UK Health Security Agency

Antimicrobial Resistance:

Open Innovation in early-stage antimicrobial discovery and evaluation

November 2024

An Open Innovation Model to support AMR research

UKHSA offers its expertise and facilities to researchers within the AMR field, in order to work openly and collaboratively towards the goal of reducing the AMR burden on public health. As part of this 'open innovation' model, UKHSA has capabilities in the following areas which can be accessed by AMR researchers:

- A screening cascade for novel antimicrobial therapies which can be accessed for the testing of traditional and non-traditional therapeutic approaches against multidrug resistant bacteria, fungi and viruses. These include antimicrobial peptides, bacteriophage, immune modulators, microbiome modulators, antibiotic resistance breakers, rationally designed small molecules, biofilm disruptors, natural products and chemical libraries.

- Studies on the role of the built environment on transmission of AMR and Infection Prevention and Control (IPC) procedures to reduce transmission and improve antibiotic stewardship

- Research using a Unified Infection Database (IUD) to study antibiotic prescribing practise and patient outcomes.

This document provides an introduction to these areas, followed by examples of projects associated with this offering, where UKHSA has worked with partners from academia and industry.

Interested parties are encouraged to discuss proposals with the UKHSA project team and should submit a brief application describing their technology/therapeutic, which will be reviewed by the project team and external advisors to amr.screening@ukhsa.gov.uk

In 2024, the UKHSA Open Innovation platform have partnered with PACE, a £30million fund founded by LifeArc, the Medicines Discovery Catapult and Innovate UK, to support innovators developing new antimicrobials. PACE-funded projects will be able to leverage the Open Innovation platform through the scheme to evaluate new antimicrobial candidates.

PACE | Pathways to Antimicrobial Clinical Efficacy

Capital procurement, hospital ward and UID funded by DHSC:
AMR Award NIHR200658

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Antibacterial and Antifungal Screening at Porton Down

Visits by students or staff to Porton Down are supported, and training is provided in the microbiological techniques used in screening therapies for antimicrobial activity against a large library of MDR priority pathogens.

Alternatively, UKHSA can support screening of therapeutics by our own staff if a visit is not practical.

Screening is possible for non-traditional therapeutics such as bacteriophage, antimicrobial peptides, microbiome modulators, antibiotic resistance breakers etc. Capabilities are also available for evaluation of novel small chemical series derived from rational design and/or library screening.

Anaerobic facilities:

- Anaerobic cabinet
- Gas controlled plate reader for fluorescence, absorbance, luminescence, polarised fluorescence assays

High throughput facilities:

- Liquid handling robot with 96 & 384 well heads under sterile conditions
- Plate readers with additional capacity to read up to 80 plates at once as endpoint or growth assays

Hollow fiber infection model:

- EMA approved model for pre-clinical PK/PD studies
- Resistance studies
- Novel therapeutic technologies and small molecules dose fractionation studies

Biofilm assays:

- Microfluidic platform for imaging and analysing biofilms under flow, allowing analysis of biofilm disruption, clearance or inhibition of growth
- Single and mixed species biofilm models
- CDC bioreactor, Calgary method, ex-vivo methods

The following page highlights a typical screening cascade for antimicrobial therapies.

To discuss visits/projects, please contact the project team at amr.screening@ukhsa.gov.uk

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Antibacterial & Antifungal screening at Porton Down; example screening cascade

Collaborations → MTA T&C → User provides information on stability and solubility

MICs for antimicrobial activity on Priority Pathogens

Primary screening

Gram-negative panel
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
Escherichia coli
Enterobacter spp.
Burkholderia spp.

Gram-positive panel
Staphylococcus aureus
(MRSA & MSSA)
Enterococcus faecalis/faecium
(VRE & VSE)
Streptococcus spp.

Candida panels
C. albicans
C. auris
C. krusei
N. glabrata
C. parapsilosis
C. tropicalis

Report to user

Anaerobic/fastidious panels
N. gonorrhoea, G. vaginalis

Intrinsic resistance?
↑cell penetration – PMBN; ↓efflux - EPIs

Lead candidate selection

Confirm activity and explore mode & mechanism of action

Hit confirmation

MICs on extended panels
of MDR isolates

Cytotoxicity/*in vivo* efficacy
Mammalian cells, Haemolysis, *Galleria mellonella*

Impedance cytometer
Electrical properties of cells - how they change over time with antimicrobial treatments

Mode of action
Time-kill assay to define bacteriostatic/cidal; Resistance emergence

Generate resistance
Mutation frequency, serial passaging, hollow fiber infection model

Efflux inhibition
Fluorescent dye accumulation

Membrane disruption
Gram-negatives.
Membrane permeabilization
Membrane depolarisation

Whole genome sequencing
of resistant strains

Report to user

Deeper exploration into mechanism of action and resistance potential

Hit validation

Synergy
With existing antibiotics – adjuvants or potentiators?

***E. coli* reporter library**
Explore mechanism of action

Single and mixed species biofilms
CDC Bioreactor
Bioflux imaging under flow
Ex-vivo models

Hollow fiber PK/PD system
Dose fractionation
Resistance emergence

Transposon mutant libraries

Mechanism of action confirmatory assays
e.g. gyrase inhibition, translation inhibition

Report to user

Abbreviations
MTA = material transfer agreement, MIC = minimum inhibitory concentration, MDR = multidrug resistant, EPI = efflux pump inhibitor

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Antiviral screening at Porton Down; example screening cascade

Direct acting antivirals and cell-targeting therapies can also be screened for anti-viral activity against a range of high consequence viruses, based on the below screening cascade.

