



Public Health  
England

Protecting and improving  
the nation's health

# Antimicrobial Resistance:

## Open-innovation in early stage antimicrobial discovery and evaluation



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Working with industry and academia



# An Open Innovation Model to support AMR research

PHE 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, PHE has capabilities in the following three areas which can be accessed by AMR researchers:

- 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 new Unified Infection Database (UID) to study antibiotic prescribing practice and patient outcomes
- A screening cascade for novel antimicrobial therapies which can be accessed for the testing of traditional and non-traditional therapeutic approaches against multidrug resistant pathogens. These include antimicrobial peptides, bacteriophage, immune modulators, microbiome modulators, antibiotic resistance breakers, rationally designed small molecules, natural products and chemical libraries.

This document provides a more in-depth introduction to the above three areas followed by examples of projects associated with this offering, where PHE has worked with partners from academia and industry.

Applications are invited to undertake studies, either for targeted projects to be carried out by PHE staff or through short research projects by visiting scientists at PHE. Non-microbiologists are welcome to visit, as training will be provided.

Interested parties are encouraged to discuss proposals with the PHE project team and should submit a brief application, which will be reviewed by the project team and external advisers to [amr.screening@phe.gov.uk](mailto:amr.screening@phe.gov.uk)



Application form available by contacting the project team.



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



Public Health England (PHE) 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 PHE Porton and will be fully commissioned during the autumn of 2021.

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 PHE Porton

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

**Environmental Test Chamber** (approximately 20m<sup>3</sup> supplied with HEPA filtered air venting the room (2 air changes per minute) 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.



# 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 PHE, returning patient-level and aggregated data outputs:

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

UID users can save and schedule queries for routine work and add transformations to the linked data outputs.

UID objectives:

- to create an automated data linkage pipeline that can be deployed on PHE 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

UID applications:

## Bacteraemia and AMR surveillance

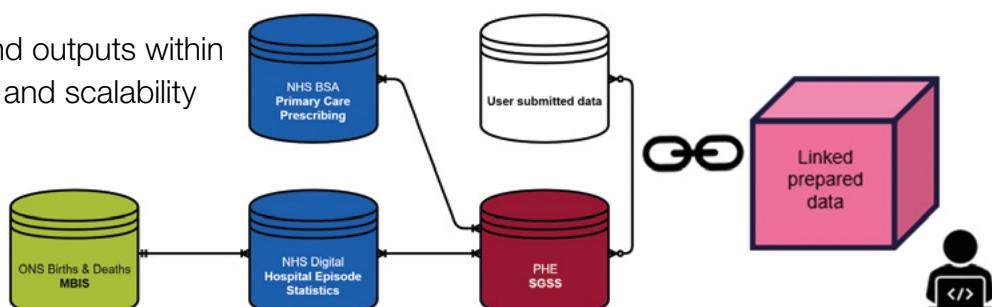
- Now: PHE scientists perform a series of manual analyses using a variety of scripts and tools
- Future: UID automates these processes, providing data outputs for surveillance report outputs (enhanced by linkage)

## Epidemiological research

- Now: PHE scientists need to extract data manually and write one-off scripts to clean, de-duplicate and link data
- Future: UID will provide a ready-linked cleaned and de-duplicated dataset

## Healthcare-associated infection

- Now: Data analysis requires manual linkage of SGSS to HES records to assign HCAI classifications
- Future: UID provides a dataset linking infection episodes with hospital spells and A&E attendances



This project is currently in the Beta development phase and will go live in Autumn 2021. Future developments include provision of a simulated (synthetic) dataset and incorporation of other core datasets including bioinformatics, reference laboratory data, disease-specific surveillance, primary care patient records and secondary care prescribing data.



