Biofilms: What are they and why do we care?

Guest speakers: Mark Webber & Freya Harrison

Moderator: Laura Piddock

21 October 2021

GARD P

Global Antibiotic Research & Development Partnership

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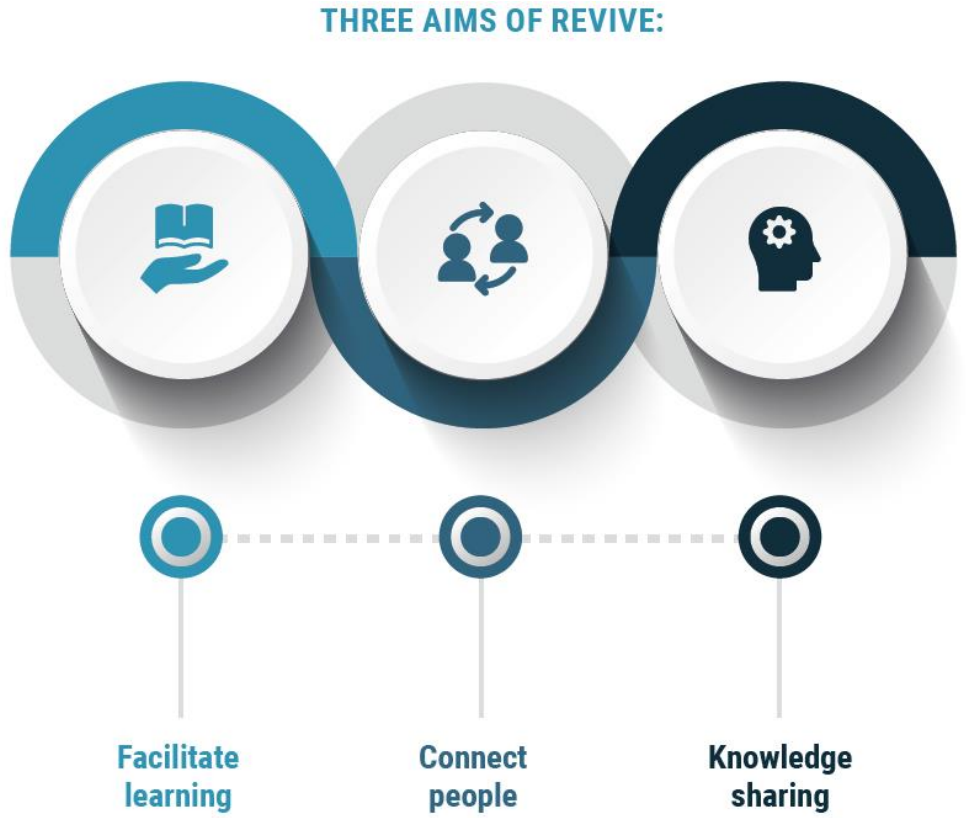

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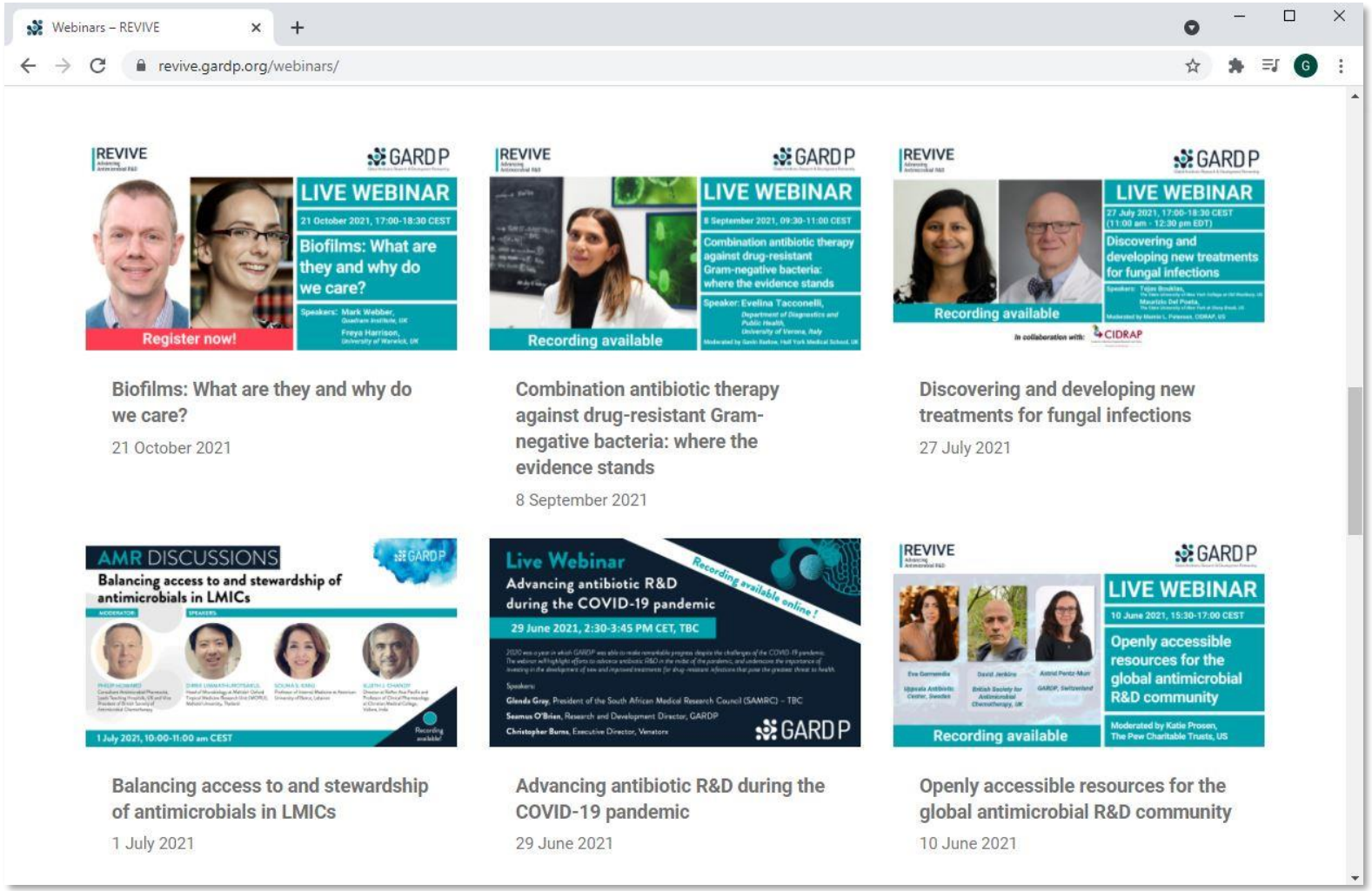

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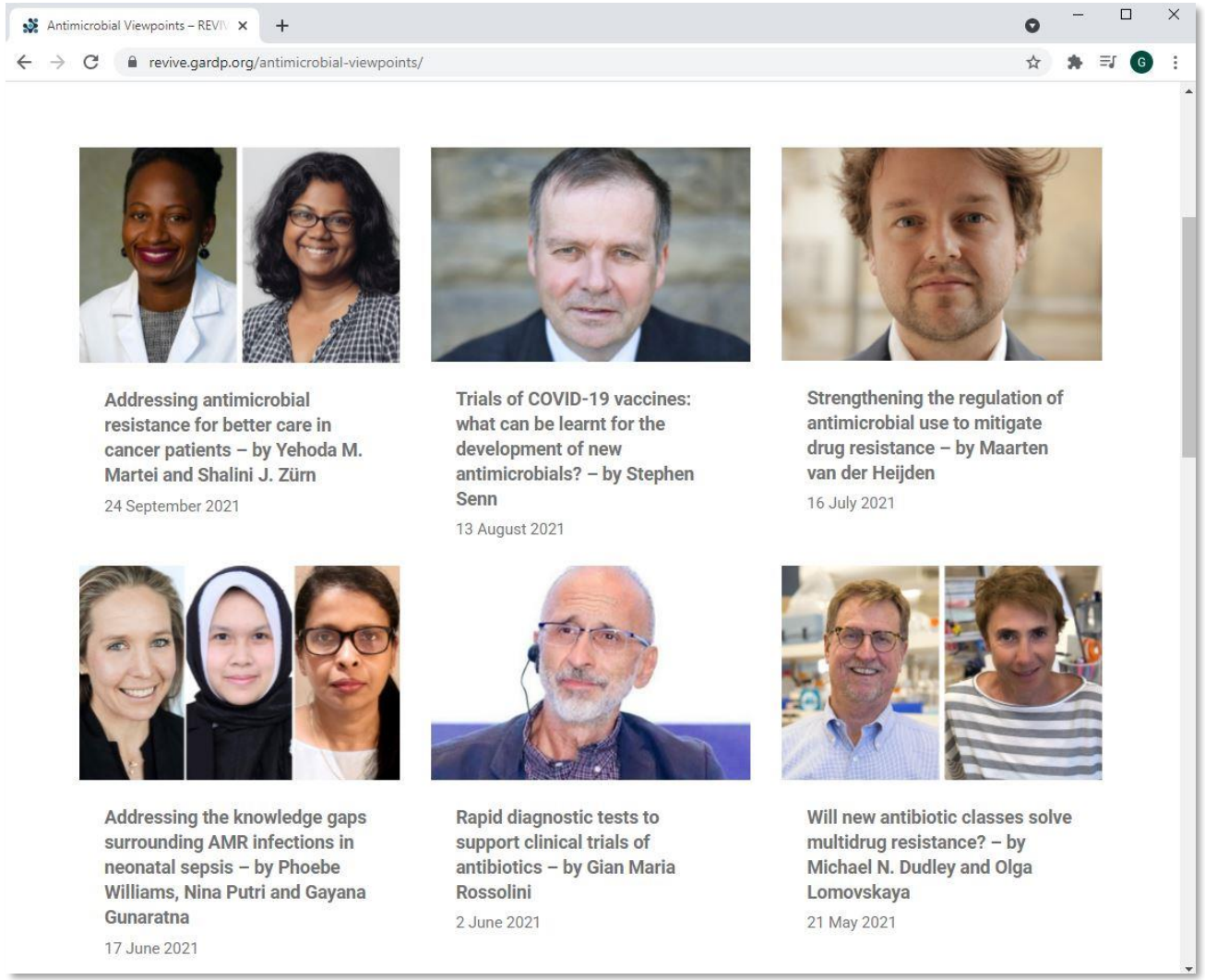

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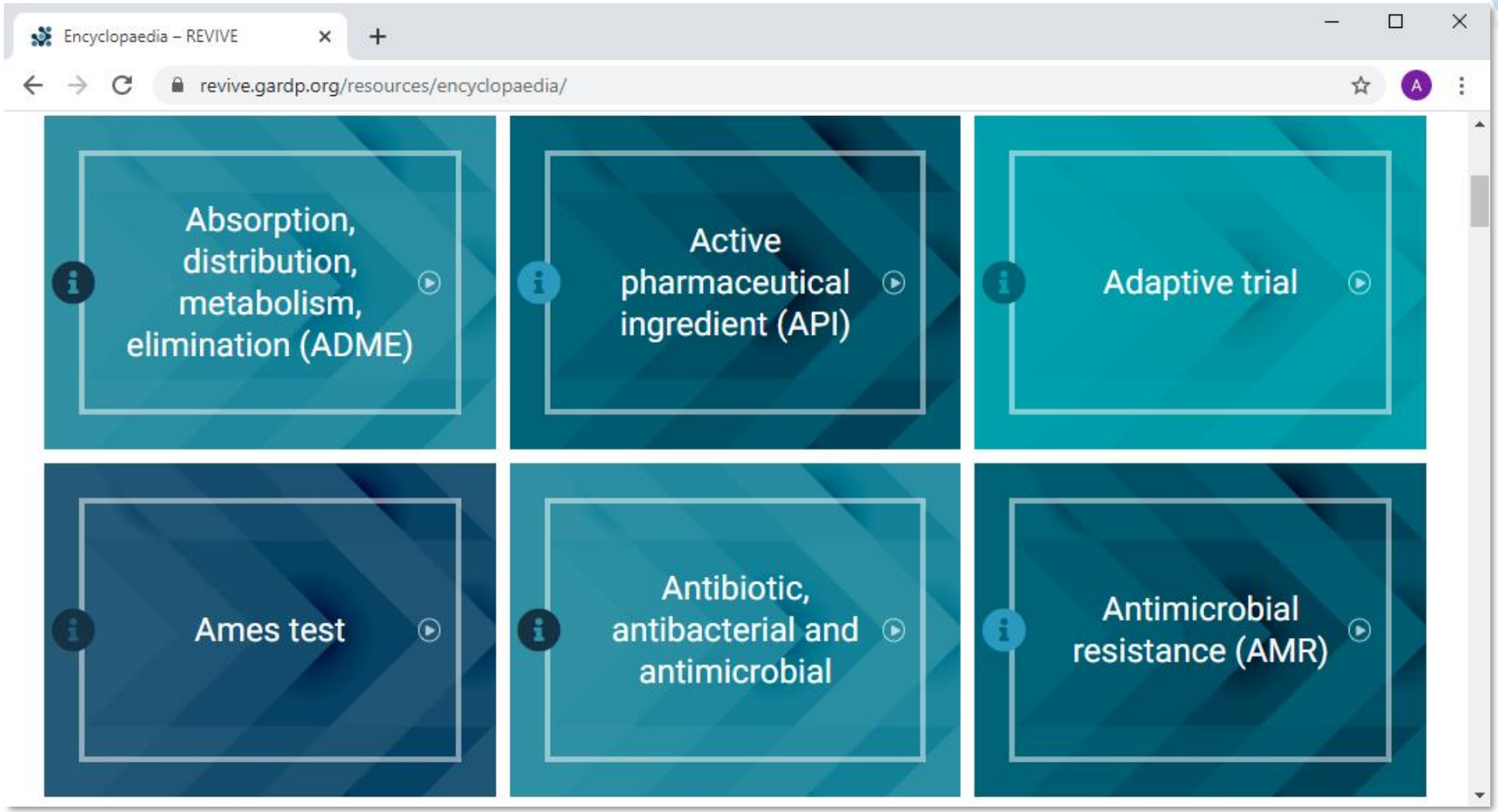
Antimicrobial Encyclopaedia



revive.gardp.org/resources/antimicrobial-encyclopaedia

Today’s speakers

Biofilms: What are they and why do we care?

