**Project Title**

Biomonitoring of Antimicrobial Resistance in UK Freshwater Ecosystems: an Integrated Microbiological and Genomic Approach

**Lead Supervisor Name**

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Institution: Cardiff

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**Full Project Description**

Successful treatment of bacterial infections is paramount in healthcare, veterinary medicine, animal husbandry and agriculture, yet extensive use of antibiotics is driving selection for antimicrobial resistance (AMR). While the AMR mechanisms per se are a natural phenomenon, spill-over from anthropogenic settings is believed to be leading to the accumulation and global spread of AMR. This can lead to further evolution of AMR strains through vertical transmission or horizontal gene transfer to previous non-AMR strains. This poses a severe threat when strains are mobilised back into healthcare, veterinary or agricultural settings. Our project will (1) utilise two bacterial culturing approaches to obtain cultures of AMR bacteria from freshwater habitats – (i) non-selective culturing and testing for antibiotic susceptibility (indicator bacteria); (ii) selective screening for *E. coli* resistant to extended-spectrum cephalosporins, and carbapenems, which reveals Extended Spectrum Beta-Lactamase- (ESBL), pAmpC-, and carbapenemase-producing *E. coli* –the most commonly involved AMR *E. coli* in healthcare settings. (2) Obtained AMR strains from (1) will undergo whole-genome sequencing for phylogenetic analysis and functional characterisation, which in part will be conducted with resources and support from our partner at Uppsala University. (3) Based on these and previously published UK AMR strains, we will develop quantitative PCR-based assays to specifically screen various environmental sources for the AMR-related genes (possibly also the strains) detected in (1-4). (4) We will collect and screen sediment samples and spatially matched fresh biological samples from benthic invertebrates, non-migratory fish (exploring collaboration with the Fish Tissue Archive), and faecal (spraint) samples from otters – allowing us to test AMR detectability (a) on three trophic levels and in animals with different movement radii, and (b) in different “compartments” of the environment (water, sediment). We will explore the efficacy of assays for archived otter faecal samples (Cardiff University Otter Project), which would allow retroactive screening of freshwater habitats across the UK based on >3000 samples collected since 1992. (5) Results will be analysed in a spatially explicit context, making use of catchment and river bed data on sediments, flow and surrounding habitats, using GIS. We hypothesise that the effect on microbial communities of runoff from animal husbandry will be detectable in adjacent freshwater habitats and their inhabitants, and perhaps especially so at higher tropic levels. A further goal contingent on obtaining additional funding is to conduct metagenomic sequencing, which will allow bioinformatic identification of resistance genes from freshwater environments. The supervisory team will ensure that outcomes are feasible and meet applied challenges. Drs F Hailer and E Chadwick (Cardiff University) provide access to otter samples and data (1992-present) and expertise in genomics, genetic assay development, and digital mapping/statistical modelling. Stakeholder Dr C Baker-Austin (CEFAS) is a research microbiologist studying waterborne AMR disease emergence. Dr A Singer, GW4 co-supervisor based at CEH specialises in drivers of environmental AMR using genomic approaches. Collaborator Dr J Järhult works at Uppsala University on AMR issues from a One Health perspective, and has previously collaborated with Dr Singer.

**Real Life challenges this project will address**

Our project contributes towards two important societal needs, (1) maintaining clean water, and (2) limiting the further evolution and spread of AMR in freshwater ecosystems. Antibiotic-resistant bacteria are projected to become one of the greatest emerging challenges to healthcare and agriculture settings worldwide. Prevalence of AMR in freshwater environments is a growing threat for human wellbeing (maintaining access to clean water for direct or indirect consumption) and healthcare (limiting the evolution and spread of AMR). Freshwater AMR is also impacting agriculture and veterinary settings, plus could have large impacts on recreation (fisheries, bathing). Despite this large societal importance, only relatively little is known about the distribution and prevalence of AMR in UK freshwaters. This knowledge gap not only limits our understanding of any naturally occurring AMR, but complicates pinpointing of anthropogenic AMR sources (i.e., urban or rural; healthcare or agricultural). The key challenge from our stakeholders is, therefore, to provide research-based evidence on the prevalence and drivers of environmental AMR, to aid them in developing evidence-based policy and environmental management strategies. Monitoring AMR in freshwater habitats is complicated by water flow and sediment transport, since both factors continuously lead to a redistribution of water and dissolved substances and particles, along with any AMR microbes found at a given site. This can lead to AMR tests of the water body or local sediment to produce patchy and hard to interpret results, with high spatial and temporal variability that may in fact be due to sampling artefacts. A biomonitoring approach, where freshwater taxa are screened for presence of AMR in their guts, may produce more consistent signal, since this would integrate across larger spatial and temporal scales. However, there is only little known about the efficacy of this approach for AMR monitoring, and little is known about which species should be chosen for such monitoring. Our stakeholders are therefore keen to explore the value of using a biomonitoring approach for AMR detection in UK freshwaters. To this end, this proposal will investigate the possibility of using various animal taxa with different mobility levels to detect AMR, in order to capture different trophic levels and scales of animal mobility.