Example projects:
- Focussed libraries based on molecular docking.
- Rational design or from existing AMR programmes.
- Repurposing of licensed antivirals

Collaborations → MTA T&C → User provides information on stability and solubility

Measure viral inhibition using a series of in vitro screening assays

Primary screening

Coronavirus panel
SARS-CoV-2 (Wuhan-like virus)
SARS-CoV-2 variants
Middle Eastern Respiratory virus (MERS)
SARS-CoV
OC43
NL63

Influenza virus panel
Influenza A (H1N1)
Influenza A (H3N2)
Influenza B

Other viruses
Respiratory Syncytial virus (RSV)

Report to user

Lead candidate selection

Confirm activity and explore mode & mechanism of action

Confirm the effect of the drug in different cell lines

Mode of action
Host and viral transcriptomics using RNAseq

Cellular innate immunity
Drug innate immunity pathways

Cell cytotoxicity assessment
Assess drug cytotoxicity

Synergy drug studies
Co-culture experiments

Drug resistance emergence experiments

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Investigating the role of the built environment:
Healthcare-Associated Infections and Antimicrobial Resistance

UKHSA has been awarded funding by the Department of Health and Social Care to help accelerate the UK's work in the global fight against antimicrobial resistance. A proportion of this funding has been invested in the design and build of a full-scale, fully functional modular ward to study how hospital facilities can be designed and operated to improve infection control and reduce transmission of antibiotic resistant infections. The facility has a 4-bed ward and isolation rooms, designed according to current UK guidelines, with dedicated heating, ventilation and air conditioning systems, realistic water and drainage systems and appropriate surfaces, fixtures, fittings and furnishings. The facility is located at UKHSA Porton Down.

The modular ward facility is available for collaborators to investigate transmission dynamics of antimicrobial resistant bacteria and other emerging pathogens through:

Understanding and interrupting potential transmission pathways, including aerosol generating procedures, taps, showers, drains, water, fomites, human behaviour

Development and evaluation of novel infection control strategies, such as surface modifications/treatments, disinfection strategies, water treatments

Development of improved sampling methods and techniques, including use of surrogate organisms, novel sampling equipment, culture-based and molecular methods for assessing microbial populations and communities

Facilities at UKHSA Porton

The modular ward facility forms part of a complementary suite designed to support research. The suite also comprises:

Environmental Test Chamber (approximately 20m3 supplied with HEPA filtered air venting the room (2 air changes/min) allows rapid removal of airborne particles, and ensures a low level of background aerosol can be achieved.

Experimental sink and drainage system allows for replicate studies on hospital taps, sinks and waste traps, designed to understand and reduce the risks of dissemination of pathogens from these sources.

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The Unified Infection Dataset (UID)

The Unified Infection Dataset (UID) is a linked data resource for the surveillance and study of healthcare-associated infection (HCAI) and antimicrobial resistance (AMR) in England. The core functionality of the UID is to link the following four existing datasets held by UKHSA, returning patient-level and aggregated data outputs:

- UKHSA Second Generation Surveillance System (SGSS)
- NHS Digital Hospital Episode Statistics (HES)
- ONS Mortality (MBIS)
- Primary Care Prescribing data (NHSBSA)

The objectives of UID development are:

- To create an automated data linkage pipeline that can be deployed on UKHSA infrastructure and comply with IG requirements
- To provide functionality and interfaces that meet the data linkage and output format needs of scientific users
- To provide linked datasets and outputs within parameters for performance and scalability

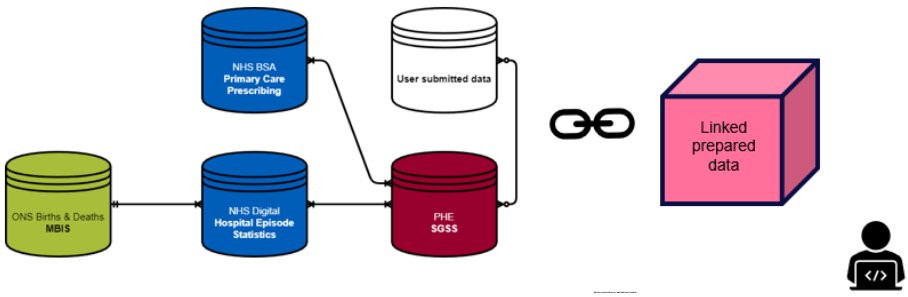
Use cases include:

Bacteraemia and AMR surveillance
Now: UKHSA scientists perform a series of manual analyses using a variety of scripts and tools
Future: the UID automates these processes, providing data outputs for surveillance report outputs (enhanced by linkage)

Epidemiological research
Past/now: UKHSA scientists need to extract data manually and write one-off scripts to clean, de-duplicate and link data
Now/future: the UID will provide a ready-linked cleaned and de-duplicated dataset

Healthcare-associated infection
Past/now: data analysis requires manual linkage of SGSS to HES records to assign HCAI classifications
Now/future: the UID provides a dataset linking infection episodes with hospital spells and A&E attendances

Since the UID's inception, scientists have increasingly been using the UID for the use cases above and we continue to see an uptake in the number of UID users.



The UID is in use within UKHSA and can be accessed via the following link: https://uid.phe.gov.uk/. Details of the relevant access requirements can be found on this page. For any issues, please contact uid@ukhsa.gov.uk.

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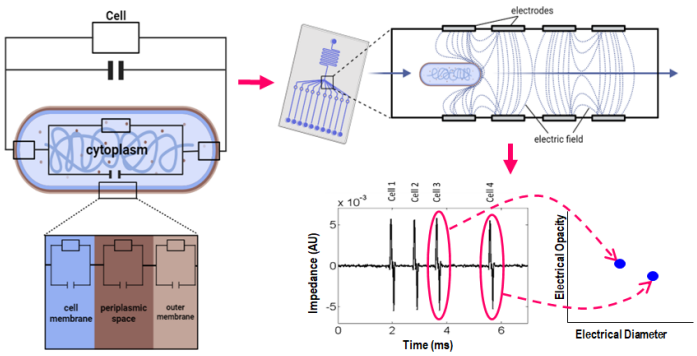
UNIVERSITY OF Southampton

iFAST

Impedance cytometry in early stage drug discovery

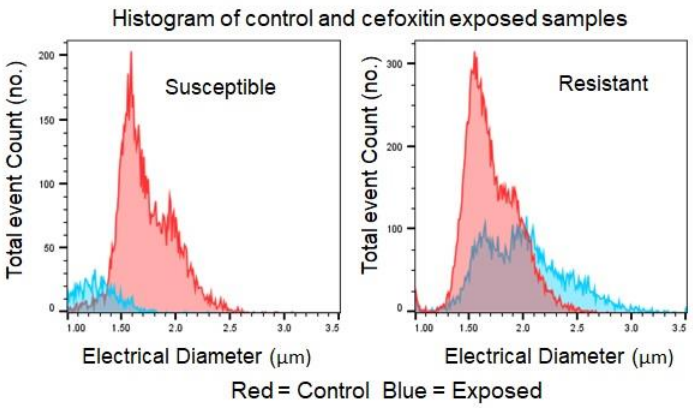
Bacterial Impedance measures the electrical impedance of individual bacterial cells as they flow through a microfluidic channel. Researchers at the University of Southampton and UKHSA have developed this methodology as a rapid antimicrobial susceptibility test, resulting in the creation of a spin-out company, iFAST Diagnostics (ifastdiagnostics.com).



