants these species may possess and express in a community-dependent manner. Metagenomics, for identification of encoded metabolic pathways present in bacterial populations, has revealed many novel, possibly dormant genes in microbial communities as well as a large discrepancy between predicted and detected resistance genes. While metagenomics has primarily focused on analysis of DNA as the source of genomic information, RNA can also serve as genetic material. Recent high-throughput RNA-sequencing (RNA-Seq) analyses of bacterial systems have made two critical discoveries. Firstly, expression of the bacterial genome is extensively regulated by non-coding (nc) RNAs, including antisense (as) RNAs (a class of ncRNA that are encoded on the opposite strand of their target genes). asRNAs regulate gene expression via RNA-RNA interactions, thus leading to modulation of mRNA translation and stability. asRNAs were firstly found on mobile elements (phages (bacterial viruses), plasmids or transposons) which are used for horizontal transfer (HT) of AR genes between different bacteria. However, the mechanism by which these asRNAs regulate expression and possibly acquisition and spread of genes involved in AR is unknown. Secondly, the microbial metatranscriptome contains double stranded (ds) RNA sequences, derived from uncharacterised phages which do not match to the corresponding DNA. Intriguingly, these dsRNAs have coding potential for a large proportion of novel proteins of unknown function.

The use of AB in medicine and agriculture triggers a number of adaptation responses in bacterial communities. Dynamics and mechanisms underlying such functional changes in microbiomes in response to AB are still elusive. This may involve activation of expression of a number of genes relevant to resistance (e.g. transposases, proteases or efflux pump genes), HT or mobilisation of genes and non-coding DNA/RNA elements on mobile structures. Thus, key questions are: How do as-metatranscriptomes respond to antibiotic treatments? Does the animal gut microflora contain dsRNAs that do not correspond to their DNA metagenomes? If yes, then what roles do these dsRNAs play in this ecosystem? Does this uncharacterised genetic information play a role in adaptation responses, including AR?

Our aim therefore is to undertake RNA-Seq of dsRNAs extracted from animal faecal samples in order to identify dsRNAs metatranscriptome in response to antibiotic therapy. The results will lead to identification of an unexplored array of novel genetic information, including non-coding regulatory elements and new open reading frames relevant to AR mechanisms. This in turn, will transform our view on the role novel dsRNAs play in the development and regulation of AR in bacterial communities. The results will also lead to detecting novel or dormant pathways which are regulated by dsRNAs for the production of secondary metabolites with low susceptibility to resistance and discovery of novel genetic information involved in development, transmission and regulation of AR. This ground breaking research project has the potential to provide a paradigm shift in the understanding of transmission and regulation of AR originated from environment and direct the future strategic development of novel antimicrobials.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Barbara Kasprzyk-Hordern	University of Bath	Impact of stereochemistry of antimicrobial agents on their environmental fate, biological potency and the emergence of resistance

Co-Investigators

University of Bath:

Professor Edward Feil
Dr Simon Lewis

Summary

This project aims to understand and address the impact of stereoisomerism of antimicrobial agents in their environmental cycle on mechanisms behind the development of antimicrobial resistance. The risk of promotion of antibiotic resistant bacteria is by far the greatest human health concern with regards to medicinal products in the environment. The continuous introduction of sub-inhibitory quantities of antimicrobial agents (AAs) to the environment is believed to be directly linked with antimicrobial resistance (AMR). Unfortunately, there is little knowledge of mechanisms in the environment and influencing factors due to the multi-dimensional nature of the AMR problem. There are several research gaps that need to be addressed including research into contaminated habitats (e.g. wastewater) where AAs, co-selecting agents, bacteria carrying resistance determinants and favourable conditions for bacterial growth prevail at the same time. Furthermore, the stereochemistry of AAs (which is key in defining their biological potency) has never been studied in the context of their environmental fate and effects. This is an oversight as changes in stereoisomeric profile of AAs throughout their environmental cycle will lead to (and be influenced by) changes in the composition and structure of microbial communities present in the environment. This might further contribute to the development of AMR, a phenomenon that has never been the subject of investigation in the context of stereochemistry of AAs.