Mark Webber
Group Leader
Quadram Institute (UK)

Freya Harrison
Principal Investigator
University of Warwick (UK)

Moderator:
Laura Piddock
Scientific Director
GARDP

Mark Webber

Mark Webber has been a group leader at the Quadram Institute in Norwich UK, since the start of 2017. His research group studies the molecular mechanisms of antibiotic resistance with focus on understanding how, where, when and why bacteria evolve antibiotic resistance.

A particular interest of his group is bacterial biofilms and how bacteria adapt to antimicrobial pressure within them. Their work employs a variety of molecular microbiology, functional genomic and bioinformatic approaches to study bacterial survival and resistance mechanisms. Mark has published over 100 articles relating to antimicrobials and has acted as an editor for various journals.

Quadram Institute
Science Health
Food Innovation

Biofilms; the good the bad and the ugly

Mark Webber

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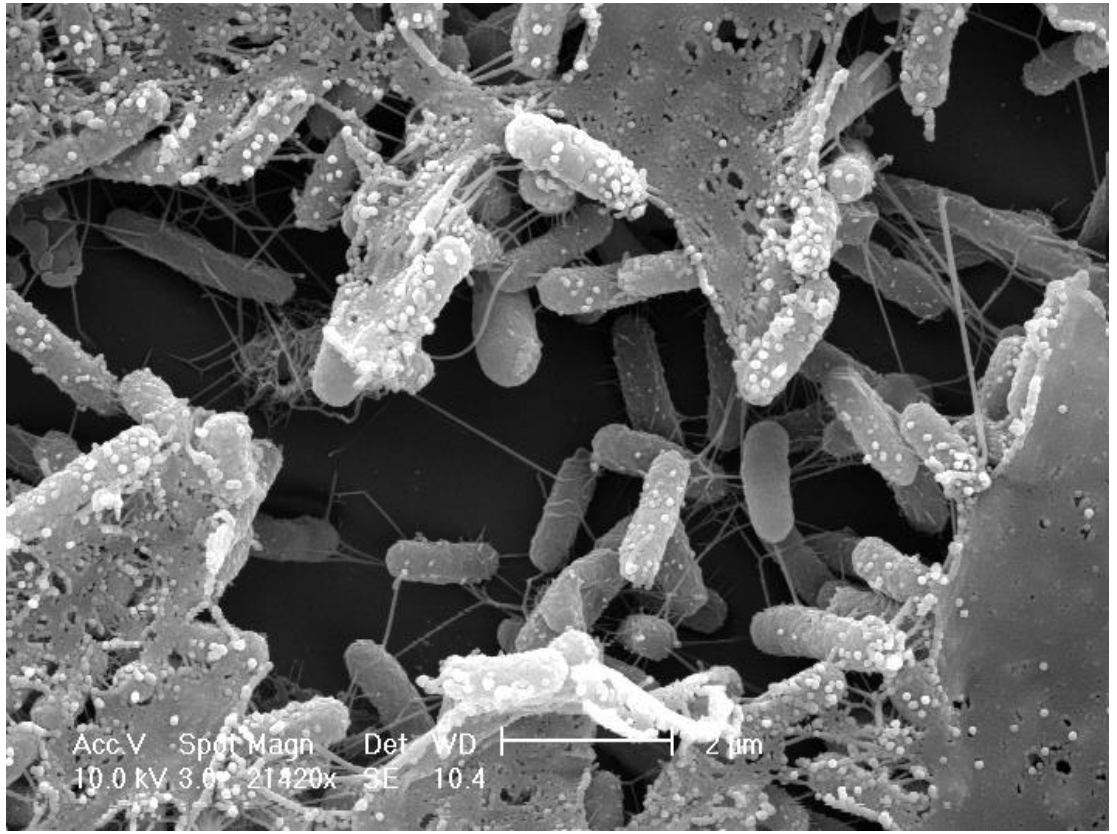
Science Health
Food Innovation

What are biofilms and why should we care about them?

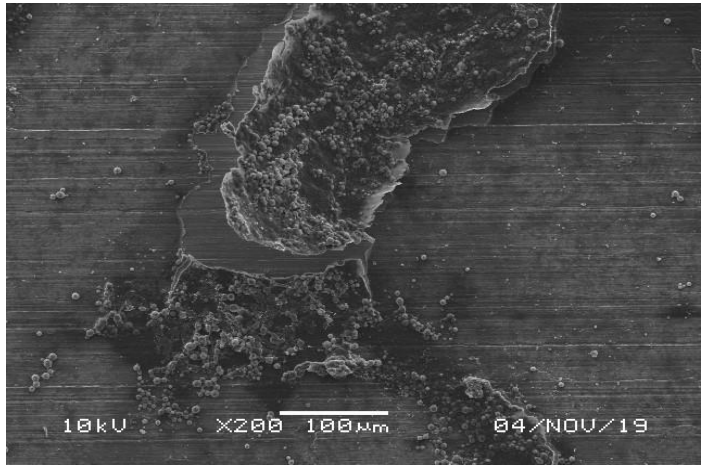
What are biofilms?

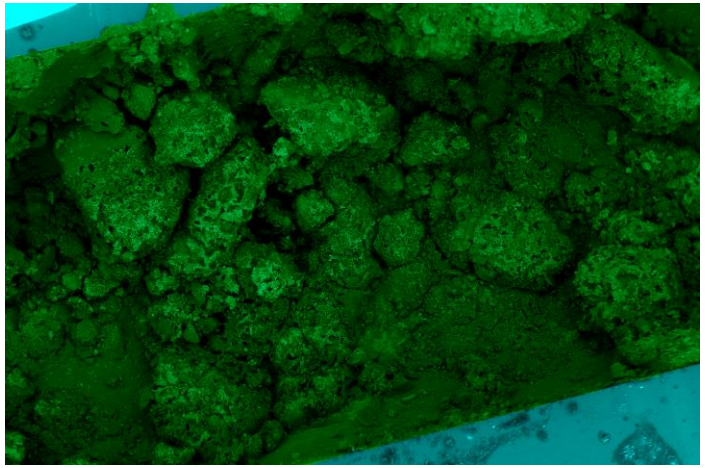
- Bacteria readily form communities of aggregated cells
- Cells forming a biofilm produce an extracellular matrix
- Often multispecies
- Found pretty much everywhere (wet, dry, biotic, abiotic)
- Clinically very important – IPC* focusses on biofilms, and cause *in vivo* and device associated infections
- Also industrially very important
- Significantly different properties compared to cells grown in liquid – a distinct lifestyle
- Lots of heterogeneity within a biofilm in cell behaviour
- **Usually highly tolerant of antibiotics**

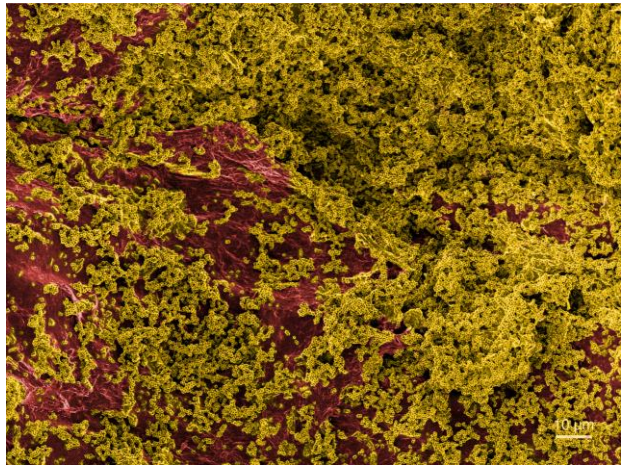


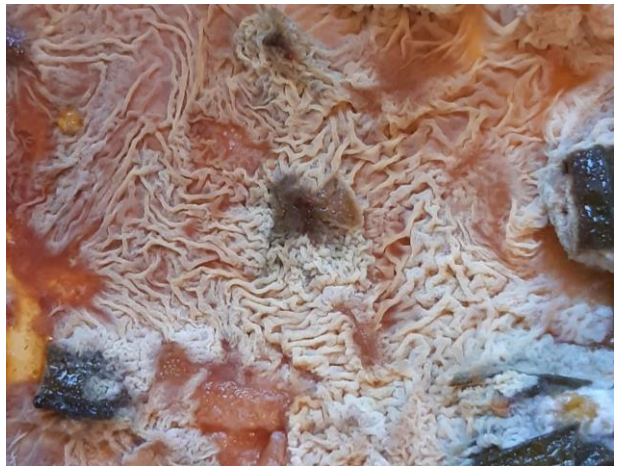
*IPC – Infection, prevention and control

The structure of a biofilm varies with conditions



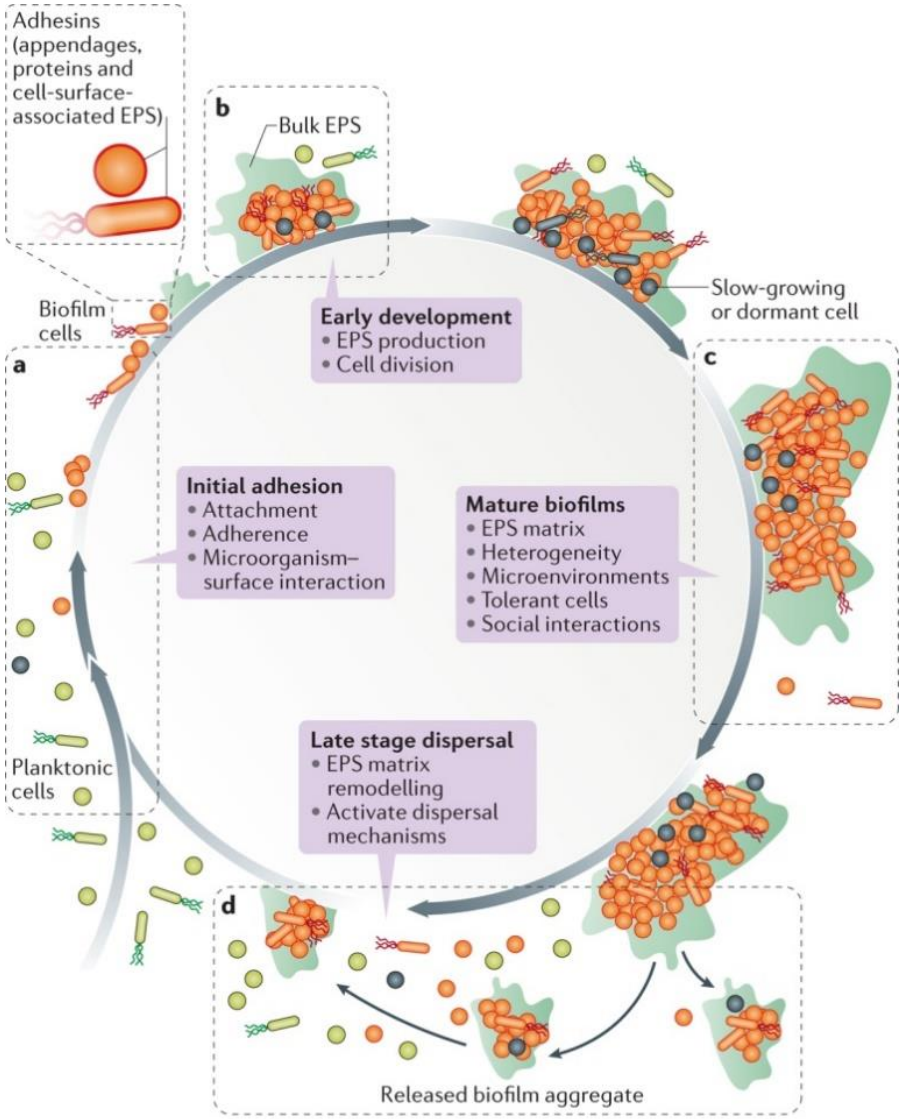






https://www.biofilms.ac.uk/biofilm-image-gallery/

How does a biofilm form?