# Facilities available through the antimicrobial screening open innovation model 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 multidrug resistant priority pathogens. Alternatively, PHE can support screening of therapeutics by our own staff if a visit is not practical or possible. Screening is possible for non-traditional therapeutics such as bacteriophage, antimicrobial peptides, microbiome modulators, antibiotic resistance breakers and immune modulators. Capabilities are also available for evaluation of novel small chemical antimicrobial 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 fibre model:**

- EMA approved model for pre-clinical PK/PD studies
- resistance studies
- novel therapeutic technologies and small molecules

## **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 and Calgary method



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



# Screening for antimicrobial efficacy

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

## MICs for antimicrobial activity on ESKAPEE and other pathogens

Primary screening	<b>Gram-negative panel</b> <i>Klebsiella pneumoniae</i> <i>Acinetobacter baumannii</i> <i>Pseudomonas aeruginosa</i> <i>Escherichia coli</i> <i>Enterobacter spp.</i> <i>Burkholderia spp.</i> (MDR & sensitive strains)	<b>Gram-positive</b> <i>Staphylococcus aureus</i> (MSSA & MRSA) <i>Enterococcus faecalis/ faecium</i> (VSE & VRE) <i>Streptococcus spp.</i>	<b>Candida panels</b> <i>C. albicans</i> <i>C. auris</i> <i>C. krusei</i> <i>C. glabrata</i> <i>C. parapsilosis</i> <i>C. tropicalis</i>	<b>Anaerobic/ fastidious panels</b> <i>N. gonorrhoea</i> <i>G. vaginalis</i> <i>Lactobacillus spp.</i>	Report to user
<b>Intrinsic resistance?</b> ↑ cell penetration – PMBN; ↓ efflux - EPIs					

## Lead candidate selection

## Confirm activity and explore mode of action

Hit confirmation	MICs on <b>extended panels</b> of MDR isolates	<b>Mode of action</b> 24h time-kill assay to define if bactericidal or bacteriostatic; resistance emergence	Plasma protease stability	<b>Synergy</b>	Report to user
	<b>Cytotoxicity / in vivo efficacy</b> Mammalian cells / Haemolysis / <i>Galleria mellonella</i>		<b>Efflux inhibition</b> Fluorescent dye accumulation	<b>Membrane disruption - G-ve</b> Potentiation of antibiotics	

## Deeper exploration into mode of action and resistance potential

Hit validation	Generate resistance Time-kill. Mutation frequency. Serial passage	Whole genome sequencing  <i>E. coli</i> reporter strains	Membrane permeability / depolarisation  Single and mixed species biofilm  Gyrase/topoisomerase inhibition assays; inc. FQ- resistant enzymes	PK/PD hollow fiber system  Bacterial Impedance Cytometer  Transposon mutant libraries	Report to user
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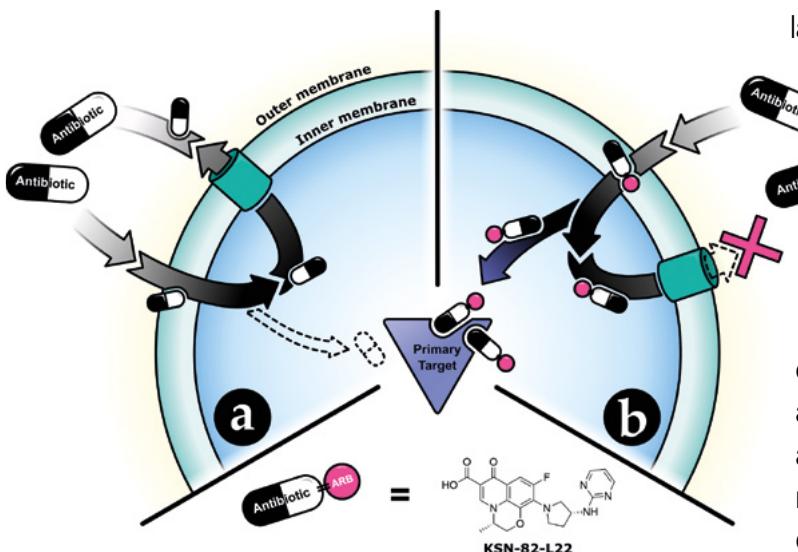
## Abbreviations

MTA = material transfer agreement, MIC = minimum inhibitory concentration,  
MDR = multi-drug resistant, EPI = efflux pump inhibitor



# Efflux-Resistance Breaker technology

Antibiotics and antifungals, cornerstones of healthcare systems worldwide, are threatened by rising levels of antimicrobial resistance (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 antibiotic and efflux pump inhibitors (EPI), 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 fluoroquinolone core scaffold. The resulting ERB-modified quinolones prevent their own efflux by inhibiting the NorA efflux pump in *Staphylococcus aureus*.

