Industry-Academia Structural Biology Alliance to Tackle Bacterial Antibiotic Resistance

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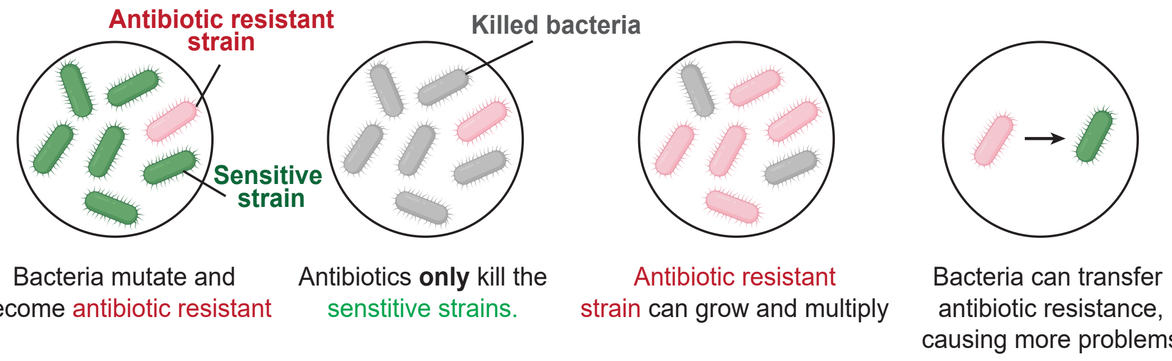
1. What is antimicrobial resistance?

Antimicrobial resistance (AMR) occurs when microbes such as **bacteria** and viruses accumulate changes over time. They no longer respond to antimicrobial treatments such as **antibiotics** and antivirals designed to kill them. **Main causes** include the misuse of antimicrobials in humans, animals and plants.

Antibiotic resistance in bacteria



Bacteria mutate and become antibiotic resistant

Antibiotics **only** kill the senstitive strains.

Antibiotic resistant strain can grow and multiply

Bacteria can transfer antibiotic resistance, causing more problems

2. Why is it a global challenge?

- AMR is one of the **top ten threats** to global public health, food security and development. It is known as a "silent pandemic" (*WHO*).
- In 2019, **bacterial antibiotic resistance** was directly responsible for 1.27 million global deaths.
- Antibiotic resistance makes medical procedures and treatments such as surgery and cancer chemotherapy riskier.

WORLDWIDE

10 million

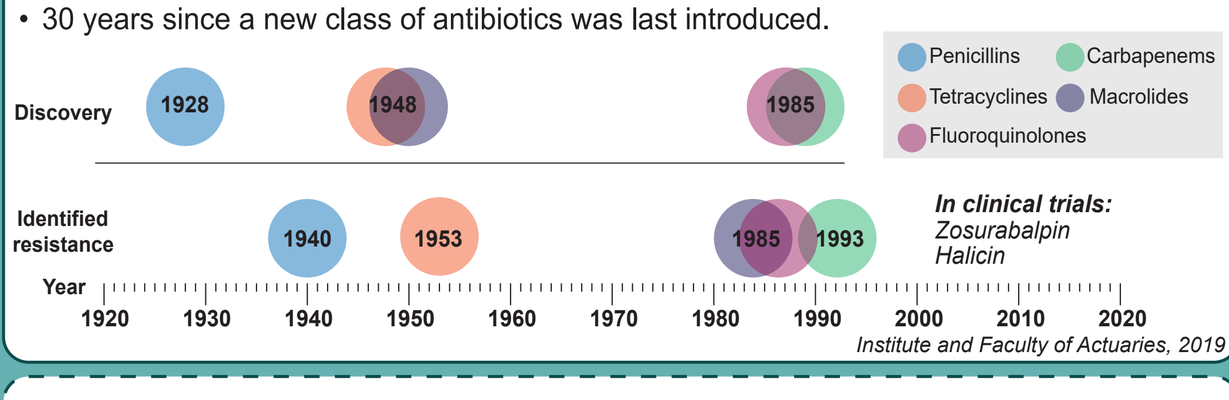
deaths per year by 2050 (greater than cancer)

Costing £66 trillion

(www.gov.uk)