- A generalised lifestyle describes:
  - initial colonisation of a site
  - commitment to a sessile lifestyle
  - production of biomass and matrix
  - release of cells allows colonisation of new environments

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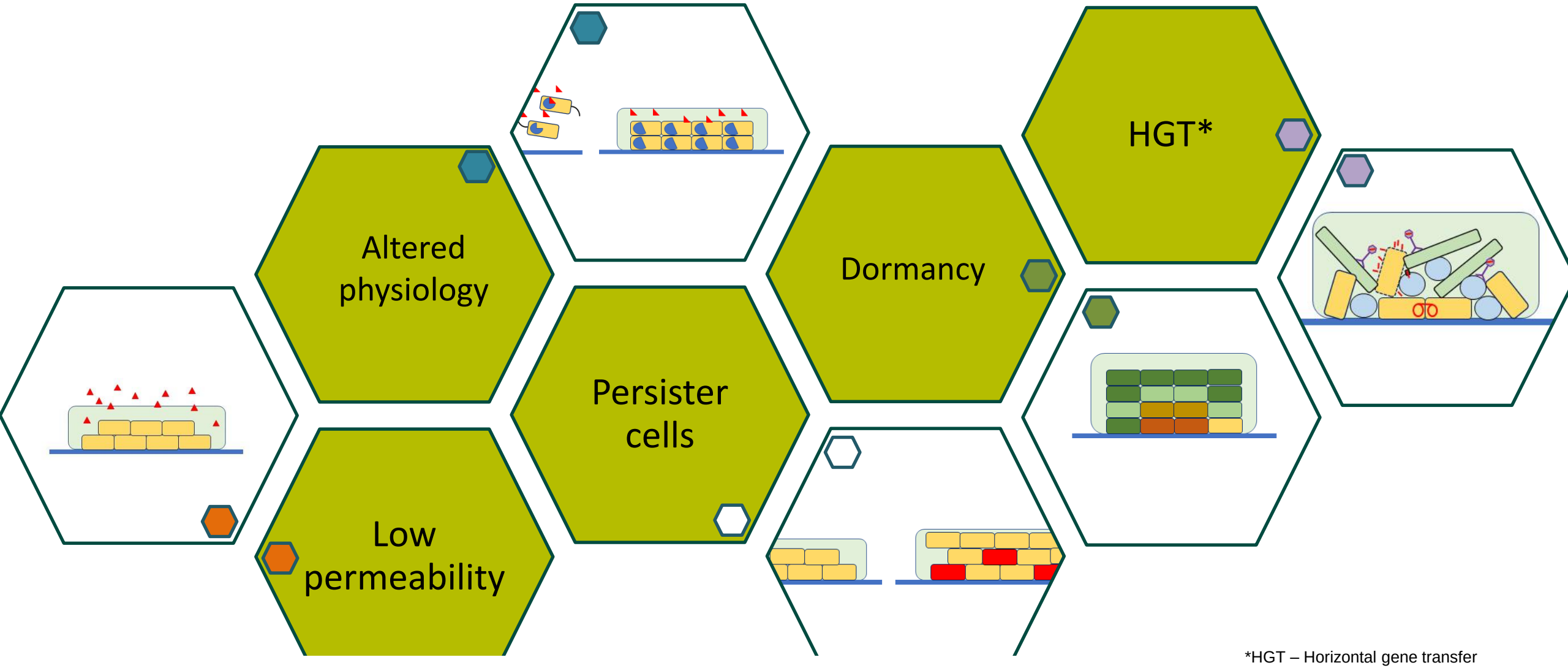
Antimicrobial resistance and biofilms

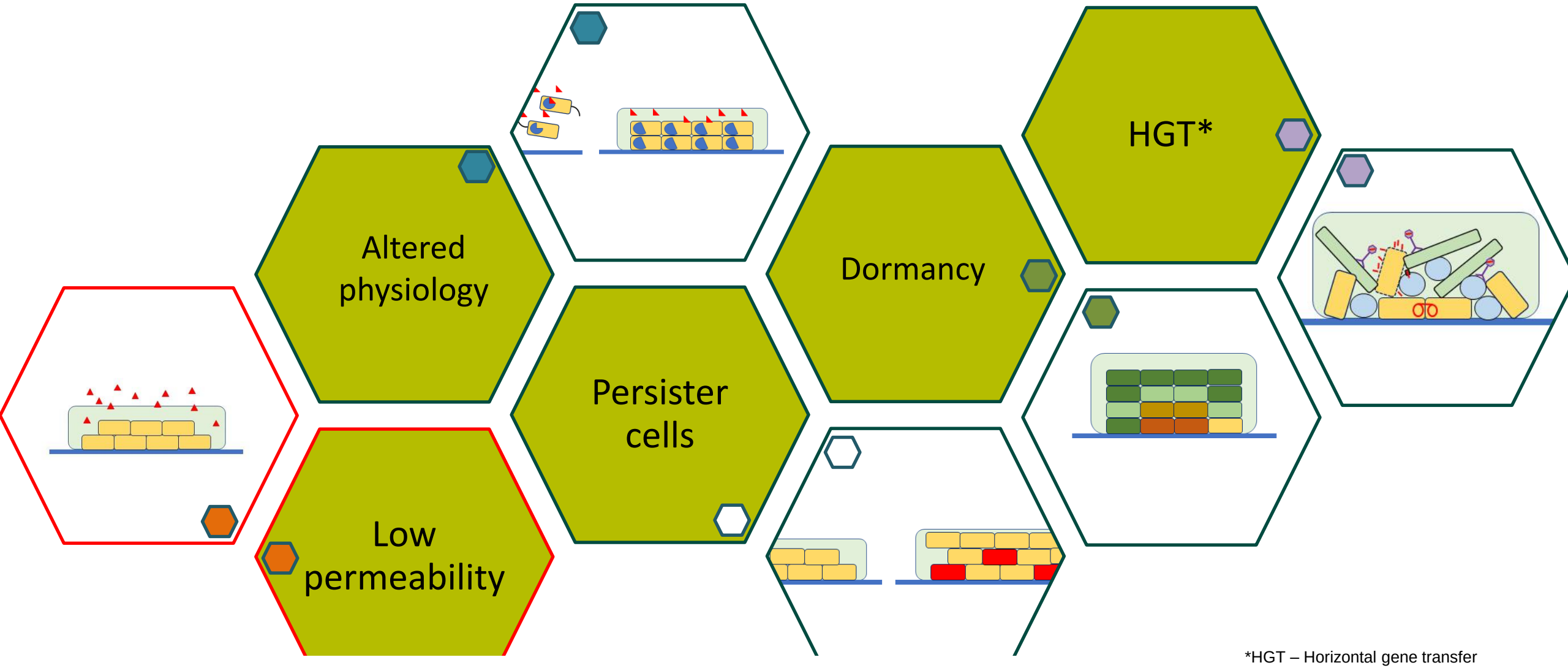
Why are biofilms so hard to kill with antibiotics?



*HGT – Horizontal gene transfer

A combination of mechanisms are commonly relevant.
Environmental and bacterial cues (quorum sensing etc) affect all these mechanisms

Why are biofilms so hard to kill with antibiotics?



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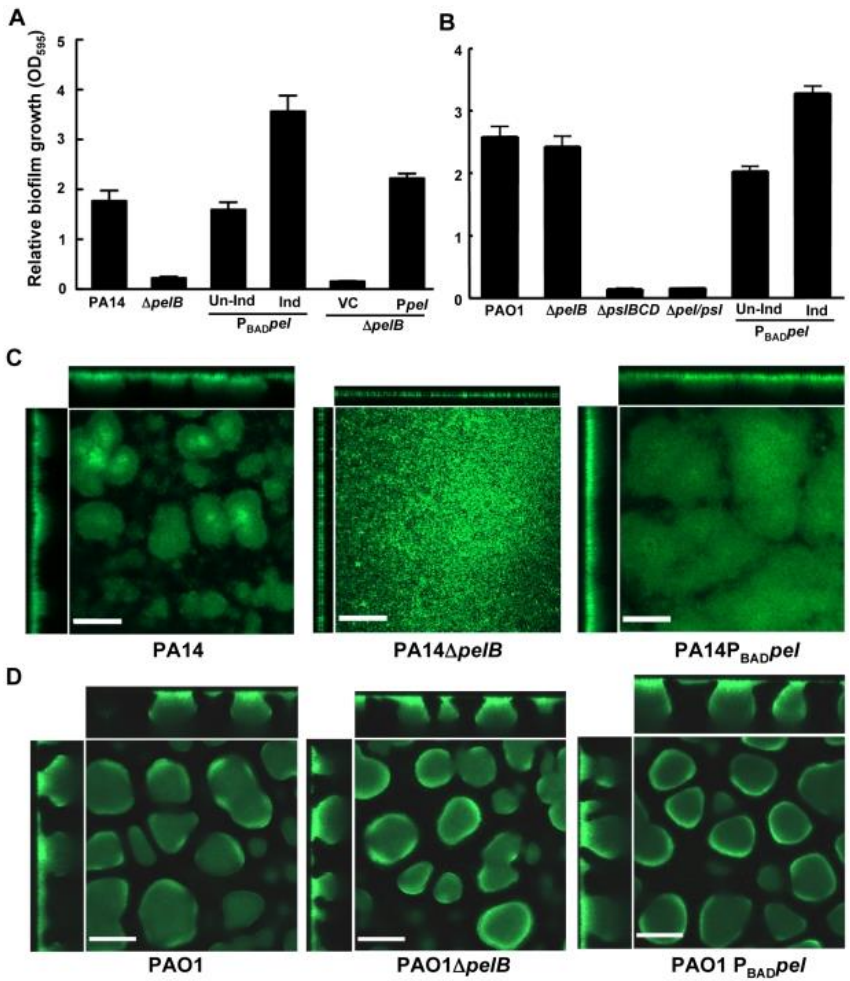
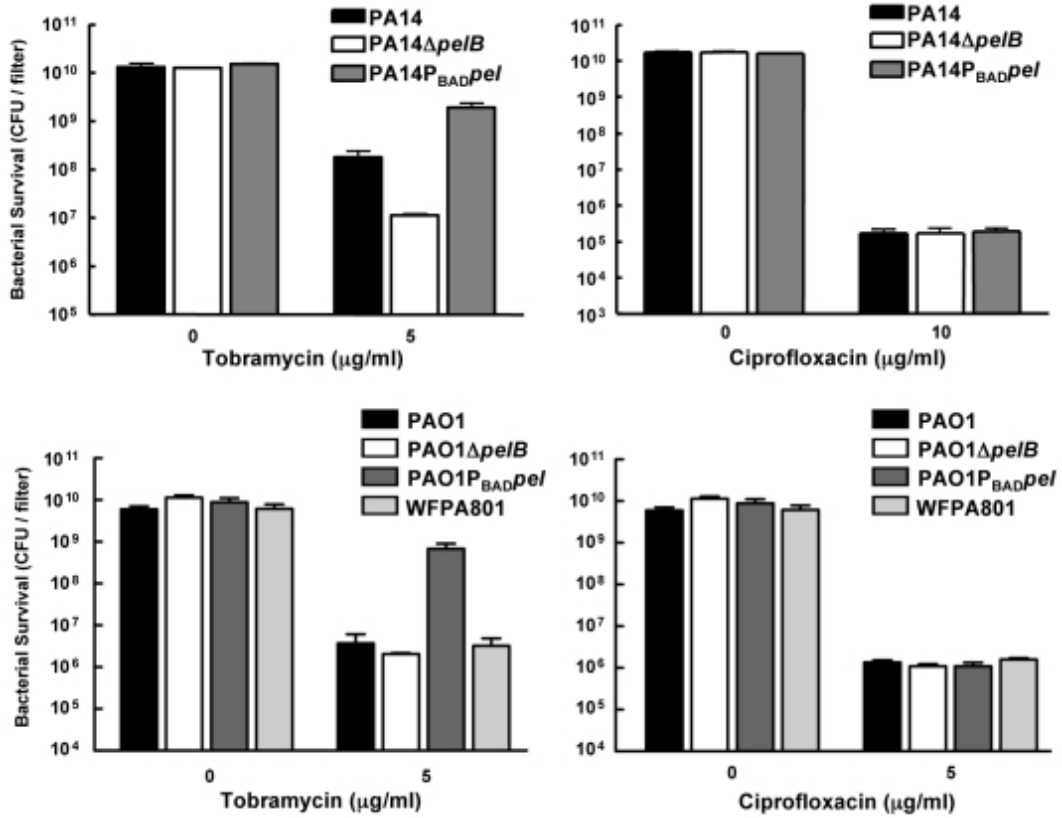
Matrix matters, but not for all drugs…..





Colvin *et al*., PLoS Pathog. 2011 Jan; 7(1): e1001264.