Our lead molecules KSN-L22 and BL-7 have been shown to be particularly effective *in vitro* against WHO and CDC priority pathogens including *A. baumannii*, *K. pneumoniae*, *E. coli*, *N. gonorrhoea*, *S. aureus* and *E. faecalis/faecium* with MIC<sub>90</sub> data <0.0625, 0.125, <2 µg/mL with large panels of *S. epidermidis*, *S. aureus* and

*N. gonorrhoea*, respectively. 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 dose level and excellent oral and IV PK/PD profiles which are comparable or more favourable than levofloxacin. This approach can theoretically be applied to any antimicrobial agent to achieve efflux resistance and is currently being used to develop efflux resistant antifungals.



## Key collaborators

KM Rahman, M Laws, K Nahar, Y Chen and S Jamshidi, King's College London

## Publications

- Chen Y, Hind C, Sutton M and Rahman KM (2020), *New Efflux Resistant Antifungal Compounds*, UK Patent Application No GB2001564
- Laws M, Jamshidi S, Nahar K, Hind C, Sutton M and Rahman KM (2017), *Antibiotic Resistance Breakers*, PCT/GB2018/051468

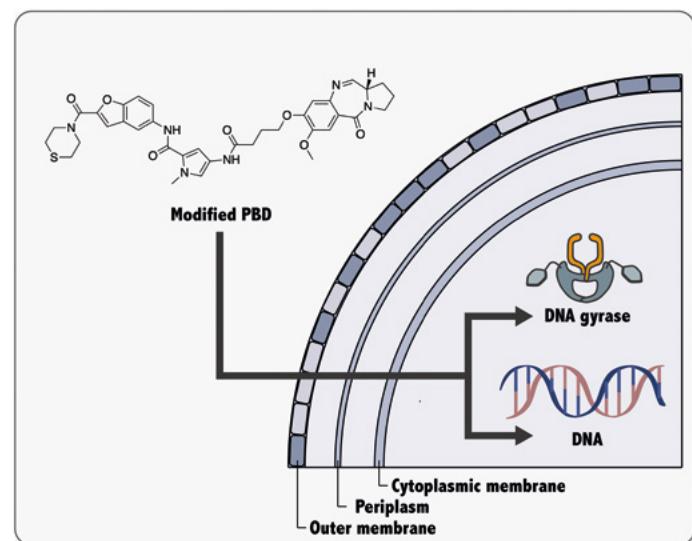


# New broad-spectrum antibiotics from an anticancer drug

This work responds to the need to find new antibiotic classes with activity against multidrug resistant (MDR) Gram-negative pathogens. Modified pyrrolobenzodiazepines (PBDs) with a C8-linked aliphatic-heterocycle are a new class of broad-spectrum antibacterial agents with activity against multidrug resistant Gram-negative bacteria, including WHO priority pathogens.

PBDs have been studied as anticancer agents and PBD-containing antibody-drug conjugates are approved for clinical use. We designed and synthesized a new generation of C8-PBD monomers with an aliphatic third ring that showed notable activity against Gram-negative bacteria. 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.



## Key collaborators

KM Rahman, P Picconi, King's College London

## Publications

- Picconi P, Sutton M, Rahman, KM (2015), *PBD Antibacterial Agents*, PCT/GB2016/053882
- Picconi P, Hind CK, Nahar KS, Jamshidi S, Di Maggio L, Saeed N, Evans B, Solomons J, Wand ME, Sutton JM and Rahman KM (2020), New Broad-Spectrum Antibiotics Containing a Pyrrolobenzodiazepine Ring with Activity against Multidrug-Resistant Gram-Negative Bacteria, *Journal of Medicinal Chemistry* 63, 6941-6958



# Supramolecular chemistry: Small Self Associating molecules as antimicrobials

On the first day of Professor Hiscock's independent academic career, she had an idea to conduct fundamental studies into the self-associative properties of a novel class of Supramolecular Self-associating Amphiphilic salts (SSAs). A few months later, with the help of some Masters' students, the initial drawings of SSA targets, were tangible compounds. The work was submitted for publication and reviewed. The reviewer's comments led Hiscock to consider further literature stating compounds such as SSAs would not exhibit any antimicrobial properties.