This project postulates that stereochemistry of AAs determines their environmental fate and biological effects. It also hypothesizes that two enantiomers of the same AA should be recognised as two different substances that can elicit different responses leading to changes in the environmental fate and effects of the drug.

The project will:
1. Verify the mechanisms of (stereoselective) transformation of chiral antimicrobial agents and their metabolites during wastewater treatment and in receiving waters
2. Identify resistant bacterial taxa responsible for (stereoselective) degradation of antimicrobial agents and to study the development of antimicrobial resistance at stereoisomeric level
3. Recommend changes to ERA via inclusion of AAs (and their stereochemistry) and ARGs as AMR indicators

The stereochemistry of AAs is complex, as many of the semi-synthetic agents are marketed as mixtures of diastereomers and a number of synthetic agents are used as racemates. In this project we will focus on ofloxacin and chloramphenicol, but we will also consider other synthetic quinolones, Beta-lactams (e.g. amoxicillin) and carbapenems (e.g. meropenem).

Considering the importance of better understanding environmental and human health impacts from chiral pollutants such as AAs and the need for the development of new solutions tackling AMR, this project has the potential to lead to groundbreaking research with long term scientific, technological and societal impact.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Alexander Corbishley	University of Edinburgh	The dynamics of antimicrobial resistance gene prevalence on a commercial pig farm: implications for policy

Co-Investigators

University of Edinburgh:

Professor David Gally

SRUC:

Professor M Hutchings

Summary

There is considerable concern regarding the increasing threat to human health from drug resistant bacterial infections. The major driver for the development of these drug resistant infections is the use of antibiotics in humans and animals. Each time an antibiotic is used, a wide variety of bacteria including pathogenic ('bad'), commensal ('good') and environmental bacteria will be killed by the drug, however some of these bacteria will survive because they will have become resistant to the antibiotic used. Bacteria can become resistant either through a random change in their genes (mutation) or by acquiring a new gene(s) from another bacteria (horizontal gene transfer). The ability of bacteria to share antibiotic resistance genes is of considerable concern as it is possible that commensal and environmental bacteria could act as a reservoir of resistance genes that could be acquired by pathogenic bacteria. The more antibiotics that are used, the more likely it is that these resistance genes will become established within a broad range of bacteria and environments.

Approximately 590 tonnes of antibiotics are used in humans and 420 tonnes in animals in the UK each year. Accurate data regarding use in animals is not available, however poultry and pig farming represent a significant proportion of this use. Whilst the use of antibiotics as growth promoters is banned in the EU, they are still used for group level treatments of farm animals. Our understanding as to how antibiotic use in farm animals relates to the levels of antibiotic resistance genes within different farming systems is very simplistic. We do not know how management decisions on farm impact on the diversity of the commensal and environmental bacteria on the farm and how this relates to the 'quantity' of antibiotic resistance genes in this system. We also do not understand what happens to these resistance genes in the face of different antibiotic treatment protocols, whether some protocols are 'worse' than others at selecting for resistance and whether the levels of resistance genes decay when antibiotic treatment is stopped. We therefore do not have a clear evidence base as to the most effective way to reduce and refine antibiotic use on farms to minimise selecting for antibiotic resistance genes.