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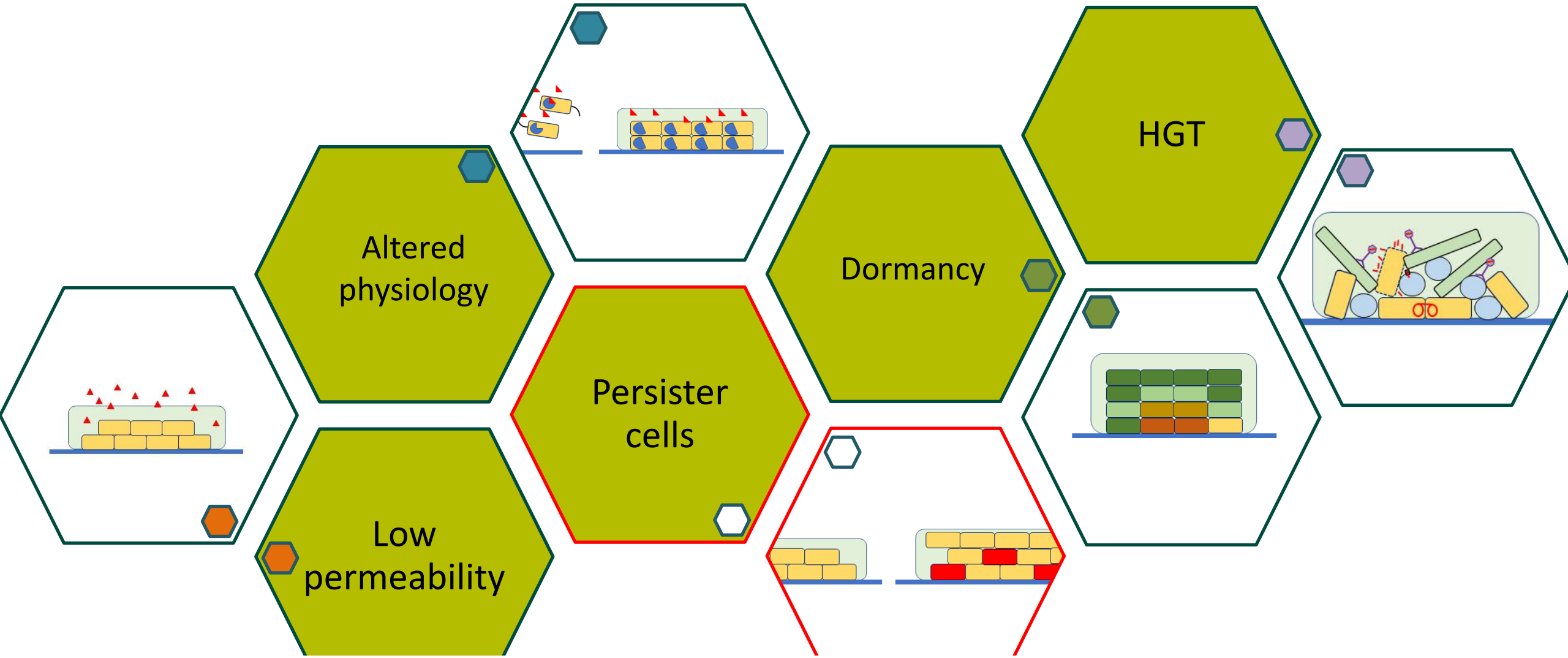

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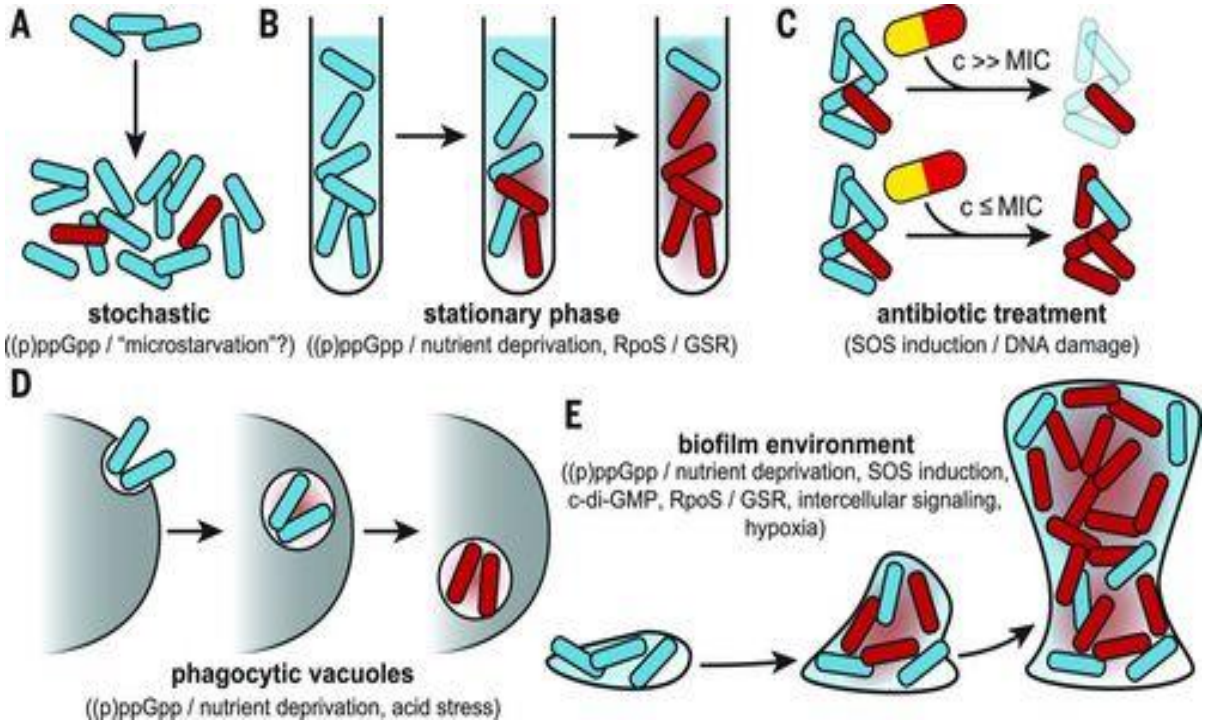
The challenge of persister cells



- Bacterial populations are heterogenous and contain persister cells
- Biofilms have particularly high fractions of persister cells
- These are often insensitive to a wide range of antibiotics

Harms *et al.*, 2016. Mechanisms of bacterial persistence during stress and antibiotic exposure, Volume: 354, Issue: 6318, DOI: (10.1126/science.aaf4268)

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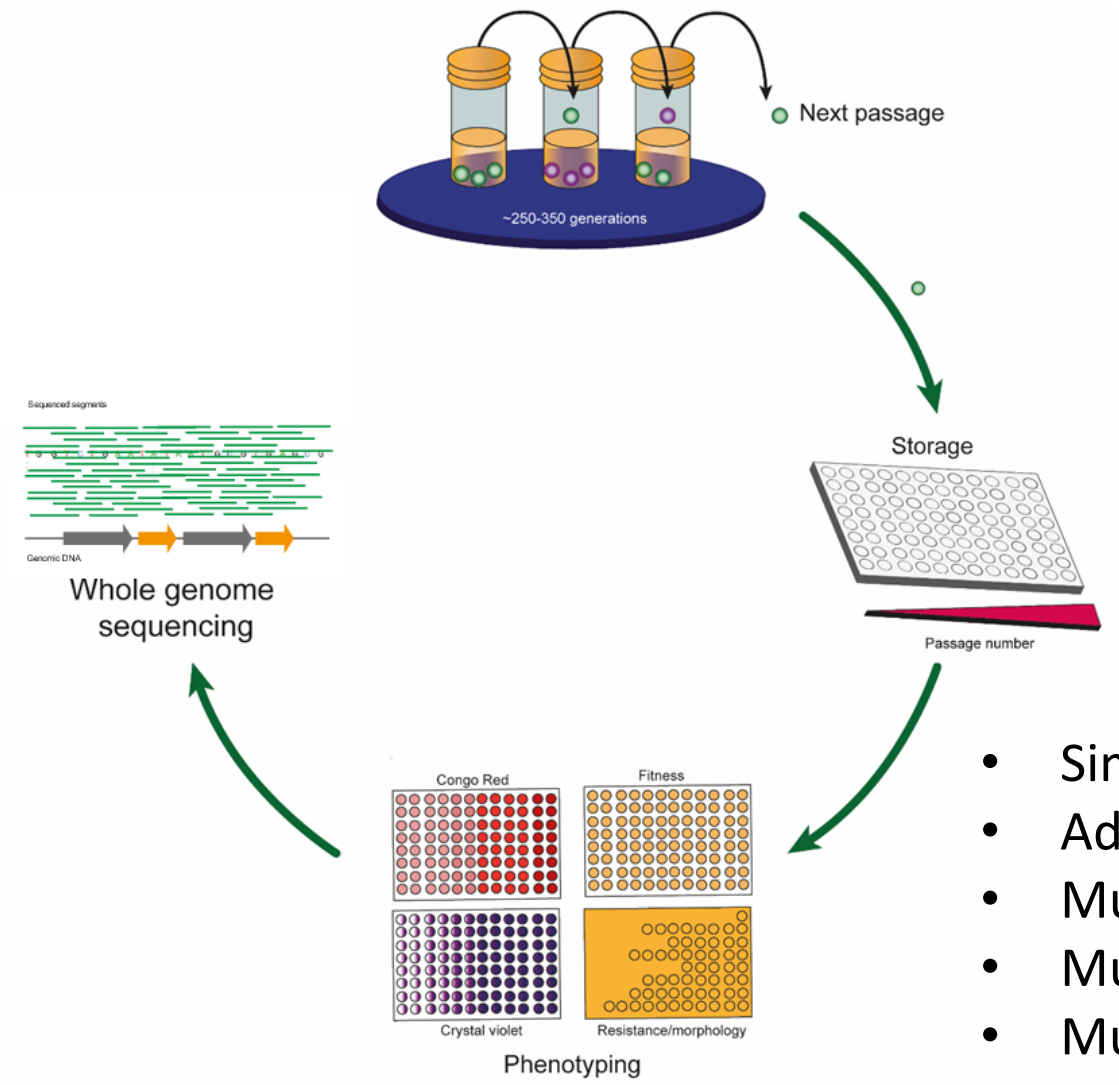
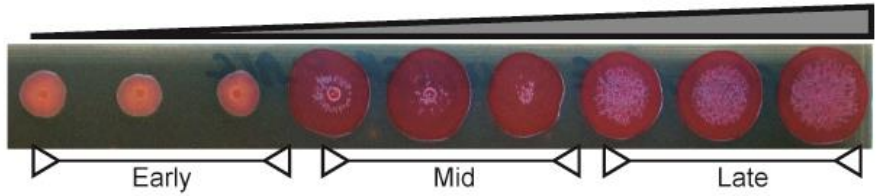
Do biofilms care about low levels of antibiotics?

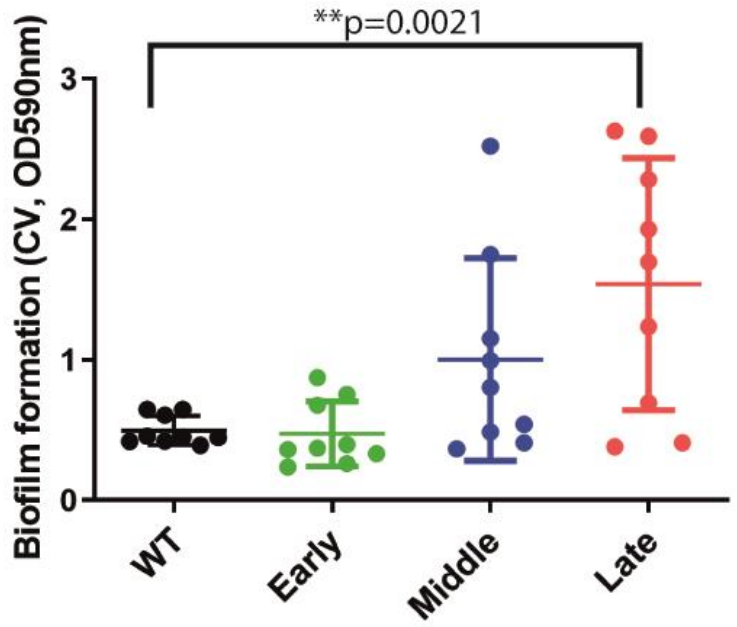
Biofilm evolution model



- Simple
- Adaptable
- Multiple lineages
- Multiple organisms
- Multiple stressors

In the absence of drug the model rapidly selects for increased biomass production