Hiscock's team in Kent did not agree, but a small group of students, led by an early career chemist with no track record in the field of antimicrobial agent innovation, initial attempts to gain the support to test their hypothesis were disregarded. This was until Prof Mulvihill (University of Kent) and Drs Sutton and Hind (Public Health England) chose to investigate the SSA innovation and test the chemistry team's hypothesis.

The SSA compound library has grown from the four original compounds (2016), to  $\approx$ 100 novel molecules (2020). Our work in this area has produced 14 peer reviewed publications and two patent applications. To date SSAs have been shown to:



- act as triggerable multifunctional materials
- act as a novel class of broad-spectrum antimicrobial agents
- selectively interact with phospholipid membranes of different compositions
- have the potential to act as drug delivery vehicles/therapeutic enhancement agents

The SSA antimicrobial development consortia has secured funding >£1.5M 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 and the support of the team at Public Health England.



## Key collaborators

J Hiscock and J Boles, University of Kent

## Publication

White L, Boles J, et al (2020), Controllable hydrogen bonded self-association for the formation of multifunctional antimicrobial materials. *Journal of Materials Chemistry*, June 7; 8(21): 4694-4700



# Can we repurpose FDA approved drugs as antimicrobials?

In 2018, 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-acting antibiotics, or compounds which sensitise resistant Gram-negative bacteria to one or more antibiotics, looking to identify 'Antibiotic Resistance Breakers' (ARBs).

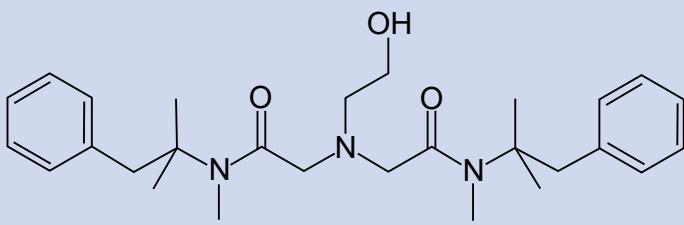
Discounting known antibacterials, the screen identified very few ARB hits, which were strain/drug specific. These ARB hits included antimetabolites (ziduvidine, floxuridine, didanosine), anthracyclines (daunorubicin,

mitoxantrone, epirubicin) and psychoactive drugs ( gabapentin, fluspirilene, oxethazaine, alexidine and chlorhexidine).

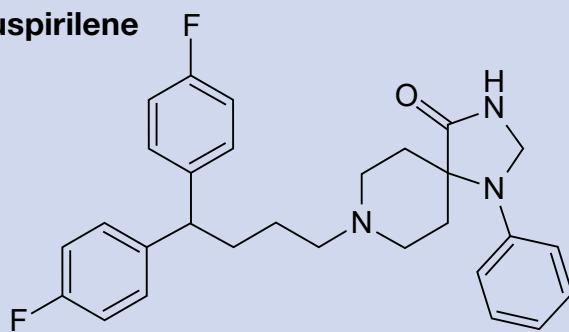
Researchers at PHE confirmed the ARB activity of the two most promising ARB candidates, fluspirilene and oxethazaine, against multiple clinical bacteria isolates. However, the results demonstrated that activity was strain-dependent even within species.

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

## Oxethazaine



## Fluspirilene



### Key collaborators

Antibiotic Research UK

### Publication

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. *Antimicrob Agents Chemother* Aug; 63(8): e00769-19

Email: [amr.screening@phe.gov.uk](mailto:amr.screening@phe.gov.uk)

Website: <https://research.phe.gov.uk/>



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

Matoke Holdings Ltd approached PHE in 2018 to explore the efficacy of its new synthetic Reactive Oxygen® (RO®) technology through a range of *in vitro* microbiology studies.