In addition, the bacterial impedance cytometer (BIC) is a powerful research tool for examining the biophysical changes that occur to bacterial, fungal and mammalian cells after treatment with traditional and non-traditional therapies, during development of resistance to therapies, during viral/bacteriophage infection, and detecting changes in cell shapes.

Alongside traditional microbiological techniques, the BIC is an exciting element of the Open Innovation screening cascade, giving insights into mechanism of action, time to kill, resistance development and much more.

An example data set is presented below. The BIC generates scatter plots, where each dot is an individual cell. These can then be converted to histograms which can demonstrate both changes in electrical diameter and changes in opacity, which is a measure of the permeability of the membranes.



Key collaborators

-Hywel Morgan and Daniel Spencer, University of Southampton

-iFAST Diagnostics

Publications

-Morgan H, Spencer D, Hind C, Sutton JM (2020) *Rapid screen for antibiotic resistance and treatment regimen.* International Patent Application: PCT/GB2021/050694

-Spencer DC *et al.* (2020) A fast impedance-based antimicrobial susceptibility test. *Nature communications.* Oct 21;11(1):5328

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Bacteriophage research at UKHSA

Phage, viruses that specifically kill bacteria, are novel antimicrobials that can be used therapeutically against established and emerging infections which are difficult-to-treat. They are also a potential weapon with which to counteract the rise of AMR.

UKHSA gave evidence to the Science Innovation and Technology Committee (SITC) Parliamentary Inquiry on the antimicrobial potential of bacteriophage (report published January 2024) which highlighted the potential of phage but recognised the need to address the "translation gap" between research and applications.

At UKHSA, researchers are working with academics and SMEs to try and address that gap, supporting the evaluation of phage and phage-based medicines as an alternative to traditional antibiotic treatment. We use a variety of classical phage test methods (e.g. plaque assays on solid media), combined with high throughput kinetic growth curve analyses, whole genome sequencing and bacterial impedance cytometry to assess susceptibility of bacteria to phage and to evaluate the potential for phage-resistance. These methods can be applied to large libraries of multi-drug resistant pathogens and bacterial isolates from patients, and with both single phage and phage-cocktails.

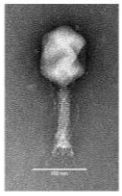
Proof-of-concept projects supported by the Open Innovation in AMR grant (NIHR200658):

- *'Using the high throughput plate stacker at UKHSA enabled me to upscale my experimental work massively. I was running phage vs bacteria kill curves on 96 well plates, trialling 468 different combinations of phages against different bacteria. With a single plate reader this would have taken months, whereas I was able to complete the work in less than 2 weeks at UKHSA.'*

- Libby Duignan, visiting researcher to UKHSA from the University of Liverpool

- *'Understanding the potential synergy and antagonism between phage and antibiotics, will be essential in the future use of phage in the clinic. We used kinetic growth curve analyses to generate heat maps showing phage – antibiotic synergy, focussing on the WHO Priority pathogen Acinetobacter baumannii'* (see below)

- with Dr Dann Turner, University West of England and Professor Ben Temperton University Exeter.



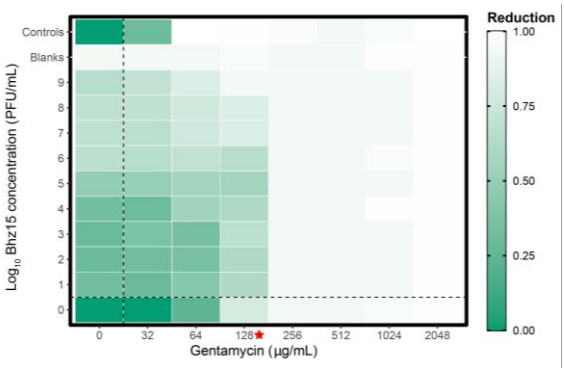


Figure provided by Dr. Dann Turner, UWE

Key collaborators

Dann Turner (UWE), Franklin Nobrega (University of Southampton), Ben Temperton (University Exeter), Jo Fothergill & Libby Duignan (University of Liverpool), SMEs

Publications

Genomic Diversity of Bacteriophages Infecting the Genus Acinetobacter

-Multiple manuscripts under preparation

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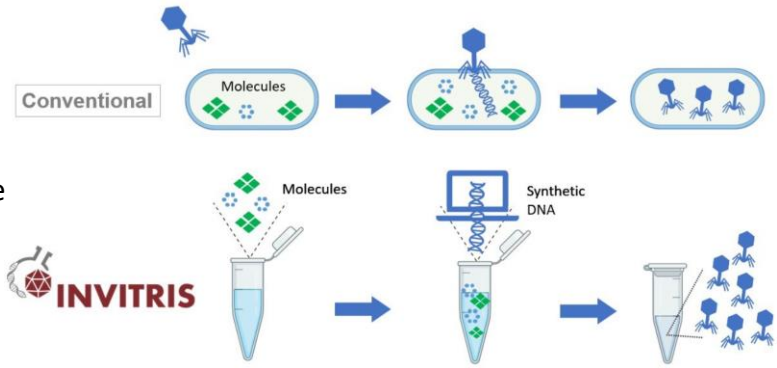
Example project; non-traditional therapies

INVITRIS

Bacteriophage produced from cell-free technology

Cell-free Technology

Researchers from Invitris have designed a cell-free technology, Phactory™, that involves creating a transcription-translation system, with added host factors and crowding reagents to generate synthetic proteins at high yield and purity. One of the applications of this platform is the production of bacteriophages that can specifically target Gram-positive and Gram-negative bacteria.



Pseudomonas aeruginosa specific phages were produced using this system and tested by UKHSA researchers against clinical strains of *P. aeruginosa* both as individual treatments and as part of phage cocktail treatments designed by UKHSA.

Single phage effectiveness

The susceptibility profile of each *P. aeruginosa* strain to the cell-free synthesised phage panel was determined by monitoring bacterial growth over 20hrs of phage exposure.

