# **Queen’s Doctoral Training Programme - Multi-dimensional approaches to understanding microbe/host interactions in the context of disease, therapeutics and community resilience**

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| **Title of studentship** | Functional metagenomics for identification of novel antimicrobial resistance genes in environmental viromes |
| **Project summary (max 250 words – this will be used to advertise the project if selected).** | Viruses are the most numerous and most diverse form of life on Earth, present in virtually all environments. The majority of viruses are bacterial viruses, or bacteriophages. Bacteriophages can transfer genes between bacteria, including virulence factors, metabolic genes, and antibiotic resistance determinants. The extent and relevance of antibiotic resistance transmission by bacteriophages is still poorly understood.  While antibiotic resistance genes are routinely detected in various viromes, the overall antimicrobial resistance potential of environmental phages remains a controversial topic. As antimicrobial resistance continues to spread, improved understanding of the potential role of bacteriophages as vectors of antibiotic resistance genes is necessary, especially taking into account that DNA fragments inside viral particles are better protected from degradation and can accumulate in the environments over time.  To address this, in this study we for the first time will explore the antibiotic resistance potential of viromes by cloning environmental bacteriophage DNA into a broad host range vector, transforming the resulting plasmid libraries into a selection of Gram-positive and Gram-negative bacteria and testing the resulting transformants against a representative selection of antibiotics of different classes for acquisition of antibiotic-resistant phenotypes. The viromes from which antibiotics resistance genes originated will be subjected to next-generation sequencing to investigate what phages the genes came from and their relative abundance in the viromes. The global distribution of these genes will be explored by searching publicly available viral metagenomes.  This study will be an exciting opportunity to gain much-needed insight into reservoir of viral genetic diversity and catalogue and characterise functionally active virome-associated antimicrobial resistance genes. This work will be particularly timely and important in light of growing interest to application of bacteriophages against pathogenic bacteria as therapeutic agents. |
| **Supervisor(s)** | Dr Timofey Skvortsov (Primary supervisor)  Prof Brendan Gilmore (Co-supervisor) |
| **What types of new collaborative relationships would this studentship support (e.g. development of national and international collaborations or industrial involvement/financial support) (100 words max)** | This studentship will support development of national and international collaborations |
| **Research centre / School** | School of Pharmacy |
| **Subject area** | Antimicrobial resistance, environmental microbiology |
| **Candidate requirements / Key skills required for the post.** Please note for the QUB-DTP awards applicants must have a 1st or 2.1 Honours degree (or equivalent) in a relevant subject. | Applicants should have a 1st or 2.1 honours degree (or equivalent) in a relevant subject. Relevant subjects include Computer Science, Bioinformatics, Molecular Biology, Molecular Microbiology, Virology, Pharmacy, Pharmaceutical Sciences, Biochemistry, Biological/Biomedical Sciences, Chemistry, Engineering, or a closely related discipline. Students who have a 2.2 honours degree and a Master’s degree may also be considered, but the School reserves the right to shortlist for interview only those applicants who have demonstrated high academic attainment to date. |
| **Relevant links for project advertisement/ more information** | 1. Olena, A. (2016) Phages Carry Antibiotic Resistance Genes. *The Scientist* <https://www.the-scientist.com/news-opinion/phages-carry-antibiotic-resistance-genes-32389>  2. Balcazar JL (2014) Bacteriophages as vehicles for antibiotic resistance genes in the environment. *PLOS Pathogens* 10(7): e1004219. <https://doi.org/10.1371/journal.ppat.1004219>  3. Enault F, Briet A, Bouteille L et al. (2017) Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. *ISME J* 11, 237–247. <https://doi.org/10.1038/ismej.2016.90>  4. Moon K, Jeon JH, Kang I et al. (2020) Freshwater viral metagenome reveals novel and functional phage-borne antibiotic resistance genes. *Microbiome* 8, 75.  <https://doi.org/10.1186/s40168-020-00863-4> |
| **Keywords for search filters** | Bacteriophage, virus, antimicrobial resistance, metagenomics |
| **Training provided through the research project** | The aim of the proposed interdisciplinary project is to investigate the contribution of bacteriophages into the spread of antimicrobial resistance in natural and built environments. During the project, the successful candidate will have an exciting opportunity to learn a variety of microbiological techniques, including standard bacteriophage isolation, purification and characterisation approaches and gain experience of working with bacterial cultures. This work will also involve molecular biology and biochemistry, including genetic engineering and molecular cloning, and protein expression and purification. The PhD student will be taught basics of next-generation sequencing and provided with necessary bioinformatics training in order to analyse viromes and identify gene sequences of antibiotic resistance genes.  Finally, the student will have an opportunity for further personal and professional development through a range of training activities available to postgraduate students at Queen’s University Belfast. |
| **Expected impact activities** | The expected outcome of the project is the creation of a catalogue of experimentally verified antimicrobial resistance genes carried by bacteriophages in Northern Ireland, both already known and novel. The sequences of these genes will be shared with the international research community via dedicated publicly accessible databases, such as FARME DB (Functional Antibiotic Resistance Element Database) or CARD (Comprehensive Antibiotic Resistance Database).  The PhD student will be encouraged to engage in a variety of impact activities, disseminate the research project findings through publications in relevant peer-reviewed journals, present the results of the study at conferences (in-person and/or online), and make them accessible to general public through broader channels, such as social media and popular science outlets. |