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 multi drug resistant strains.

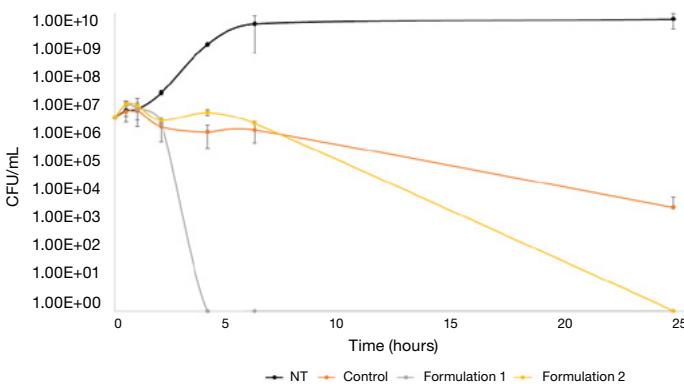
Matoke's first synthetic formulation, a gel, seeks to mimic the widely demonstrated efficacy of its CE marked Class IIb medical device, SurgihoneyRO™. Clinical evaluations of SurgihoneyRO™ which

is based on the same RO® technology, have consistently demonstrated excellent antimicrobial efficacy and pro wound healing characteristics.

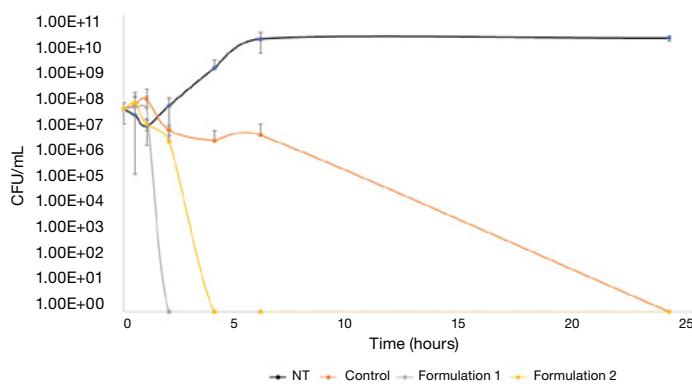
Matoke's patented RO® technology differs from other compounds in the novel way it's active ingredient, Reactive Oxygen Species, are generated over time. Our scientists at PHE were able to adapt standard processes to conduct the required studies effectively.

The graphs below outline the time kill test results showing rapid and complete eradication of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains.

**A. baumannii**



**P. aeruginosa**



## Next steps:

The development of a synthetic topical wound treatment provides an opportunity to formulate a range of RO® 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.

Email: [amr.screening@phe.gov.uk](mailto:amr.screening@phe.gov.uk)

Website: <https://research.phe.gov.uk/>



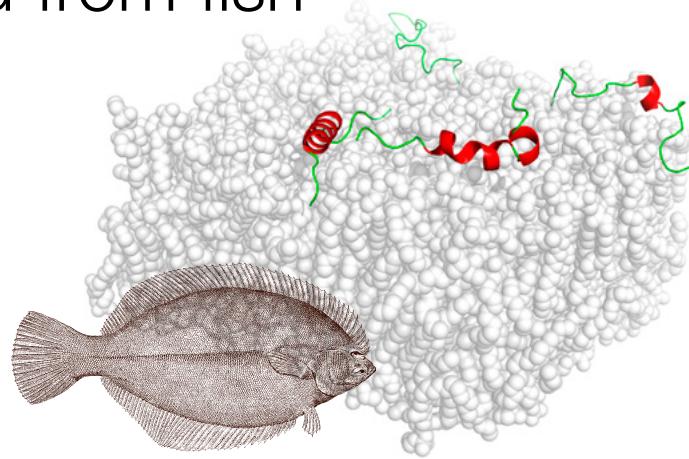
# Influenced by nature: Antimicrobial Peptides (AMP) derived from fish

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 plasma membranes and seek intracellular targets while also causing membrane damage.