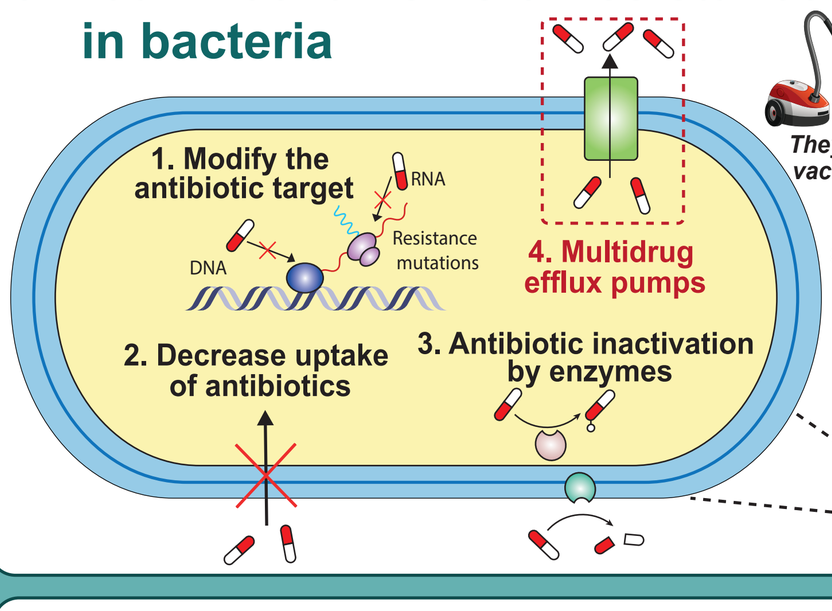
Antibiotic discovery and resistance timeline

- Bacteria are getting faster at developing resistance to antibiotics.
- 30 years since a new class of antibiotics was last introduced.



Institute and Faculty of Actuaries, 2019

3. Four mechanisms of resistance in bacteria



Multidrug efflux pumps are proteins that **expel antibiotics** out of the bacteria

They are bacterial vacuum cleaners!

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This reduces the amount and effects of the antibiotics

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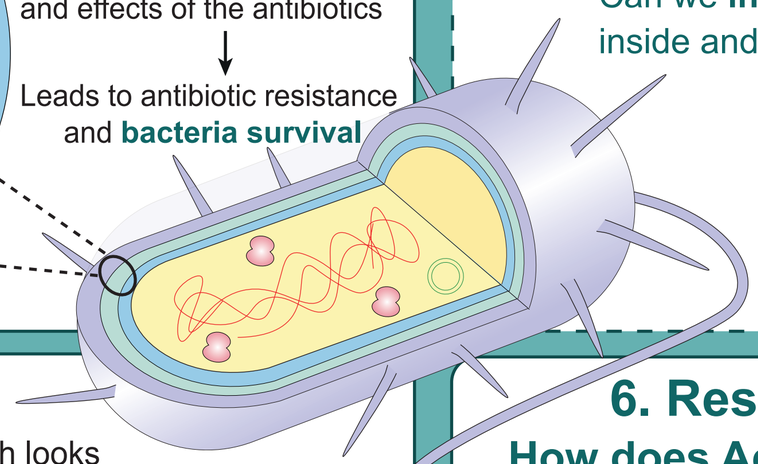
Leads to antibiotic resistance and **bacteria survival**



4. Aims

How do **multidrug efflux pumps** work to expel antibiotics?

Can we **inhibit (stop)** that happening to allow antibiotics to remain inside and kill bacteria?

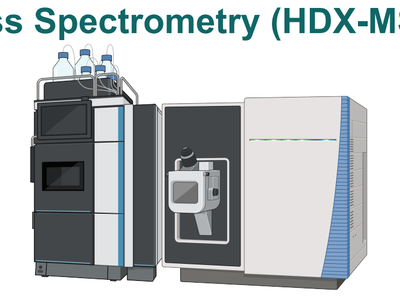
<u>To tackle this biological problem we:</u>

- Establish a **multi-disciplinary academia /industry partnership**
- Focus on two multidrug efflux pumps: **AcrB and MdtF**
- Use state-of-the-art **structural biology methods**

5. Structural biology methods

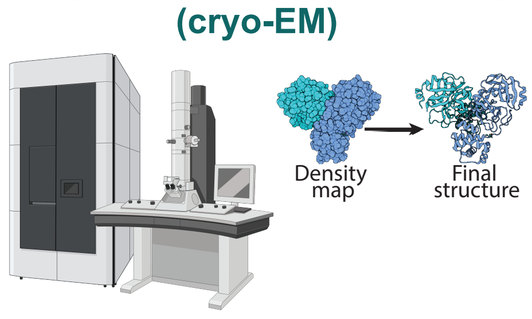
Structural biology is a key tool in **drug discovery** which looks at proteins at a molecular level, unlocking secrets of structure, function and interactions.

Hydrogen/Deuterium eXchange Mass Spectrometry (HDX-MS)



Tool for analysing structural features and **dynamic (movement)** properties of proteins

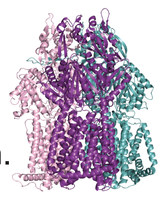
Cryogenic-electron microscopy (cryo-EM)



Super microscope to visualise how atoms of a protein and a drug are arranged in **3D space**