The aim of this work is therefore to demonstrate that changes in the diversity of bacteria and 'quantity' of antimicrobial resistance genes within pig faeces and their environment can be measured and related to one another, antibiotic use and management changes on the farm. The application of this work will be to develop a framework with which changes to both management practices and antibiotic use on farms can be proposed that minimise the selection for antibiotic resistance. This will benefit farmers by reducing the likelihood of selecting for resistant bacteria that infect farm animals and society more generally by reducing the likelihood that antibiotic resistant infections in humans will develop as a consequence of antibiotic use in farm animals.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Jennifer Ritchie	University of Surrey	A quantitative method to evaluate AMR distribution in complex communities based on methylome profiling

Co-Investigators

University of Surrey:

Dr Jose Jimenez

Summary

Antimicrobial resistance (AMR) inhibits our ability to deal with once easy-to-treat bacterial infections. AMR can be acquired by disease-causing bacteria from other organisms living in the same environment that do not pose a threat to our health e.g. in our intestines, which is home to trillions of bacteria. The acquisition of AMR by some harmful bacteria will enable these organisms to survive antibiotic exposure whereas as sensitive organisms, both beneficial and harmful to us, are killed. This means that the use of antibiotics, even at low levels, can promote the expansion of resistant populations in niches where there is little competition from other microorganisms.

Understanding the chain of events taking place during transmission of AMR is essential to inform more effective treatments and to rationalise use of our current repertoire of antibiotics. Unfortunately, methods for studying transmission are largely based on being able to grow the organism in the laboratory, something that is only possible for a relatively small number of species. As a consequence, all the events in which non-culturable species - those that cannot be grown in the lab - are involved are missing from our studies. Only recently, methods based on the production of fluorescent proteins have been used to understand transmission events in the environment and they have already given insights into previously unknown bacterial interactions. These methods are however limited to those conditions in which the fluorescent proteins work, which are largely dependent on the presence of oxygen. Specific anaerobic niches crucial in antibiotic treatments such as the intestine cannot be analysed using this methodology.

We propose an alternative approach to monitor transmission of AMR in complex bacterial populations like the gut microbiota. This method is based on the analysis of changes in the DNA of resistant populations in response to changes in environmental conditions such as during treatment with antibiotics. We will take advantage of the presence of other genes that can be transferred together with AMR to do this. These genes encode for enzymes called methyltransferases that produce permanent modifications in the DNA of the recipient cell. Monitoring the presence or absence of those modifications by a new sequencing technology will be used as proof of the acquisition of AMR. Since this method works in a high-throughput fashion, we can monitor thousands of species in a single experiment. In that way we will generate a complete dynamic picture of the main AMR interactions in the community and the rate at which these occur.

If successful, this new technique will help us to determine the flow of AMR in the natural environment, identifying potential reservoirs that favour the development of resistance. These results could be used to design tailored-made treatments to optimise the way that we use antibiotics to minimise the spread of AMR.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Fiona Henriquez	University of the West of Scotland	Genes of past, present and future: does legacy pollution contribute to antibiotic resistance in industrialised estuaries?

Co-Investigators

University of the West of Scotland:

Professor Andrew Hursthouse
Dr Roderick Williams

University of Strathclyde:
Dr Charles Knapp

Summary

Development of antibiotic resistant (AR) bacteria diminishes the efficacy of antibiotics to the point that difficult-to-treat pathogens are encountered in water, beaches and seafood. Although the inappropriate use of antibiotics in medicine and agriculture contributes to the problem, there is evidence that industrial pollution (e.g., organics and heavy-metal pollutants) have a strong role in AR development.

Can residual pollution in the environment from past industrial pollution continue to threaten present and future public/environmental health? Many chemicals do degrade in nature; however, certain signatures, e.g., persistent polycyclic aromatic hydrocarbons (PAH) and heavy metals can remain in sediments providing continual stress to resident microorganisms.

Bacteria have the ability to acquire and disseminate mechanisms to deal with chemical stress; some of these mechanisms are either analogous or closely-linked (genetically) to genes to that provide antibiotic resistance. Therefore, the presence of metals (for example) can cause bacteria to harbour and spread these stress-response genes.