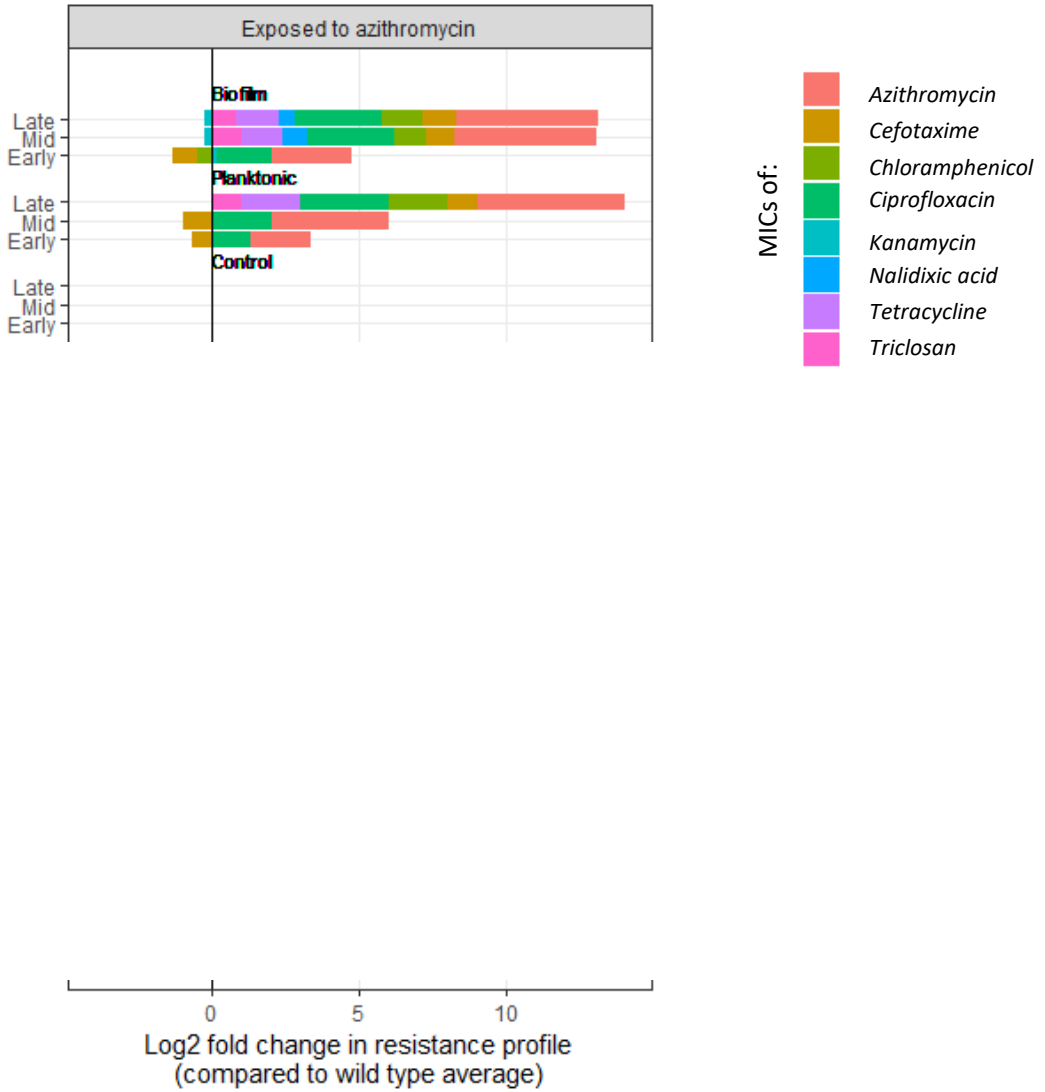




(Trampari *et al.* npj Biofilms and Microbiome 2021)

Biofilms rapidly evolve resistance

- *Salmonella* biofilms exposed to *sub-lethal* cefotaxime, azithromycin or ciprofloxacin
- Resistance emerged in all cases
- Patterns were similar to planktonic controls although there were differences in rates and cross resistance



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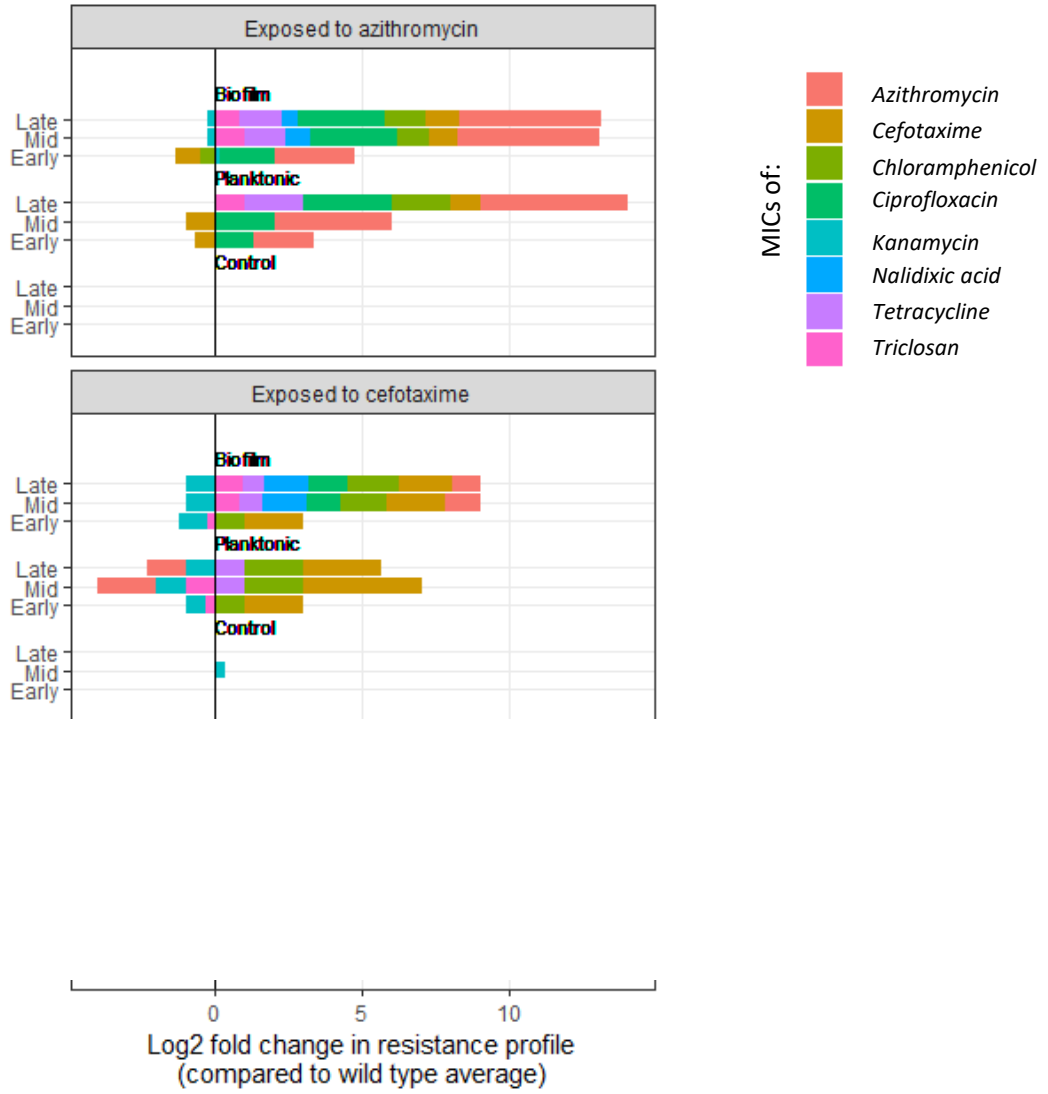

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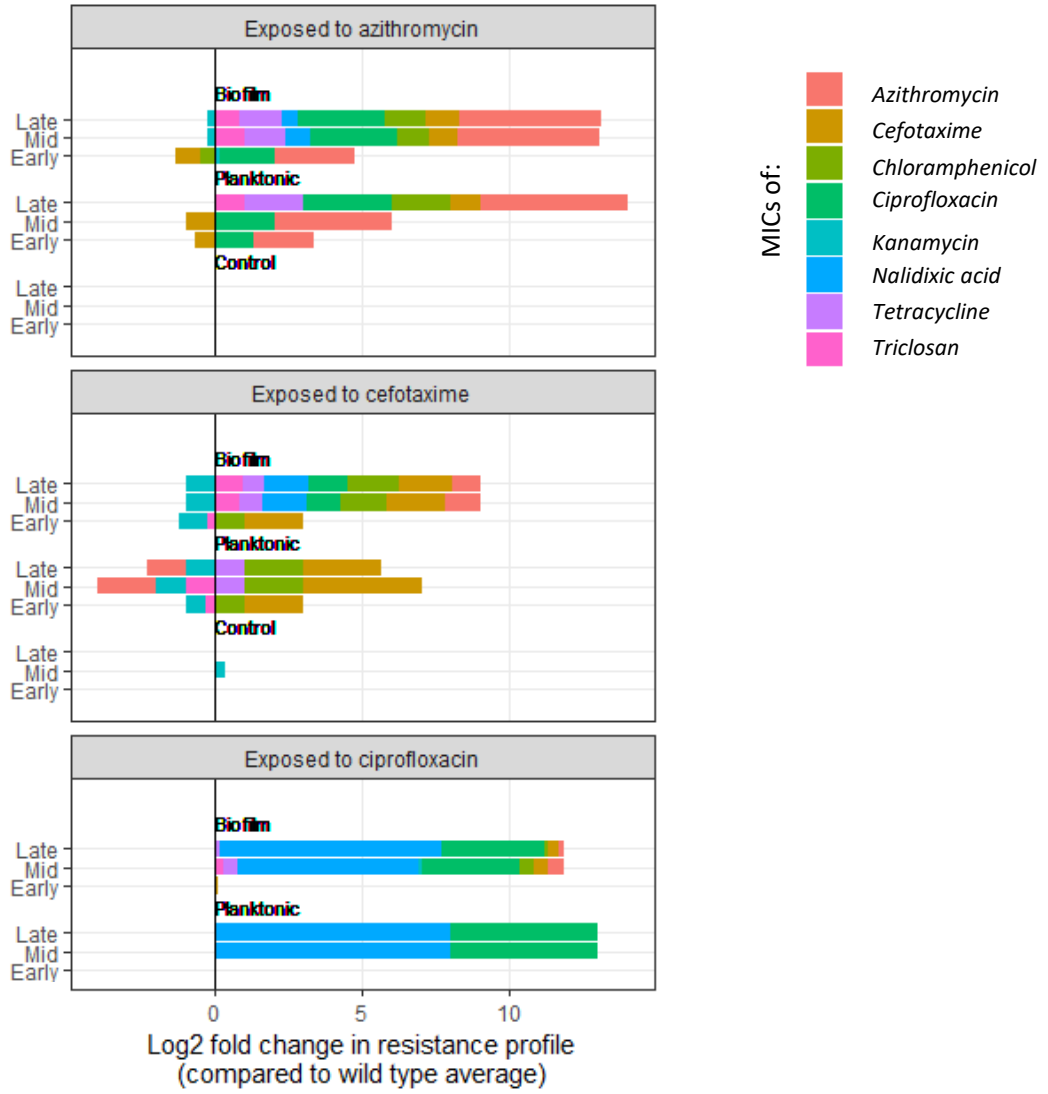
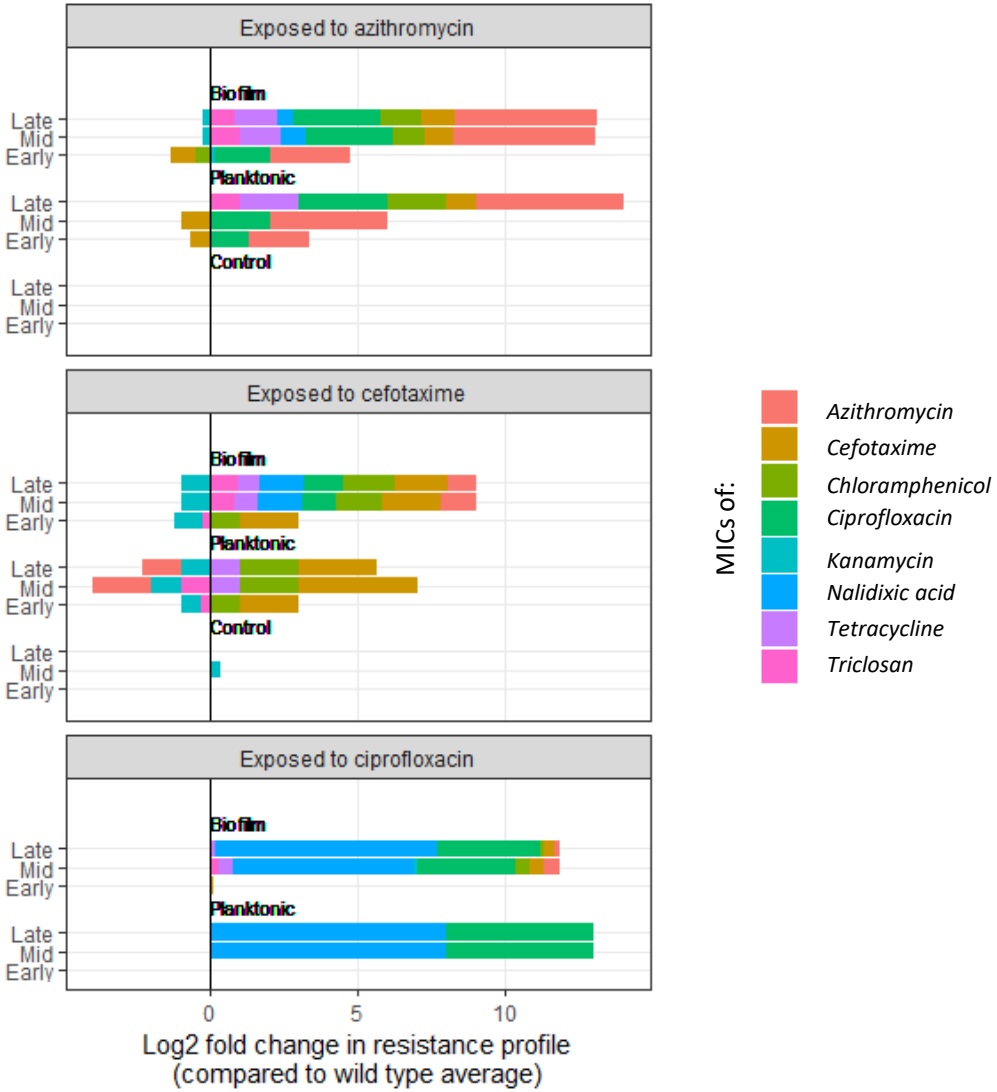