Researchers from PHE and King's College London designed analogues of pleurocidin with 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. King's researchers specialise in combining time-resolved computational and electrophysiology experiments with spectroscopic (NMR and CD) measures or peptide structure, conformation and membrane disorder.



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

Giorgia Manzo, Charlotte K Hind, Philip M Ferguson, Richard T Amison *et al* (2020), A pleurocidin analogue with greater conformational flexibility, enhanced antimicrobial potency and *in vivo* therapeutic efficacy. *Communications Biology* Nov; 3, 697  
Sutton JM, Mason AJ, Amison RT (2020) *Antimicrobial Peptides*, PCT/GB2020/053078

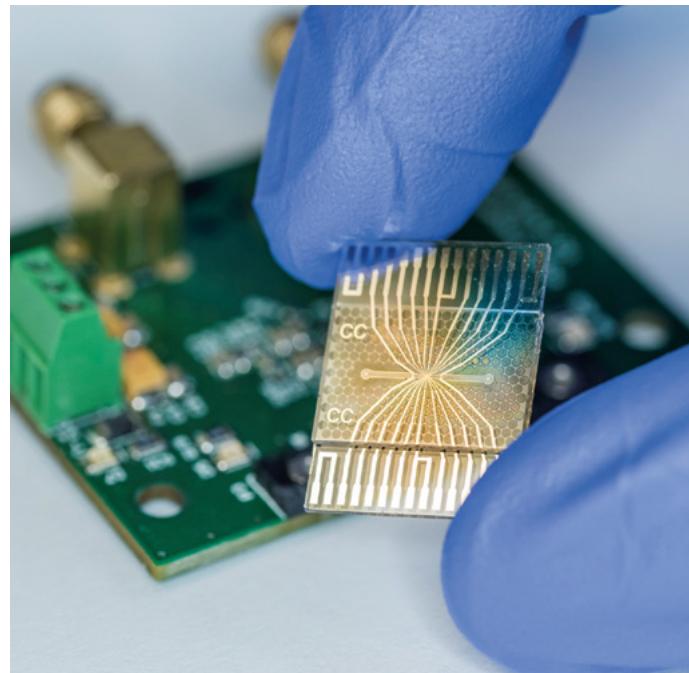


# Electrical Cytometry for Rapid Antimicrobial Susceptibility Testing

Engineers at the University of Southampton have developed a novel microchip to measure the electrical properties of single bacteria at high speed. The system has been utilized to measure the susceptibility profile of a range of gram-positive and gram-negative bacteria to a wide variety of antibiotics. The assay is based on the gold-standard broth microdilution susceptibility test and can provide an accurate result in as little as 30 minutes.

Starting from an actively dividing culture, a small sample of bacteria is incubated with antibiotics for 30 min. The electrical properties of the bacteria are measured with the microchip, a process which only takes 2-3 minutes. The results are compared with a measurement of the same bacteria without antibiotics. The measured electrical and morphological characteristics reflect the phenotypic response of the bacteria to the mode of action of a particular antibiotic.

The difference between antibiotic-exposed and untreated cells is used to discriminate between sensitive and resistant strains. Different



signatures in the phenotypical and electrical response give indications of mode of action of antibiotics and non-traditional therapeutic agents, including bacteriophage and/or of mechanisms of resistance of the cells. Working with Public Health England, the system has been evaluated for all major classes of antibiotic with Gram-negative and Gram-positive bacteria.