25 out of 33 phage demonstrated significantly high infectivity of at least 1 of the 9 strains tested, with 2 phages having the highest broad host range of 5. Some strains developed resistance to individual phages after ≤10hrs of exposure.

Cocktail Design

Phage-resistant isolates were collected and retested for cross-resistance against other phage using phage titration assays. Using the resistance assay and susceptibility profile data, 7 cocktails (4-5 phages) and 12 two-phage cocktails were designed.

The cocktails were tested against the original 9 strains and an extended panel of clinical strains, by monitoring bacterial growth (20hr) upon phage exposure. 16 out of 24 strains showed high susceptibility to one cocktail but some strains showed reduced susceptibility to the cocktails compared to the singular phages. However, one clinical strain showed no susceptibility to any singular phage but a high susceptibility to one of the two-phage cocktails. These cocktails are now being tested against singular and mixed biofilms.

Key collaborators
Invitiris (invitris.com)
Publications
-Manuscripts in draft

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Example project;
non-traditional approaches

QUEEN MARY UNIVERSITY OF LONDON

Cell-Free Synthetic Biology for Antimicrobials and Antimicrobial Resistance

Cell-free synthetic biology is an emerging research field within academic and industry for the bottom-up study of gene expression and chemical biology from outside of a living cell. Researchers at Queen Mary University of London & UKHSA have developed a cell-free protein synthesis system from *Klebsiella pneumoniae*, a Containment Level 2 pathogen. Once processed, a defining feature of this technology is that this cell-free system is safe to study at Level 1.

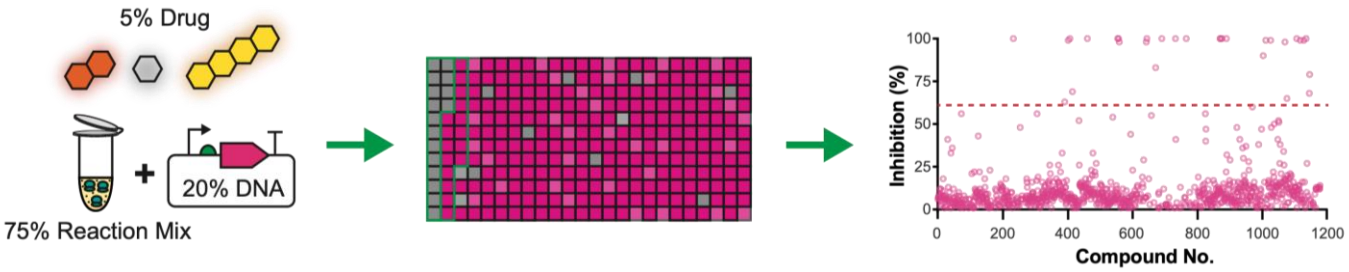
Mix for rapid results:
Bacterial extracts host 1,000-2,000 proteins, crucial for transcription, translation, and metabolism.

Multiple targets:
Highly active cell-free protein synthesis systems enable automated, high-throughput screening for compounds inhibiting gene expression (e.g., ribosomes) or metabolic pathways.

Applications:
(1) Antimicrobial discovery
(2) Structure-activity studies
(3) Antimicrobial resistance

Key Features:
- Low cost <£0.01 per drug
- Fluorescence tags (RNA / protein)
- Real-time or end-point
- 384-well plates – 2-10 µL volume
- Activity detected within 30-60 min
- Signal-to-noise ratio >150-fold
- Z-factor score >0.54
- DMSO tolerance – up to 5% (v/v)
- Extracts stable at RT for up to 4 hours
- Protocol generalisable
- Automation compatible

Measurable Impact
Collaboration with UKHSA has led to over £1 million grant funding from Royal Society, Biochemical Society and the BBSRC, as well as five international talks for QMUL researchers.



Key collaborators:
S Moore (QMUL), M Smales (University of Kent)

Key Outputs:
-Chengan *et al* (2023), A cell-free strategy for profiling intracellular antibiotic sensitivity and resistance. *Npj antimicrobials and resistance* 1:16

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Example project;
Rationally designed small molecules

KING'S College LONDON

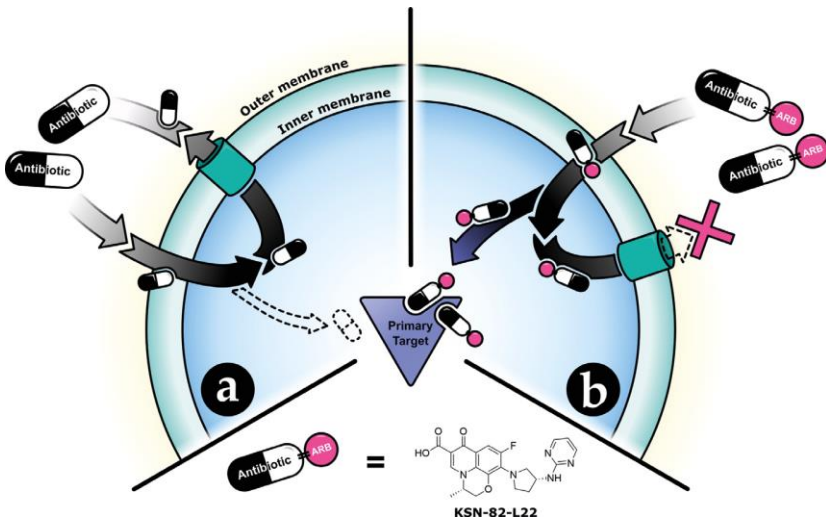
Efflux-Resistance Breaker Technology; Antibacterials

Antibiotics and antifungals, cornerstones of healthcare systems worldwide, are threatened by rising levels of AMR brought about by their overuse and misuse. This project employed state of the art computational studies to understand the interaction between different types of antimicrobial and efflux pump inhibitors (EPIs), and some of the key efflux pumps that mediate resistance. This helped us to design modified antibiotics, using a proprietary efflux resistant breaker (ERB) technology, that retain their ability to interact with the target while interacting with the inhibitor-associated hydrophobic pockets within the efflux pumps.