Biofilms rapidly adapt to antibiotics



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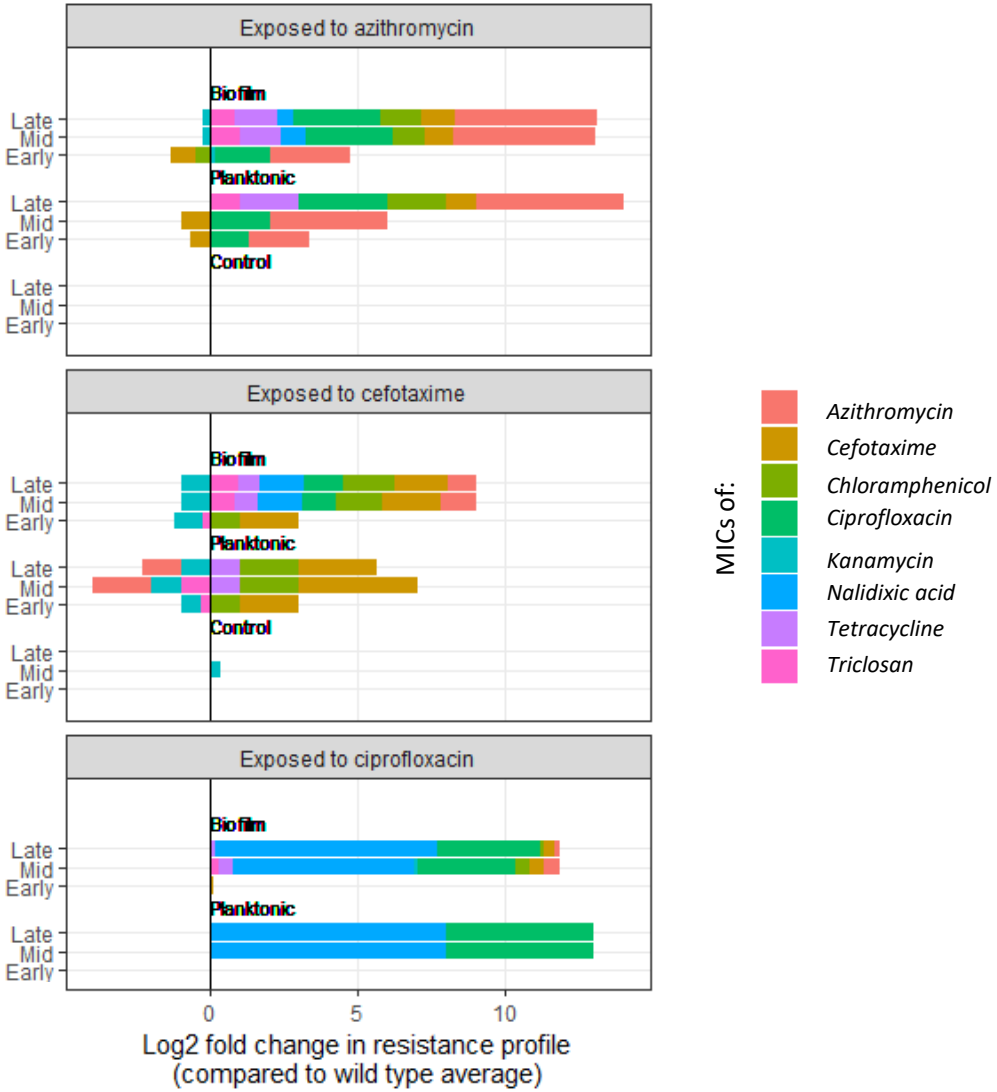
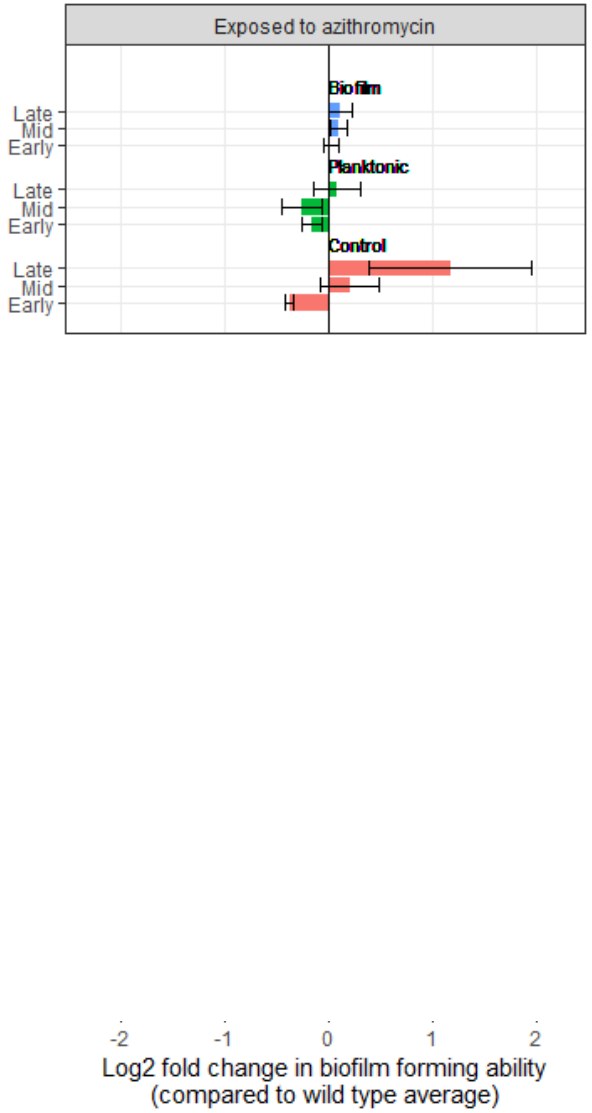


BUT….. Resistance comes at a cost to biofilm formation



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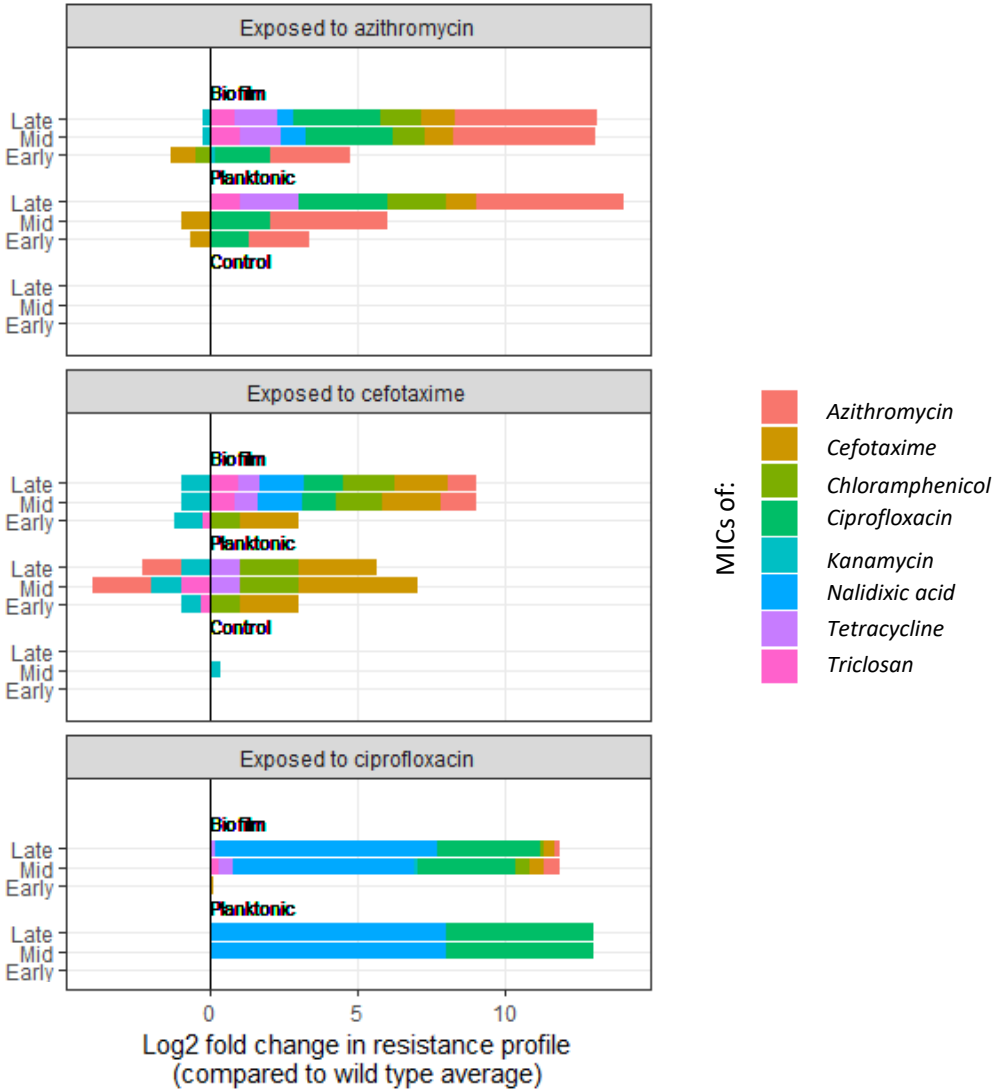

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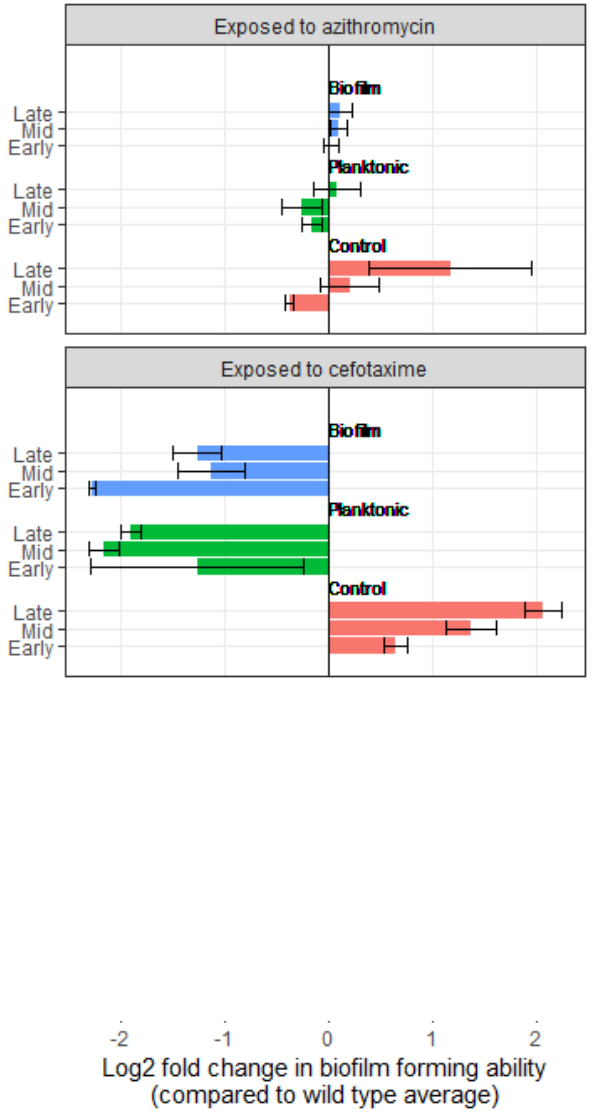