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**Project details including expertise in area and workplan (max 1200 words)**

**Project background**

Antimicrobial resistance (AMR) is a global crisis, threatening to nullify the modern health accomplishments achieved through the use of antibiotics. The excessive and inappropriate use of antibiotics imposes selective pressure on bacteria, resulting in proliferation and spread of drug-resistant strains, from which antibiotic resistance genes (ARGs) can be laterally transferred to other bacteria by mobile genetic elements, including bacterial viruses (bacteriophages).

Many studies have been dedicated to the investigation of occurrence, distribution and abundance of antibiotic resistance genes (ARGs) in natural environments. Despite the ever-increasing threat posed by the spread of AMR, surprisingly little is still known about the mobilisation and transmission of ARGs in nature by bacteriophages: while conjugation and transformation have been recognised as important mechanisms in the spread of ARGs, the role of transduction (transfer of bacterial DNA via virus particles) remains relatively understudied. DNA fragments encapsulated in viral capsids are better protected from degradation than naked DNA and can accumulate and spread more efficiently. In a recent study 33 viromes of different origins were analysed and it was found that the non-human viromes contain a large ARG reservoir, suggesting that bacteriophages could play an important role in the spread of resistances in natural environments, especially in aquatic ones [[1]](#footnote-1).

Nevertheless, the real extent of ARG transmission by virus-like particles (VLP) remains unknown, as the majority studies of viromes have so far focussed on identification of ARGs exclusively through sequence similarity searches. While the proportion of ARG-like sequences in viral particle-sized fractions is often substantial, whether or not these genes are functionally active remains to be elucidated. Moreover, novel antimicrobial resistance genes that do not have significant sequence similarity to known ARGs cannot be identified through bioinformatics analyses of metagenomic data alone. This approach allows only for identification of already known ARG genes, but not novel ones.

Two limited attempts to functionally characterise putative ARGs from viromes found through sequence similarity searches have been made, but protein products of these genes were not active *in vitro*[[2]](#footnote-2),[[3]](#footnote-3). Finally, in a study published this year in *Microbiome* journal[[4]](#footnote-4), two new types of β-lactamases were identified via functional assays of ARGs recovered from viral contigs, which is the first convincing evidence of functionally active ARGs transferred by phages.

The proposed PhD project will address the problem of functional ARGs transfer by phages and will be specifically focussed on understanding of the role of bacteriophages in the spread of antimicrobial resistance genes in Northern Ireland. This will be achieved by performing profiling of several environmental viromes for genes encoding active determinants of antibiotic resistance using a functional metagenomics approach.

**Research workplan**

**Phase 1. Collection and processing of samples**

During the initial phase, liquid samples will be collected from both potential antimicrobial resistance hotspots (e.g. wastewater treatment plants outflow, slurry tanks) and freshwater sources (Lough Neagh, River Lagan). Virus-like particle (VLP) fraction will be isolated and concentrated using tangential-flow filtration and the obtained VLP concentrates will be stored at 4°C until further use. Viral DNA will be extracted using standard procedures, as described previously[[5]](#footnote-5),[[6]](#footnote-6). The obtained viral metagenomic DNA samples will be used for subsequent experiments.

**Phase 2. Functional metagenomics**

Viral DNA samples will be amplified by using the whole genome amplification approach (GenomiPhi V2 DNA Amplification Kit), fragmented by sonication and size-selected using agarose gel electrophoresis. After DNA end-repair, plasmid libraries will be constructed. As only about 40% of genes can be functionally expressed in *Escherichia coli*, the broad-host range plasmid pBAV1K will be used as a vector. The purified plasmid libraries will be transformed into *E. coli* and amplified by plating on solid media. The resulting libraries will be transformed into a number of bacterial species, including, in addition to *E. coli, Bacillus subtilis* and *Pseudomonas putida*. The transformed cells will be used for functional screening by plating them on selective media containing different antibiotics. The presence of ARGs will be confirmed by extracting plasmid DNA from colonies formed on antibiotic-containing media, retransforming the plasmids into respective bacteria and re-plating them on antibiotic-containing media again.

**Phase 3. Experimental validation**

Minimal inhibitory concentrations (MICs) of antibiotics against antibiotic-resistant transformants and growth rates under non-selective conditions will be measured and compared to those of wild-type strains. Plasmids validated as containing ARGs will be sequenced, and the presence of these genes in the original virome samples will be confirmed by PCR. Additionally, to confirm that the transfer of ARGs can occur in nature, VLP concentrates from which novel ARGs are isolated will be used to attempt to induce antimicrobial resistance in bacteria by plating bacterial cultures spiked with VLP concentrates on antibiotic-containing solid media. Any surviving colonies will be analysed for the presence of ARGs.