In our proof-of-concept work, advanced computational tools were used to design the new generation compounds, with ERB fragments containing hetero-aliphatic spacers and a heteroaromatic ring added to the core fluoroquinolone scaffold. The resulting ERB-modified quinolones prevent their own efflux by inhibiting the NorAefflux pump in *Staphylococcus aureus.*

Our lead molecules have been shown to be particularly effective *in vitro* against WHO and CDC priority pathogens including *A. baumannii, K. pneumoniae, E. coli, N. gonorrohoea, S. aureus* and *E. faecalis/faecium.* This data extends to demonstration of *in vivo* efficacy in a standard mouse thigh infection model, with 5-log reduction of bacterial load at both 10 and 50 mg/kg doses and excellent oral and IV PK/PD profiles comparable to levofloxacin.

This technology is currently being applied to an antifungal scaffold (see next slide) as well as a novel scaffold directed towards Gram-negative priority pathogens, and could theoretically be applied to any antimicrobial small molecule to achieve efflux resistance.



Key collaborators
KM Rahman, M Laws, K Nahar, T Al-adhami, S Jamshidi; KCL

Publications
-Laws, Jamshidi, Nahar, Hind, Sutton, Rahman (2017), *Antibiotic Resistance Breakers,* PCT/GB2018/051468
-manuscript in preparation

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Example project;
Rationally designed small molecules

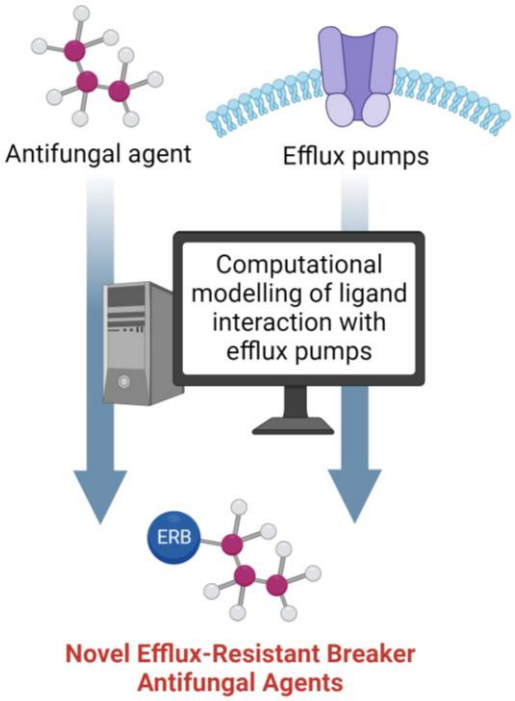
KING'S College LONDON

Efflux-Resistance Breaker Technology; Antifungals

The ERB technology, detailed on the previous page, has also been applied to the azole class of antifungal agents. Azoles are subject to efflux through multiple efflux pumps found in fungi, including CDR1 and MDR1, which are both constitutively overexpressed in the WHO Priority Pathogen, *Candida auris*.

ERB-azoles, designed to be resistant to efflux through CDR1 and MDR1, have shown excellent efficacy in *C. auris* clinical isolates, with MICs as low as <1ng/mL. This excellent activity was reflected across a broad panel of additional Candida species, including azole-resistant *C. albicans, C. krusei, C. tropicalis* and *N. glabrata.*

In vivo efficacy of these azole derivatives has been demonstrated against *C. auris,* in a *Galleria mellonella* model, and against *C. albicans,* in a murine septicaemia lung model. No toxicity was observed at 400mg/kg in mice and off-target toxicity screens did not reveal any issues; hERG channel inhibition and cytochrome p450 profiles were similar to existing azoles.



ERB-azoles continue to be developed against *C. auris* and an extended spectrum of fungal pathogens.

Projects such as this one respond to the call from the WHO to AMR researchers to 'Improve existing therapies…to prevent further resistance, enhance efficacy and minimise toxicity' (1).

Pre-clinical work was supported by NIAID under an agreement (NCEA-2019-79) between KCL and NIAID

(1) WHO fungal priority pathogens list to guide research, development and public health action, 2022

Key collaborators
KM Rahman, Y Chen, Y Li, B Panaretou, M Hassan; KCL

Publications
-Chen, Hind, Sutton and Rahman (2020) *New Efflux Resistant Antifungal Compounds,* UK Patent Application No GB2001564
-Manuscript in preparation

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Example project; non-traditional therapies

University of Kent

Supramolecular chemistry: Small Self Associating molecules as antimicrobials

Novel approaches to the discovery of non-traditional antimicrobial therapies can emerge from anywhere, including the field of supramolecular chemistry. This is one of the youngest branches of chemistry, only becoming recognised in 1987, and includes the study of interactions between molecules.

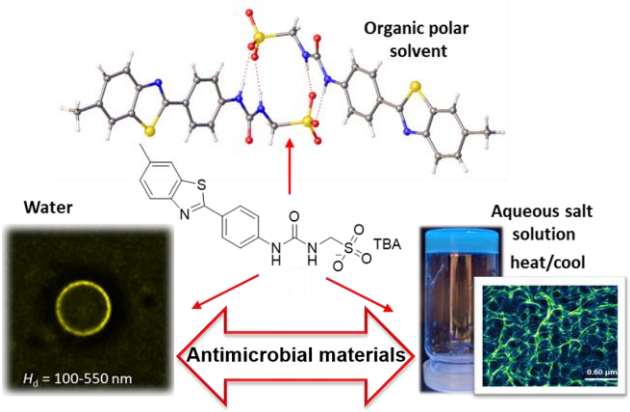
Supramolecular Self-associating Amphiphiles (SSAs) are a novel class of organic salts and related compounds designed by Prof. Hiscock's group at the University of Kent. In collaboration with UKHSA, these compounds are being developed to enhance their antimicrobial properties and explore their use as antibiotic efficacy enhancement agents.

To date, SSAs have been shown to:

- Act as triggerable multifunctional materials
- Act as broad-spectrum antimicrobial agents
- Selectively interact with phospholipid membranes of different compositions
- Enhance and reactivate the antimicrobial properties of antibiotics.

The SSA compound library has grown from the 4 original compounds (2016) to ~200 novel molecules (2024). Our work in this area has produced >6 peer reviewed publications and two patent applications.

The SSA antimicrobial development consortia has secured >£3M and is supported by an international team of chemists, drug development specialists, biologists, clinicians and materials scientists from Australia, UK, S. Africa, Nigeria, Germany, Italy and America. The development of this SSA technology was only made possible through the open innovation approach.