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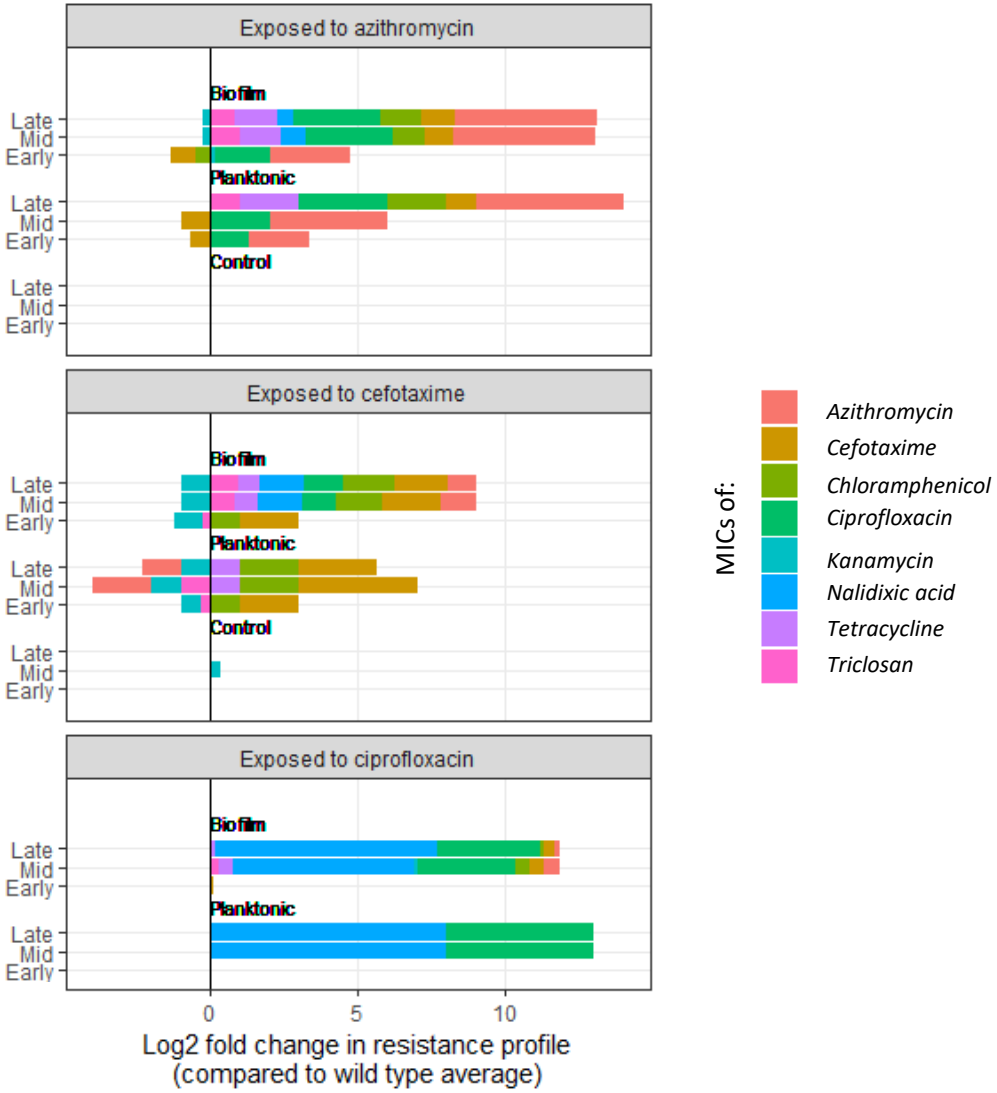

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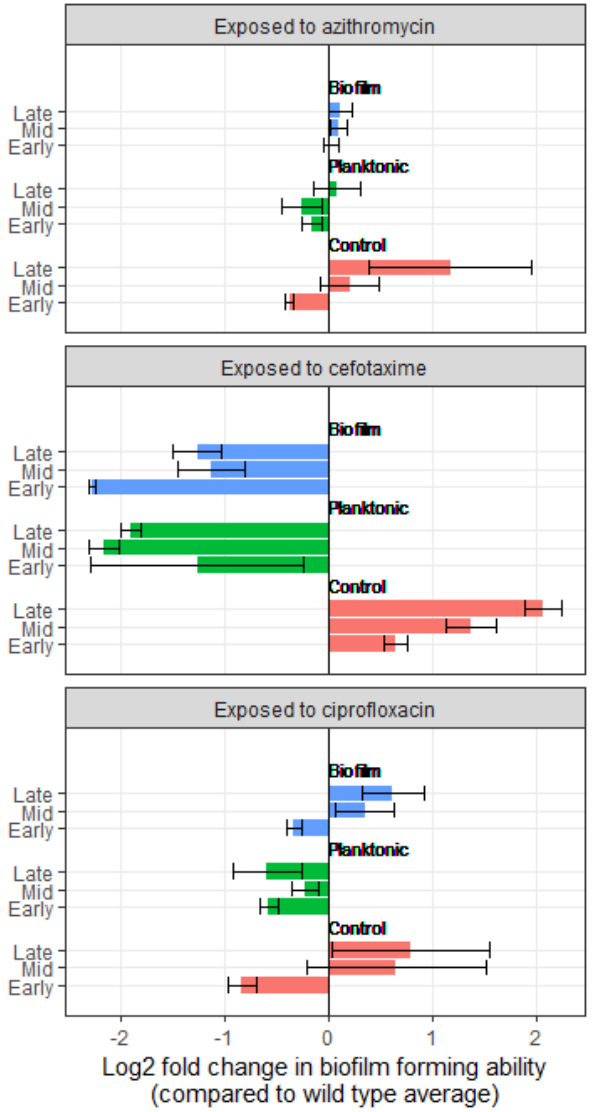
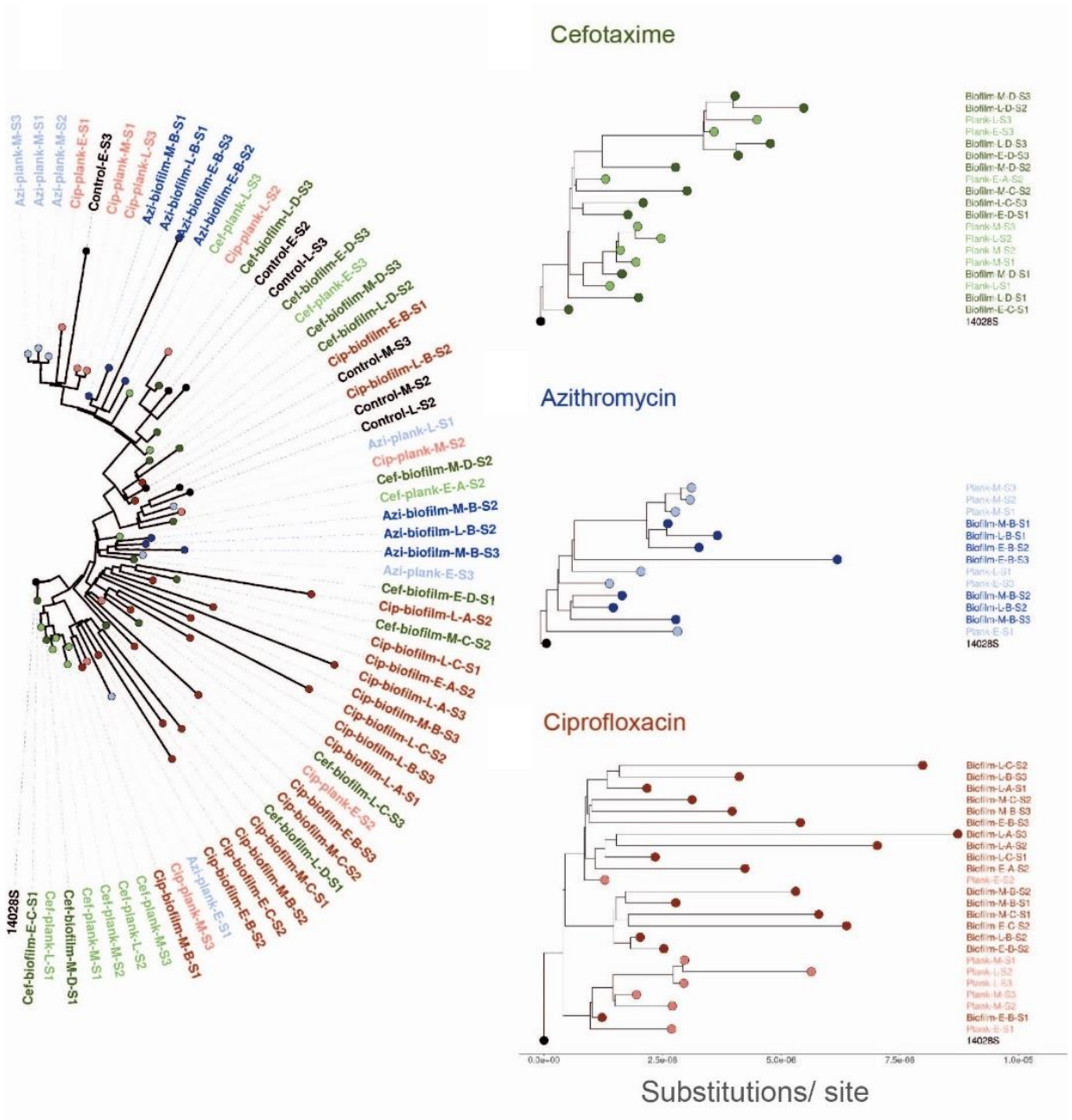
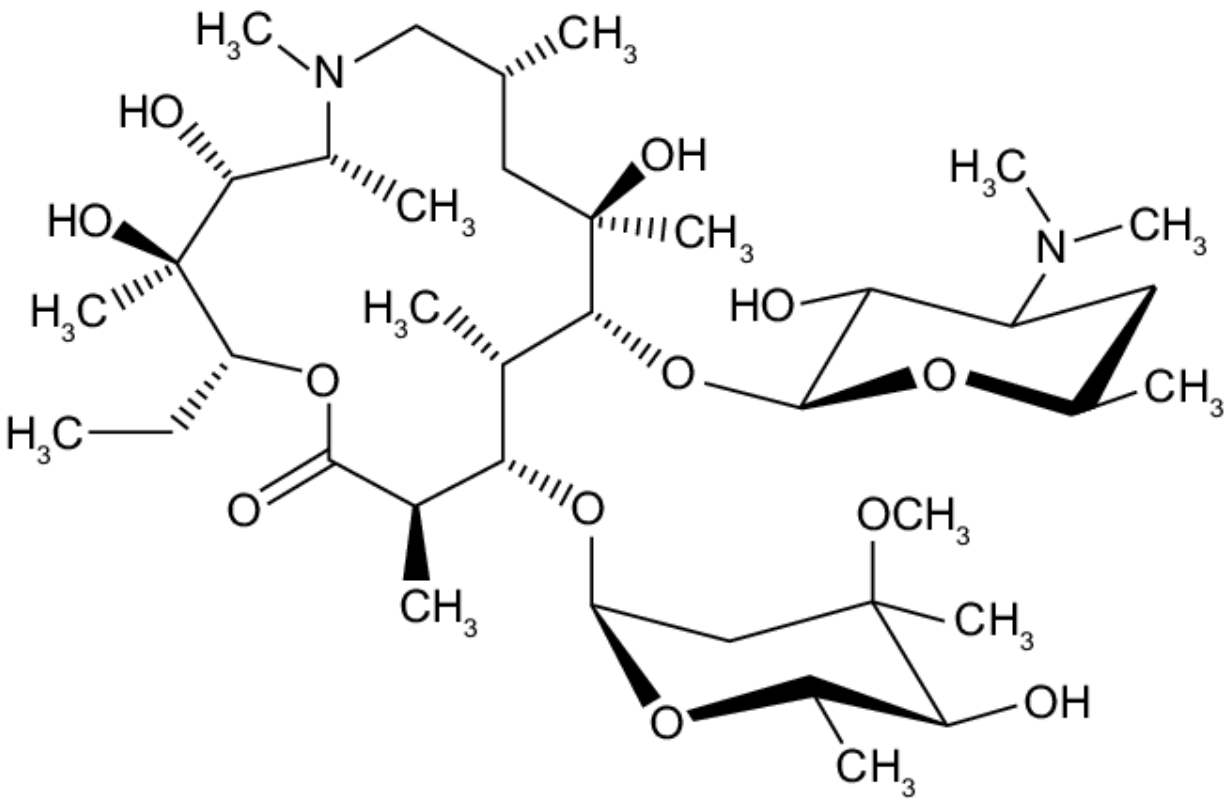




Analysis of *Salmonella* mutants:

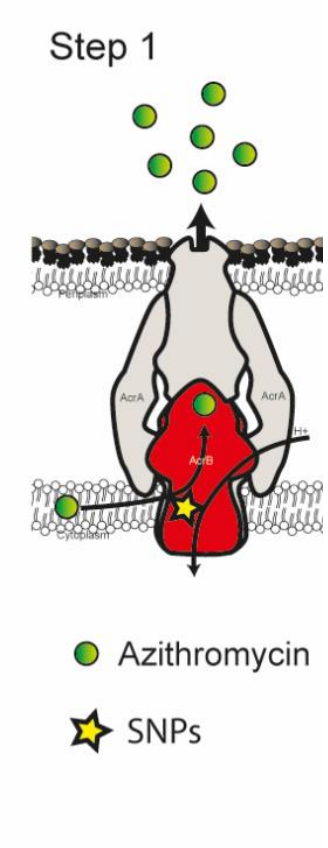
- Each drug selected distinct mutations
- Biofilm and planktonic lineages segregate
- Similar numbers of mutations for each drug
- No ‘universal’ mechanism of resistance was seen
- But some targets repeatedly seen: ***acrB, ramR, envZ***

Azithromycin as an example



- Macrolide antibiotic
- Good activity
- Stops protein synthesis
- Has to get into the cell to be active
- Important for treatment of *Salmonella*

Novel mechanism of azithromycin resistance



(Trampari *et al*. *in revision*)