In this project, we will obtain sediment cores in former/current industrial areas along the Clyde estuary near Glasgow. We will determine chemical signatures in sediment layers and use radiometric (lead and caesium) assessment to determine "pollution age". Simultaneously, we will characterise the resident bacterial populations (via 16S-rRNA analysis) and types of resistance genes (metal and antibiotic) that they harbour to determine whether risk exist in zones of pollution.

Having the ability to compare different pollution conditions among stratified layers in the sediment and among different locations along the estuary; it will allow us to determine contributing factors towards resistance traits in microbial communities. This research will better inform us the risks associated with industrial pollution, and it can influence pollution-control and remediation strategies.

Theme 3 Pump Priming Grant		
Grant Holder	**Institution**	**Title of Award**
Dr Jennifer Dungait	Rothamsted Research	Does the potential for AMR selection differ between common UK cattle grazing systems?

Co-Investigators

University of Exeter:

Dr William Gaze
Dr Mark Van Der Giezen

Rothamsted Research:

Dr Christopher Hodgson

Summary

Antimicrobial resistance (AMR) occurs because repeated exposure to antimicrobial drugs kills susceptible bacteria leaving the resistant types to multiply. Recent high-profile reports of the devastating consequences for human health caused by the resistance of disease-causing bacteria to antibiotics have emphasized the need to understand the processes that drive increasing prevalence of antimicrobial resistance in human and animal pathogens.

Farm animals are considered to be a major source of AMR because of the large amounts of antibiotics used both to treat infection (therapeutic use) and to prevent infection (prophylactic use). However, little is known about the background levels of resistance in farming systems, even when animals are only treated with antibiotics when they need them (therapeutically). For instance, the sharing of genetic material by bacteria that are in close proximity is another route where AMR may emerge unexpectedly in otherwise non-related bacteria. AMR transmission between cattle is likely to be greater when they are close together, for example during the winter housed period. In this proposal we will use an experimental farm where cattle being raised for beef production only receive minimal antibiotic treatment described as 'best practice' by vets. We will monitor dynamics of AMR bacteria and genes in cattle dung in summer when the cattle are grazing in the field, and in the winter when they are housed together. We will carefully monitor cattle who have been sick and have been given antibiotics and those in the rest of the herd.

The diet of farm animals may also effect increased AMR in their gut flora. Even common plants ('forage') like grass and clover that cattle commonly eat when they are out grazing produce natural antimicrobial compounds that may continue to be active in the stomach (rumen) where millions of bacteria thrive and multiply. In the winter, cattle are brought indoors and fed silage which is grassland plants that have been fermented to conserve them. Undigested diet and lots of bacteria from the rumen, and therefore perhaps AMR, are excreted in cow dung. We will analyse the dung of cattle that eat different forage types to find out if what they eat affects the likelihood of AMR arising in cattle fed one diet compared to another.

Most cattle farming in the UK is situated in the hilly 'wet West' where the potential for rain to runoff fields into waterways is high. Recent increases in storms that cause lots of runoff and flooding may be due to climate change, are predicted to continue. At the same time, in recent years there has been an increasing recognition that AMR may arise in farm animals and be released from the farm environment to the natural environment in water and might therefore end up in drinking water, bathing water or in seafood. Normally, pollution from cattle dung in water is monitored using faecal indicator organisms (FIOs), which are bacteria that do not usually cause disease but which correlate with those that do (pathogens), and can be handled safely by scientists. It would be useful to know what proportion of FIOs carry AMR, because they are routinely tested for, facilitating estimates of AMR transmission to be estimated in the future. We will sample water flowing from fields that have been grazed by beef cattle or spread with their manure, and analyse the bacteria in the water including the FIOs to see if they are resistant to antibiotics.

The results of this study should help us to understand whether AMR that arises in cattle herds may be transferred to the environment, whether management can limit AMR and its transport, and inform approaches for assessing risks of new antibiotics and managing adverse effects that might be occurring. It is important work for addressing risk in our complex agro-food system that is clearly important for both consumers and farmers.