**Phase 4. Next-generation sequencing and bioinformatics data analysis**

Experimental studies will be complemented by the bioinformatics analyses, during which publicly available genomic and metagenomic databases will be searched for the presence of newly identified ARGs. Finally, next-generation sequencing of the viromes where functional ARGs are detected will be performed (BGI Genomics). Subsequent bioinformatics analysis (metagenome assembly and annotation) will be conducted to identify phages carrying the novel ARGs and estimate their relative abundances.

**Supervisors’ expertise and relevant publications**

Dr Timofey Skvortsov has extensive theoretical and practical knowledge of viral and microbial metagenomics. One of his recent projects was an extensive analysis of temporal dynamics of Lough Neagh viromes (1, 2), during which more 9000 novel viral genomes were assembled, characterised taxonomically and functionally and their abundance was tracked throughout the period of one year. As a leading specialist in research on antimicrobial resistance, Professor Gilmore will provide guidance on all aspects of this study related to antibiotics and antibiotic resistance genes.

* [Dr Timofey Skvortsov](https://pure.qub.ac.uk/en/persons/timofey-skvortsov) (MRSB) is a Lecturer in Microbial Bioinformatics at the School of Pharmacy. Dr Skvortsov’s research interests lie in the areas of bacteriophage biology and viral metagenomics. His research is currently focussed on development of bacteriophage-derived antimicrobial agents for the detection, control and elimination of clinically important pathogens. This work is interdisciplinary in nature and relies heavily on application of methods of molecular biology, microbiology and bioinformatics approaches. Dr Skvortsov published 40 articles in high profile scientific journals. He currently supervises one and co-supervises three PhD students.
* [Prof Brendan Gilmore](https://pure.qub.ac.uk/en/persons/brendan-gilmore) (FRSC FLS FRSB) is a Professor of Pharmaceutical Microbiology and President of President of the Society for Applied Microbiology. Prof Gilmore’s research team are focused on understanding the processes which govern bacterial biofilm formation and tolerance to antibiotics, and the discovery of novel antibiotics and disinfectant approaches. His recent work has included the application of cold plasmas for biofilm decontamination, discovery of novel antibiotics and biocatalytic enzymes from extremely halophilic microorganisms (using both culture-based and metagenomic approaches) and uncovering novel druggable targets in bacterial biofilm formation among the ESKAPE pathogens using molecular tools to identify and inhibit proteolytic enzymes involved in biofilm development, as adjuvants to conventional antimicrobial agents. Professor Gilmore is an author of more than 200 papers in high impact journals and an author and editor of a key text on Pharmaceutical Microbiology (Hugo & Russell's Pharmaceutical Microbiology 8th Edition).

1. Arkhipova K, Skvortsov T, Quinn JP, McGrath JW, Allen CC, Dutilh BE, McElarney Y, Kulakov LA. 2018. Temporal dynamics of uncultured viruses: a new dimension in viral diversity. ISME J 12:199–211.

2. Skvortsov T, Leeuwe C de, Quinn JP, McGrath JW, Allen CCR, McElarney Y, Watson C, Arkhipova K, Lavigne R, Kulakov LA. 2016. Metagenomic Characterisation of the Viral Community of Lough Neagh, the Largest Freshwater Lake in Ireland. PLOS ONE 11:e0150361.

1. Lekunberri I, Subirats J, Borrego CM, Balcázar JL. 2017. Exploring the contribution of bacteriophages to antibiotic resistance. Environ Pollut 220:981–984. [↑](#footnote-ref-1)
2. Enault F, Briet A, Bouteille L, Roux S, Sullivan MB, Petit M-A. 2017. Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. ISME J 11:237–247. [↑](#footnote-ref-2)
3. Parsley LC, Consuegra EJ, Kakirde KS, Land AM, Harper WF, Liles MR. 2010. Identification of Diverse Antimicrobial Resistance Determinants Carried on Bacterial, Plasmid, or Viral Metagenomes from an Activated Sludge Microbial Assemblage. Appl Environ Microbiol 76:3753–3757. [↑](#footnote-ref-3)
4. Moon K, Jeon JH, Kang I, Park KS, Lee K, Cha C-J, Lee SH, Cho J-C. 2020. Freshwater viral metagenome reveals novel and functional phage-borne antibiotic resistance genes. Microbiome 8:75. [↑](#footnote-ref-4)
5. Skvortsov T, Leeuwe C de, Quinn JP, McGrath JW, Allen CCR, McElarney Y, Watson C, Arkhipova K, Lavigne R, Kulakov LA. 2016. Metagenomic Characterisation of the Viral Community of Lough Neagh, the Largest Freshwater Lake in Ireland. PLOS ONE 11:e0150361. [↑](#footnote-ref-5)
6. Arkhipova K, Skvortsov T, Quinn JP, McGrath JW, Allen CC, Dutilh BE, McElarney Y, Kulakov LA. 2018. Temporal dynamics of uncultured viruses: a new dimension in viral diversity. ISME J 12:199–211 [↑](#footnote-ref-6)