Key collaborators

J Hiscock, J Boles, K Hilton, L Thompson, L White, University of Kent

Publications

-L White *et al* (2020), Controllable hydrogen bonded self-association for the formation of multifunctional antimicrobial materials. *Journal of Materials Chemistry*

-J Boles *et al* (2022), Establishing the selective phospholipid membrane coordination, permeation and lysis properties of a series of 'druggable' SSAs. *Chemical Science*

-J Boles *et al* (2022), Anionic Self-assembling supramolecular enhancers of antimicrobial efficacy against Gram-negative bacteria. *Advanced Therapeutics*

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Example project; Antivirals

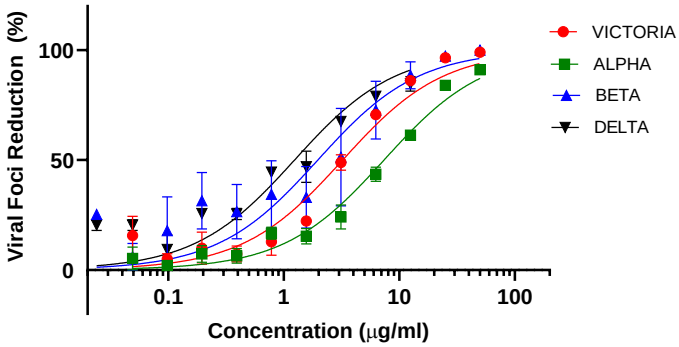
UNIVERSITY OF LIVERPOOL

SARS-CoV-2 antiviral screening identified heparin and heparin mimetics

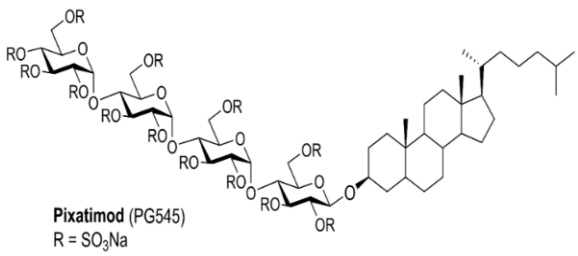
During the COVID-19 pandemic UKHSA collaborated with many partners including Professor Jerry Turnbull of the University of Liverpool to help identify/quantify the antiviral activity of a range of heparins and heparin mimetics.

Commercially prepared low molecular weight heparins (LMWH) and different unfractionated heparin (UFH) preparations were tested for antiviral activity against live wild-type SARS-CoV-2, in vitro, at high containment, at Porton Down. Unfractionated heparins were found to be more potent than LMWHs (Tree *et al,* 2021). These results provided additional supportive information in favor of trialing nebulized unfractionated heparin, for the treatment and management of COVID-19, in clinical trials around the world.

In collaboration with Professor Jerry Turnbull we also tested a synthetic heparin mimetic called pixatimod (PG545), a cancer drug candidate, for inhibitory activity against SARS-CoV-2, in vitro.



Pixatimod had potent inhibitory activity against live wild-type SARS-CoV-2 virus (Victoria). As the pandemic progressed, we also tested pixatimod against various SARS-CoV-2 variants as they emerged (Guimond *et al,* 2022). The graph above shows the inactivation curves for different concentrations of pixatimod against the percentage reduction in live virus, for different SARS-CoV-2 variants of concern, when compared to the control.



Key collaborators

Dr Jerry Turnbull, University of Liverpool, UK. Dr Clive Page, King's College, London

Publications

- Tree, *et al.* Unfractionated heparin inhibits live wild type SARS-CoV-2 cell infectivity at therapeutically relevant concentrations. Br J Pharmacol. 2021 Feb;178(3):626-635.
- Guimond, *et al.* Synthetic Heparan Sulfate Mimetic Pixatimod (PG545) Potently Inhibits SARS-CoV-2 by Disrupting the Spike-ACE2 Interaction. ACS Cent Sci. 2022 May 25;8(5):527-545.

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UK Health Security Agency

Example project; non-traditional therapies

KING'S College LONDON

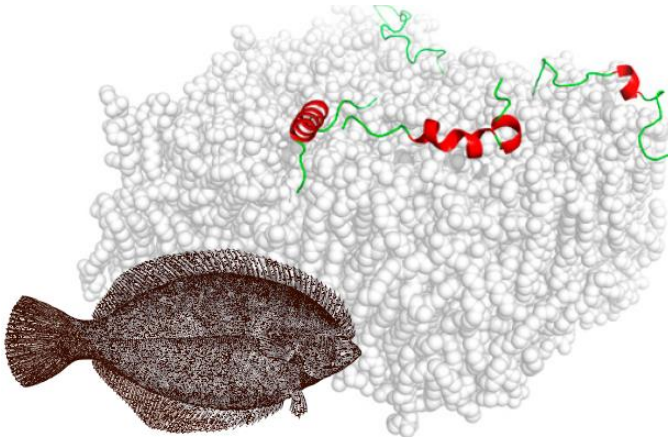
Influenced by nature: Antimicrobial Peptides (AMPs) derived from fish

Antimicrobial peptides (AMPs) are a potential alternative to classical antibiotics that are yet to achieve a therapeutic breakthrough for treatment of systemic infections. This substantially limits the scope of infection settings that are tractable to AMPs and hence their future development.

Identified in the Winter Flounder, *Pleuronectes americanus,* pleurocidin is a potent AMP with broad spectrum anti-bacterial activity. Its high potency is linked to its ability to cross bacterial plamsa membranes and seek intracellular targets while also causing membrane damage.

Researchers from KCL designed analogues of pleurocidin, which UKHSA demonstrated had substantially improved, broad spectrum, antibacterial properties, which are effective in murine models of bacterial lung infection.

A key part of the design process is understanding how pleurocidin and its analogues bind to and penetrate the target bacterial plasma membrane. KCL's researchers specialise in combining time-resolved computational and electrophysiology experiments with spectroscopic (NMR and CD) measures or peptide structure, conformation and membrane disorder.



CGI image supplied by: © King's College London
Winter Flounder image: © Mayer – stock.adobe.com

They found that increasing peptide-lipid intermolecular hydrogen bonding capabilities enhances conformational flexibility, associated with membrane translocation, but also membrane damage.