6. Research findings

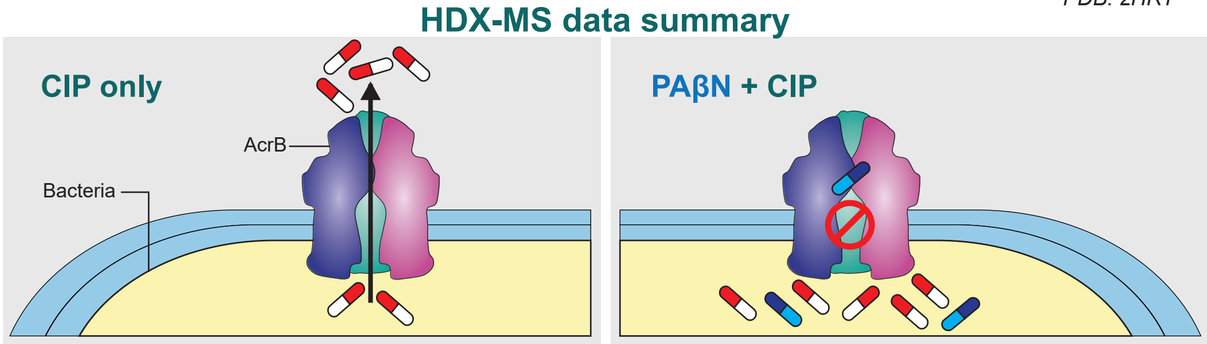
How does <u>AcrB</u> expel antibiotics?



Published AcrB Cryo-EM structure *PDB: 2HRT*

- Although structures exist for AcrB, its movement and function are unknown.
- We used HDX-MS to explore how the **antibiotic (CIP)** and the efflux pump **inhibitor (PAβN)** affect AcrB movements.

HDX-MS data summary



AcrB **movement increases**

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Antibiotic **CIP is expelled out** of the bacteria

↓

Lower cell concentration of CIP

↓

Bacteria is antibiotic resistant and **survives**

PAβN restricts AcrB **movement**

↓

Antibiotic **CIP remains inside** the bacteria

↓

CIP can perform its role in killing bacteria

↓

Drug efflux is inhibited and **bacteria dies**

Antibiotic susceptibility assays:
PAβN enhances CIP activity

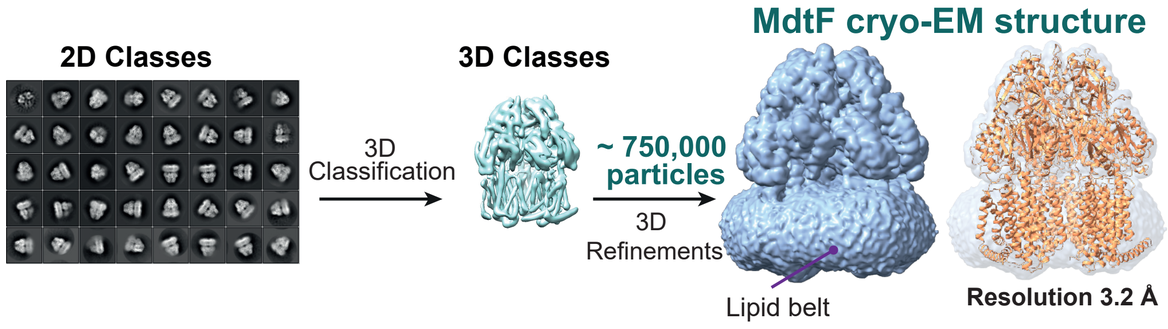
Microbial strain		Minimum Inhibitory Concentration (μg/ml)
		CIP
E. coli ATCC 25922		0.008
PAβN addition	+ 16 μg/ml	**0.004**
	+ 32 "	**0.002**
	+ 64 "	**0.001**
	+128 "	**≤0.00012**

The efflux pump inhibitor PAβN can stop AcrB from expelling the antibiotic CIP, preventing bacterial resistance.

Reading, E, Ahdash, Z et al. Nature Commun, 2020

What is the structure of <u>MdtF</u>?

- Same class of protein as AcrB, but currently no structural information is available.
- Increased expression and efflux in anaerobic/acidic conditions (e.g. mammalian gut).



- We solved the **first structure of MdtF** using cryo-EM.
- Cryo-EM structure reveals three monomers (similar to AcrB).

Future work

- Perform HDX-MS to investigate movement and function.
- Find inhibitors that restrict the movement of MdtF and stop the removal of antibiotics, consequently prevent bacterial resistance.

Impact

- Efflux pumps are also found **in human cells** (not just in bacteria).
- The leading cause of **cancer chemotherapy failure** is the development of multidrug resistance by **efflux pumps** e.g. P-glycoproteins **in human cancer cells**.
- Overall, understanding **how inhibitors of efflux pumps work** can:
  - overcome bacterial resistance and allow us to **reuse antibiotics**.
  - restore the sensitivity of human cancer cells toward chemotherapy drugs.

ucb Inspired by **Patients** Driven by **Science**

University of Southampton

King's College London

References

Antibiotic Resistance: Modelling the Impact on Mortality and Morbidity A report by the Antibiotic Resistance Working Party. Institute and faculty of Actuaries, 2019.

Antimicrobial Resistance Collaborators. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet; 399(10325): P629-655.

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