Prevalence and diversity of antimicrobial resistance genes in wild fish gut microbiota linked to diffuse and point source river pollution

Supervisors

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Project description

The problems posed by widespread antimicrobial resistance (AMR) has recently been acknowledged by the UK Prime Minister, Chief Medical Officer of England, WHO, academics, and clinicians. Much of the problem is linked to transmission of antibiotic resistance genes within the human population, such as in the hospital environment. However, a proportion of the antibiotics given to humans and farm animals are excreted as intact parent molecules and can escape treatment in sewage treatment plants (STPs) or the soil and enter water courses. This is also possible for antibiotic resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) excreted by patients and farm animals.

River sediment downstream of STPs has revealed much higher prevalence of ARGs and ARBs than a sample just upstream of the STP outfall [15]. Some freshwater fish populations are doing well in areas of high sewage effluent exposure raising the possibility they harbour a high prevalence of ARBs—a feature well characterised in aquaculture [3] and wild marine fish species [2,9]|. This research will investigate both the use of fish microbiomes as indicators of environmental AMR pollution and also the potential risks posed by fish-associated bacteria to human health. If sewage-exposed freshwater fish are caught and eaten by one of the estimated 3 million anglers within the UK, there is the potential for the 'antibiotic use – environmental resistance – exposure' cycle to be closed and ARB and ARGs returned to human hosts [14]. Increased AMR colonisation of the fish microbiome may be predictive of exposure to microbial pollution caused by a range of human or animal husbandry activities.

Lowland UK rivers are among the most sewage-impacted (as a proportion of total river flow) in Europe [12]. It follows that the most abundant fish, by weight, in lowland UK rivers, the roach (Rutilis rutilis) [7], would be highly tolerant of wastewater effluent. However, it remains unclear whether freshwater fish carry ARB in response to sewage effluents and their antibiotic and ARG discharge [13]. An important part of their diet, particularly in their first year is detritus and biofilms [8], a characteristic that will ensure high exposure to ARG and ARBs in polluted areas. Another important feature of the roach is that they tend to remain largely in one territorial area [1] which has been confirmed by wide genetic diversity of sub-populations from one another in the Thames [4]. Thus, the roach would be most likely to reflect their local environment in terms of

environmental contamination and would serve as an ideal model species for the proposed PhD project.

Since 2007 the Co-I has been collecting and archiving whole roach from several different locations in the Thames catchment as part of the fish tissue archive project done in collaboration with the Environment Agency. These locations go from the headwaters to the tidal limit [6]. The fish are preserved in vacuum packed bags and held at -80 Celsius, ideally suited for the preservation and recovery of DNA from the gut microbiome. The archive now holds over 2000 individual fish. Exposure for any location to sewage effluent can be precisely modelled for any point in time [5, 10]. Geographic based modelling of exposure to resistance genes is currently being developed in a new NERC Environmental Microbiology & Human Health (EMHH)-funded project (NE/M01133X/1), involving the PI and Co-PI at the University of Exeter, can be used to estimate the prevalence of resistance genes at any location in a catchment with a minimum of metadata [11].

To our knowledge no studies around the world have yet examined antibiotic resistance in freshwater fish in a systematic way. Thus, an opportunity exists with the proposed studentship to examine whether (a) antimicrobial resistance occurs in fish-associated microflora, (b) whether there is a temporal trend to the AMR observed, and (c) whether the AMR community fingerprint contained within the fish microflora reflect that of its environment, such as: 1) wastewater exposure; 2) proportion and type of animal husbandry; 3) Septic tanks (feature of small headwaters).

Analysis will initially focus on the historic archived fish samples, where priority will be given to fish that co-locate with the 77 sampling locations of the new EMHH project. For the final stage, in collaboration with the EA fisheries teams, a wider geographic spread of sites will be examined to more fully capture the breadth of sampling locations within the EMHH project.

Analysis of the fish microbiome will employ all of the standard methodologies, including: 1) culture-based work (i.e., specific antibiotic resistant microbes); 2) qPCR, i.e., specific primers to provide a quantitative analysis of antibiotic gene prevalence; and 3) metagenomes, i.e, supplying a detailed unbiased analysis of the full resistome, thereby capturing the sequences for all major resistance genes within the sample. The molecular and bioinformatic methodologies will mirror those chosen for the EMHH project, thereby offering confidence in the quality and timeliness of the data generated. Fish microbiome analysis will be directly compared to AMG and AMB in the sediment recovered from the respective EMHH study sub-catchments.

Through leveraging the wealth of data produced from the existing EMHH project, the student will be able to focus on the question of how well the fish microbiome reflects that of its environment particularly where affected by point and diffuse pollution sources.