These analogues were more potent against a panel of Gram-positive and Gram-negative bacteria including multi-drug resistant strains. *In vivo* therapy was successful with an analogue comprising D-amino acids. It was well tolerated at an intravenous dose of 15 mg/kg and similarly effective as vancomycin in reducing EMRSA-15 lung CFU. This highlighted the therapeutic potential of systemically delivered, bactericidal AMPs. Next generation analogues are now being screened to identify a lead for pre-clinical development.

Key collaborators
J Mason, G Manzo, King's College London

Publications

- Clarke *et al* (2023) Synergy between Winter Flounder Antimicrobial Peptides. *Npj Antimicrobials and Resistance*
-G Manzo *et al* (2020), A pleurocidin analogue with greater conformational flexibility, enhanced antimicrobial potency and *in vivo* therapeutic efficacy. *Communications Biology* Nov; 3, 697
-JM Sutton, AJ Mason, RT Amison (2020) *Antimicrobial peptides,* PCT/GB2020/053078

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UK Health Security Agency

Example project;
Repurposing

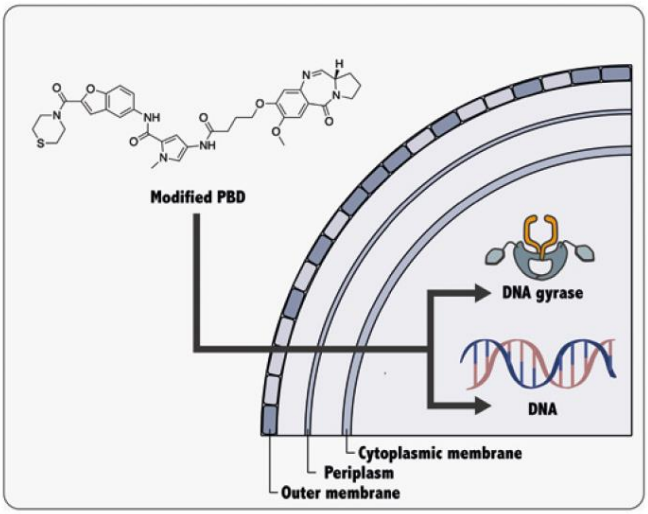
KING'S College LONDON

New broad-spectrum antibiotics from an anticancer drug

One avenue to explore for novel antimicrobial compounds is to modify existing drug scaffolds for antimicrobial activity. Pyrrolobenzodiazepines (PBDs) have been studied as anticancer agents and PBD-containing antibody-drug conjugates are approved for clinical use.

Modified PBDs with a C8-linked aliphatic-heterocycle are a new class of broad-spectrum antibacterial agents with activity against MDR Gram-negative bacteria, including WHO priority pathogens. Medicinal chemists at KCL designed and synthesised a new generation of C8-PBD monomers with an aliphatic third ring that showed notable activity against Gram-negative bacteria when tested at UKHSA. The aliphatic third ring improved the prokaryotic selectivity and reduced eukaryotic toxicity of the molecules, as it interfered with their DNA binding ability.

The synthesized compounds showed broad-spectrum activity against MDR and PDR clinical ESKAPE strains with MICs of 0.03 – 1 mg/L against Gram-positive species, and 0.125 – 32 mg/L against Gram-negative species.



The C8-PBD monomers demonstrated a rapid bactericidal mode of action against both Gram-positive and Gram-negative species. Lack of DNA binding, combined with an absence of eukaryotic toxicity highlights the potential therapeutic value of this new type of C8-linked PBD monomers as antibacterial compounds.

This project demonstrates that medicinal chemistry projects on existing drug scaffolds hold promise for developing novel antimicrobial compounds.

Key collaborators
KM Rahman, P Picconi, KCL

Publications
-Picconi, Sutton, Rahman (2015), *PBD Antibacterial Agents* PCT/GB2016/053882
-Picconi P, Hind CK *et al.* (2020), New Broad-Spectrum Antibiotics Containing a Pyrrolobenzodiazepine Ring with Activity against MDR Gram-negative Bacteria, *Journal of Medicinal Chemistry*

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UK Health Security Agency

Example project; Repurposing

Antibiotic RESEARCH UK
FIGHTING ANTIBIOTIC RESISTANCE TODAY AND TOMORROW

Can we repurpose FDA-approved drugs as antimicrobials?

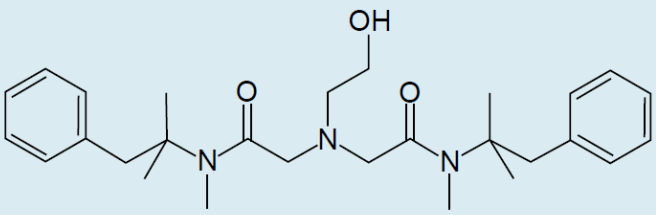
In 2018, Antibiotic Research UK (ANTRUK), the UK's first and only charity dedicated to tackling drug-resistant infection, commissioned a study to explore a library of 1200 FDA-approved drugs for potential antibiotic compounds. The study aimed to identify either direct-activing antimicrobials, or compounds which sensitise resistant Gram-negative bacteria to one or more antibiotics, looking to identify 'Antibiotic Resistance Breakers' (ARBs).

Discounting known antibacterials, the screen identified very few ARB hits, which were strain/drug specific. These ARB hits included antimetabolites (ziduvidine, floxuridine, didanosine), anthracyclines (daunorubicin, mitoxantrone, epirubicin), psychoactive drugs (gabapentin, fluspirilene, oxethazaine) and biocides (alexidine and chlorhexidine).

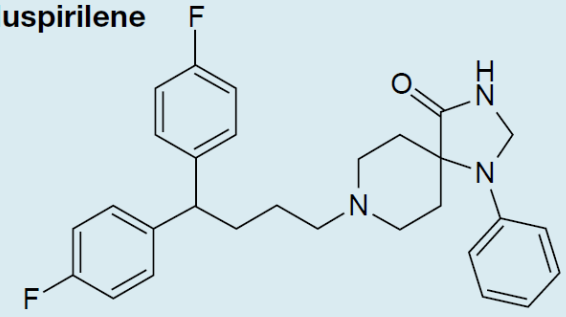
Researchers at UKHSA confirmed the ARB activity of the two most promising ARB candidates, fluspirilene and oxethazaine, against multiple clinical bacterial isolates. However, the results demonstrated that activity was strain-dependent even within species.