## Key collaborators

H Morgan, D Spencer and R Mingels, University of Southampton

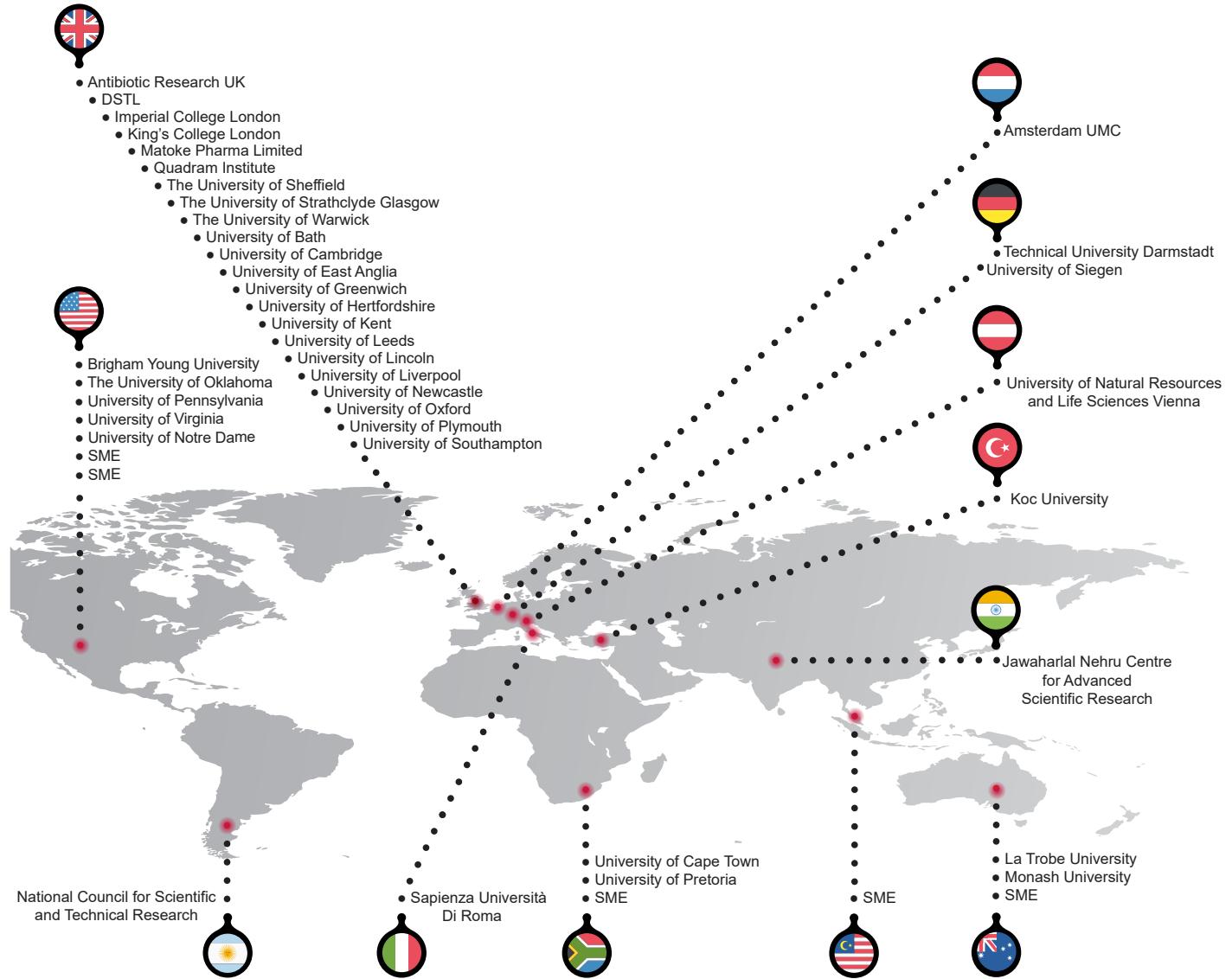
## Publication

Morgan H, Spencer D, Hind C, Sutton JM (2020) *Rapid screen for antibiotic resistance and treatment regimen*. International Patent Application No. PCT/GB2021/050694  
Spencer DC, Paton TF, Mulroney KT, Inglis TJJ, Sutton JM, Morgan H (2020) A fast impedance-based antimicrobial susceptibility test. *Nat Commun.* Oct 21;11(1): 5328



# Collaborator map

## Showing our partners across the globe



## Next steps

To find out more about accessing support and expertise for public health systems please contact:

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## About Public Health England

Public Health England exists to protect and improve the nation's health and wellbeing, and reduce health inequalities. We do this through world-class science, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. We are an executive agency of the Department of Health and Social Care, and are a distinct delivery organisation with operational autonomy to advise and support government, local authorities and the NHS in a professionally independent manner.

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