**What you should know about this project**

Our project will pursue an integrated microbiological and genomic biomonitoring approach, surveying UK freshwater environments for anti-microbial resistance (AMR). First, we will conduct broad-target screening to compare the prevalence of live AMR cultures in freshly collected animal guts with that in sediments at the same location. Biomonitoring will be done at three trophic levels: benthic invertebrates, non-migratory fish, otters. Second, obtained live AMR strains will be tested phenotypically and undergo whole-genome sequencing for phylogenetic and functional analysis. Third, we will develop and apply high-throughput environmental screening approaches (based on the obtained knowledge of UK freshwater AMR strains), testing a larger sample of fresh environmental samples across the UK. Fourth, we will apply these screening methods to archived animal gut samples, exploring trends in freshwater AMR across time and space. Sampling will focus on being able to determine the impact of AMR deriving from agriculture and animal husbandry, since the other main source for freshwater AMR, human wastewater, has already been studied previously at much greater detail. Specifically, we will investigate areas with runoff from high- and low-intensity livestock farming. The research team unites expertise in (i) genomics, development & application of molecular methods for screening of environmental samples (1st supervisor Dr Hailer, Cardiff University, currently recipient of NERC funding to study microbiota in the Arctic), (ii) ecosystem scale modelling approaches, and extensive sample archive provided by the Cardiff University Otter Project (CUOP) and co-supervisor Dr Chadwick, with (iii) internationally renowned microbiological and metagenomics expertise by stakeholders Dr Baker-Austin (Centre for Environment, Fisheries and Aquaculture Science; CEFAS) and Dr Singer (GW4, NERC Centre for Ecology and Hydrology) and collaborator Dr Järhult (Uppsala University, Sweden), as well as (iv) key stakeholder support (provision of advice, data, and dissemination routes) provided by the project partners at CEH and CEFAS. Considerable added value is provided by the availability of >3000 pre-collected otter faecal samples in the holdings of CUOP, which will be tested for suitability for AMR monitoring of freshwater ecosystems across the UK.

**What expertise you will develop**

The PhD student will spend ca. 1 month/year with partner organisations (CEH, Uppsala University), undertaking all research steps from field collection of abiotic and biological samples (Cardiff University & CEH) to analytical microbiology (Cardiff, CEH), genomics and development of genetic screening methods (Cardiff, CEFAS, Uppsala University), spatial analysis and statistical modelling (Cardiff, CEH). Field, laboratory and data analysis skills: The PhD student will be trained in sample collection in the field, including sediment, benthic invertebrates, fish, and fresh otter spraint samples. Microbiology laboratory skills for culturing and isolating AMR bacteria, phenotypic classification, next-generation whole-genome sequencing of resistant isolates to conduct phylogenetics and sequence annotation. Development and application of PCR-based screening of environmental samples for strains detected in the previous steps. Analysis of landscape / river catchment data using GIS, and statistical modelling in ‘R’. Transferable skills: Effectively interacting with the team of supervisors will train the student in key skills of time and people management. Visits to stakeholders will entail interactions with and seminars for local staff, training the student in aspects of outreach and science communication, as will participation in normal ongoing outreach events by CUOP. Further, the PhD will cover a breadth of societally relevant topics, including healthcare and policy, river catchment and livestock management, equipping the student with detailed insights into these disciplines.

**Why this project is novel**

Previous research has demonstrated the presence of AMR bacteria in freshwater ecosystems, and has used sediment, water (e.g. work by partners Singer/CEH and Baker-Austin/CEFAS) and aquatic (or associated) organisms such as wildfowl (e.g., work by collaborator Järhult at Uppsala University), to map AMR occurrence and abundance in freshwater systems. However, screening of environmental samples has typically been focused on (1) a relatively narrow target group of live bacteria that were tested for AMR, or (2) screened for AMR genes (using PCR or metagenomics approaches), which cannot conclusively reveal whether AMR is in fact present in live strains in the watercourse, or whether the AMR genes derive from bacterial DNA remains in wastewater effluents. Furthermore, effluents from healthcare, animal husbandry and agricultural settings into freshwaters complicates the identification of any normally occurring AMR. A more comprehensive understanding of AMR in the UK would therefore greatly benefit from a biomonitoring approach, both because larger areas could be screened more efficiently, and because of environmental archives of animal guts, that would allow looking back in time. Our proposed project addresses both these aspects in a novel and exciting way.

**Rest of Supervisory Team:**

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