The results of this study suggest that there are very few approved drugs which could be directly repositioned as adjunct-antibacterials and these would need robust testing to validate efficacy.

Oxethazaine



Fluspirilene



Key collaborators
Antibiotic Research UK; www.antibioticresearch.org.uk

Publications
-Hind CK *et al* (2019) Evaluation of a library of FDA-approved drugs for their ability to potentiate antibiotics against multidrug-resistant Gram-negative pathogens. *Antimicrobial Agents Chemotherapeutics*

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UK Health Security Agency

Example project; non-traditional therapies

Matoke Holdings Limited

Testing a novel, topical antimicrobial formulation to combat the rise of MDR

Unresolved chronic wounds are a huge burden on healthcare settings, with over 2 million unresolved chronic wounds in the UK alone. Patented technology by Matoke Holdings Limited (Matoke) aims to revolutionise wound care by reducing and in some cases replacing the need for antibiotics - resulting in better antimicrobial stewardship in the clinic.

Matoke's patented RO® technology differs from other compounds in the novel way it's active ingredient, Reactive Oxygen Species, are generated over time. At UKHSA, standard efficacy assays were adapted to conduct the required studies effectively.

Matoke have been collaborating with the UKHSA since 2018 to explore the efficacy of its new Reactive Oxygen® (RO®) technology against pathogens of interest, including highly drug resistant strains.

Matoke's next generation of wound gel formulations, seek to improve upon the widely demonstrated efficacy of its UKCA marked Class IIb medical device, SurgihoneyRO™ (SHRO). Clinical evaluations of SHRO, which is based on the same RO® technology, have consistently demonstrated excellent antimicrobial efficacy and pro wound healing characteristics. The next generation of RO® products seek to address the limitations of SHRO's honey-based preparation.

There are multiple clinical case studies which have demonstrated the efficacy of Matoke's RO technology in a wide variety of clinical settings, including leg ulcers, neonate surgery sites and necrotising fasciitis infected wounds.

The creation of a new range of safe and effective wound treatment products will provide an alternative to antibiotics, effective against both Gram-positive and Gram-negative pathogens, including MDR strains.

Next steps:
The development of next generation topical RO® wound treatments provides an opportunity to formulate a range of products that can be precisely tailored to meet the needs of clinicians and patients alike, for a wide range of clinical indications, including acute, traumatic, surgical and chronic wounds.

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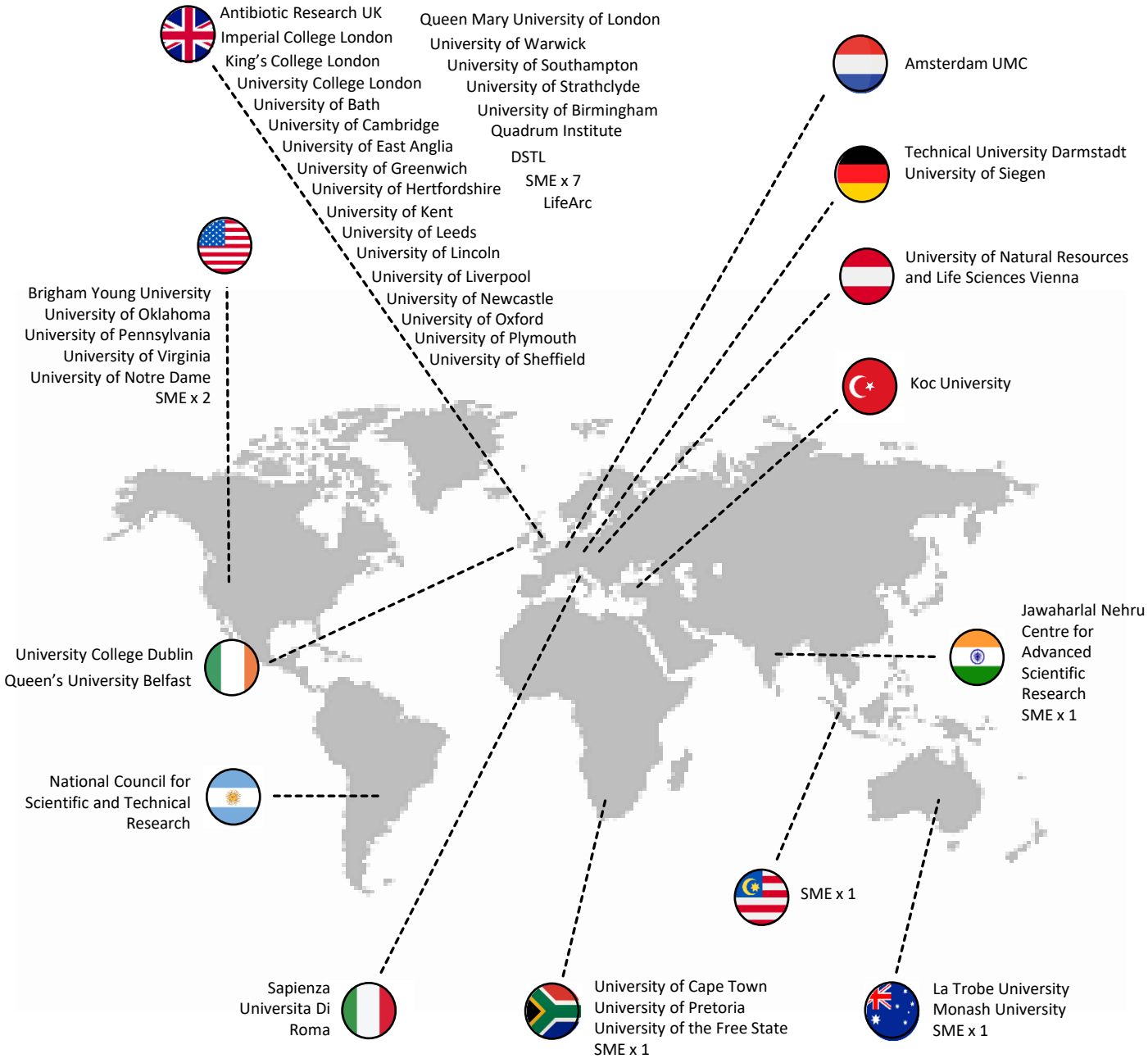
Collaborator map

Showing our partners across the globe



*SME (Small and Medium Enterprises) are anonymised for commercial confidentiality reasons

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