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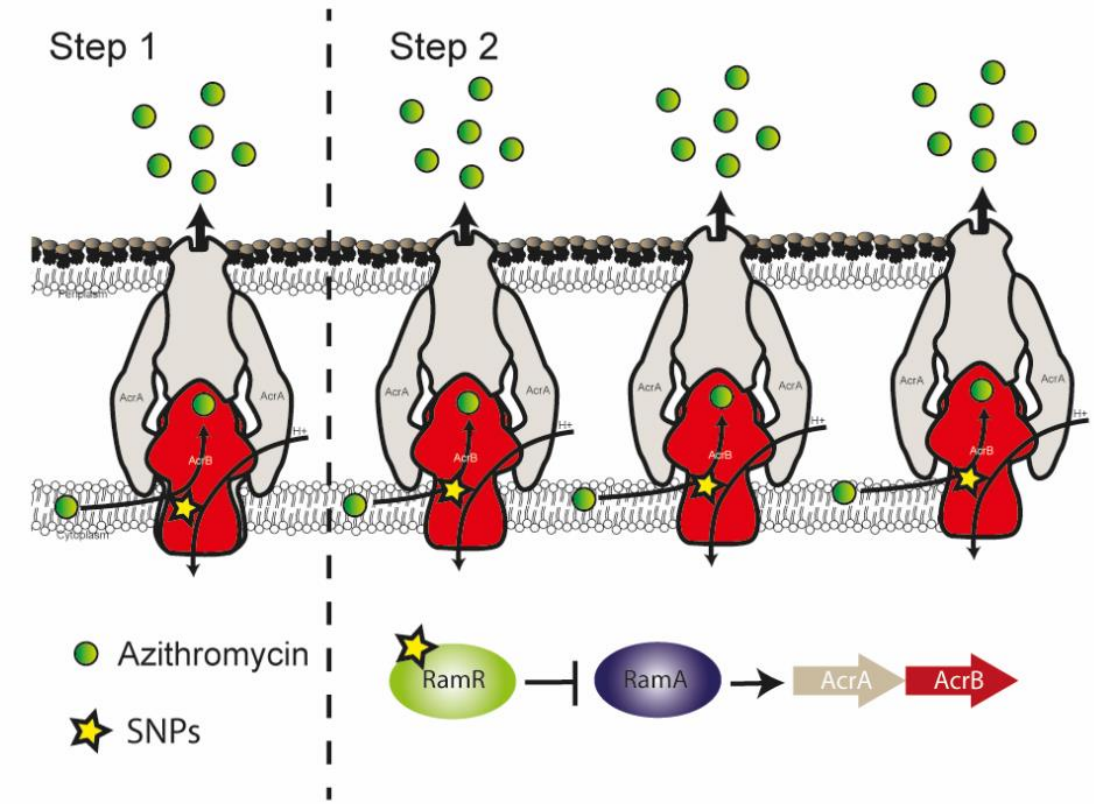

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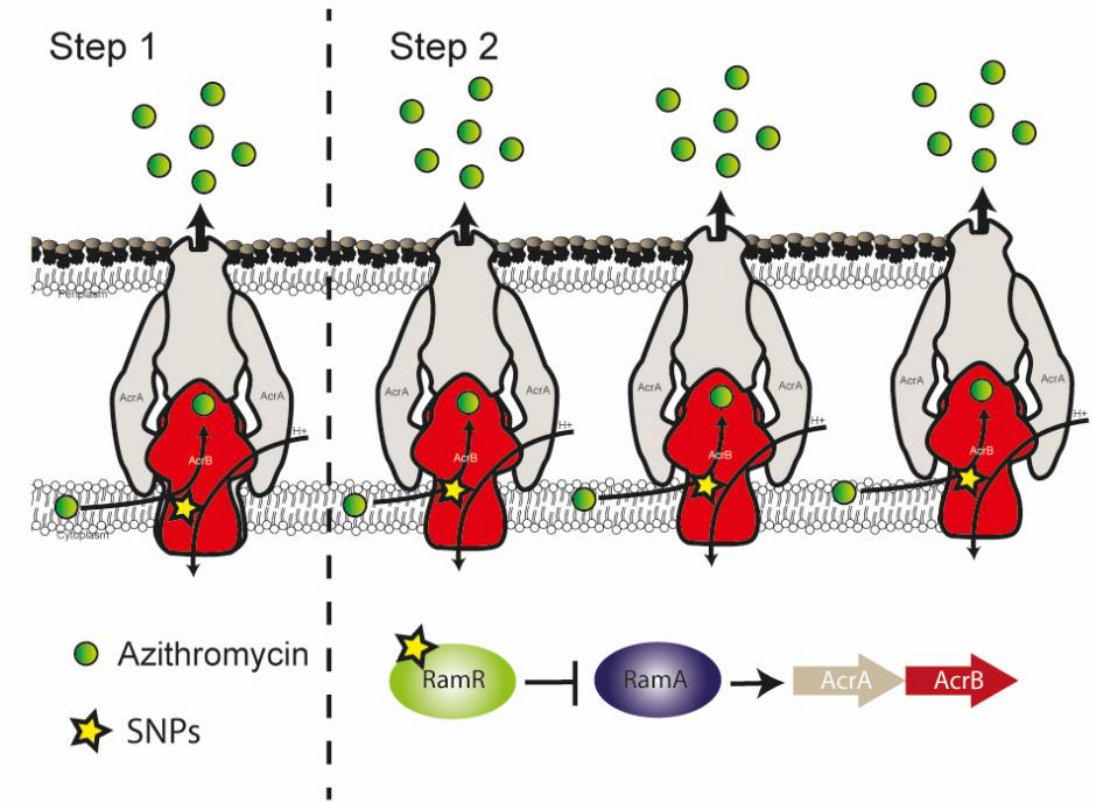
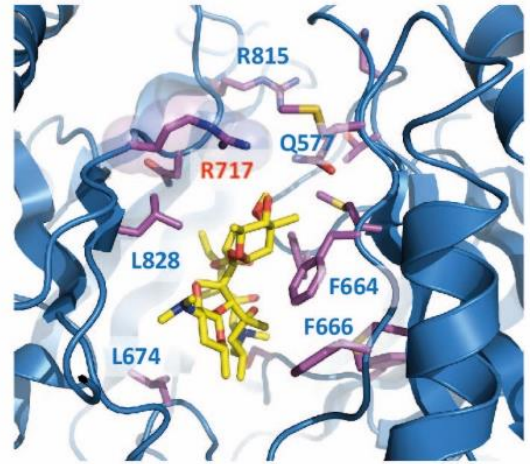


R717 WT- Azithromycin in macrolide site A (proximal pocket)



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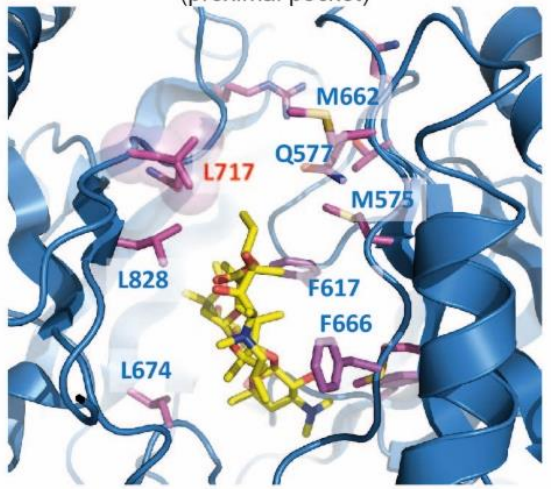
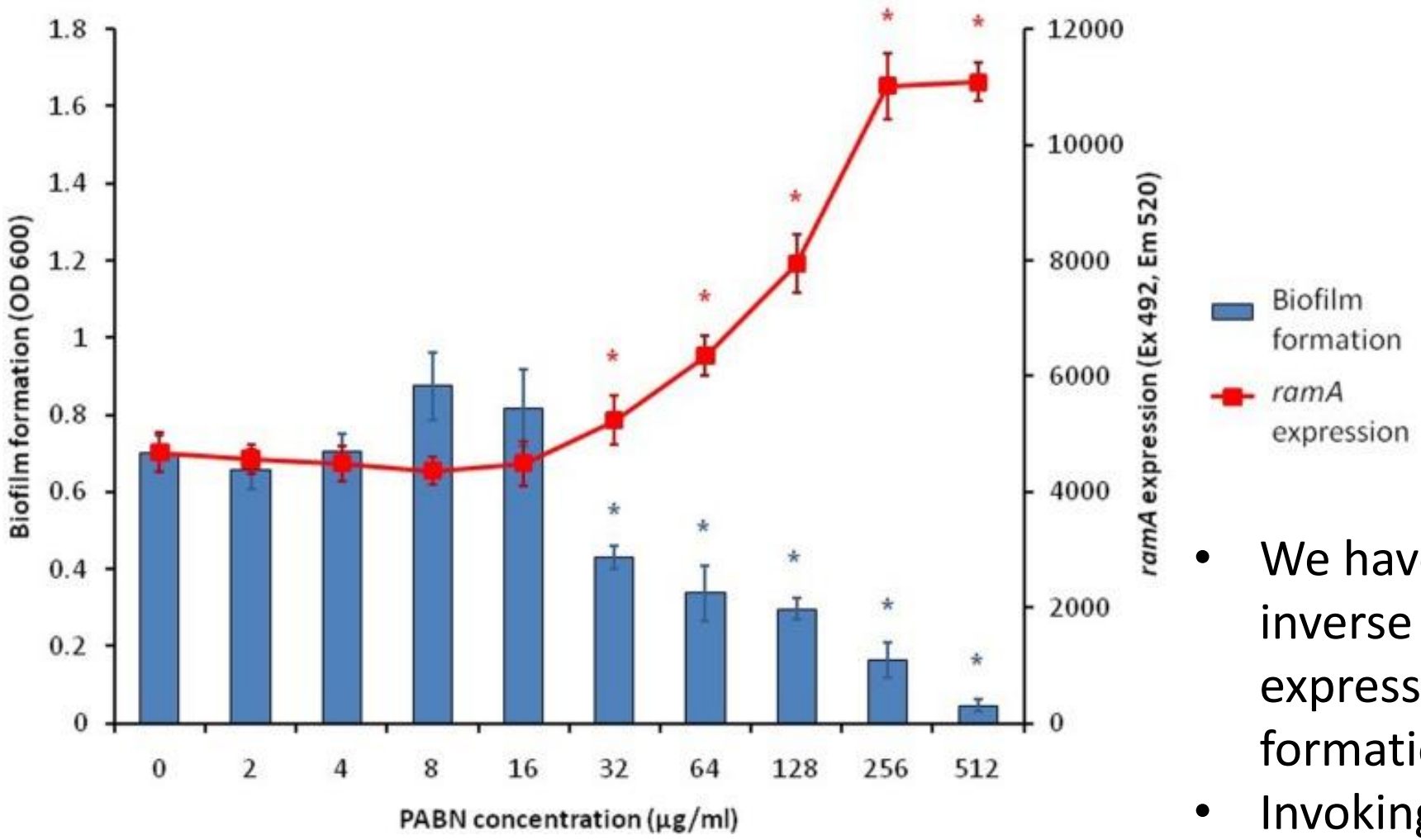


(Trampari *et al. in revision*)

Why does developing resistance impair biofilm?



- We have previously seen an inverse relationship between expression of *ramA* and biofilm formation.
- Invoking *ramA* to gain resistance has a cost to biofilm.

Baugh *et al.*, 2014, Holden *et al.*, 2020

Conclusions

Biofilms are everywhere!

Biofilms matter!

Biofilms are drug resistant as a result of multiple factors

Biofilms do care about low concentrations of drugs

There is no universal mechanism of AMR in biofilms

Understanding fundamental biology is important in developing ways to control biofilms

Acknowledgments

UKRI Biotechnology and Biological Sciences Research Council

gama healthcare

Unilever

Nestlé

P&G

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Freya Harrison

Freya Harrison is a Principal Investigator in the School of Life Sciences at the University of Warwick, UK with an interest in how the development of biofilm infection is influenced by interactions between infecting bacteria, and between bacteria and their environment.

Freya's research group builds and employs high-validity models of biofilm infections to understand how specific host environments can alter bacterial physiology and result in highly antibiotic-resistant phenotypes. Her group also uses bespoke models to test the activity of new antibacterial agents, including natural products derived from historical infection remedies. This ethnopharmacological approach to antimicrobial drug discovery is fueled by collaborations that bridge the traditional sciences and humanities divide.

Building & using models of lung and wound biofilms

Dr Freya Harrison

THE UNIVERSITY OF WARWICK

Harrison Lab @ Warwick

Lab alumna

Dr Blessing Anonye, University of Central Lancashire

Funders

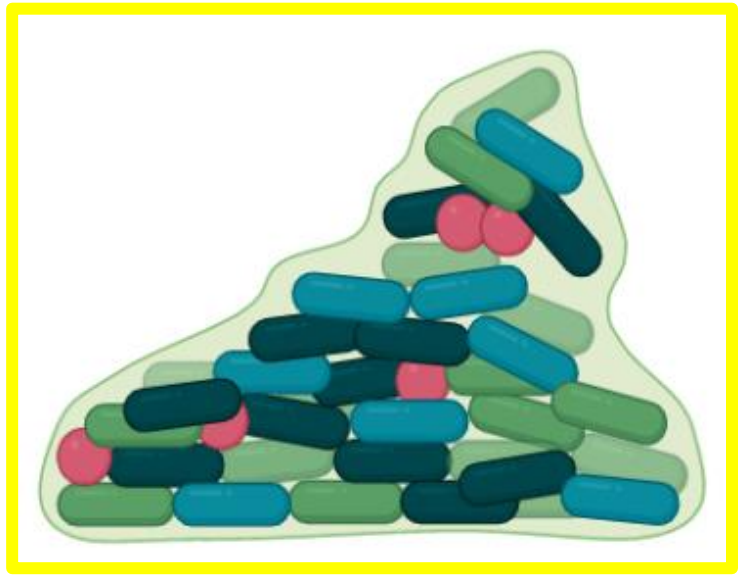
UKRI Medical Research Council

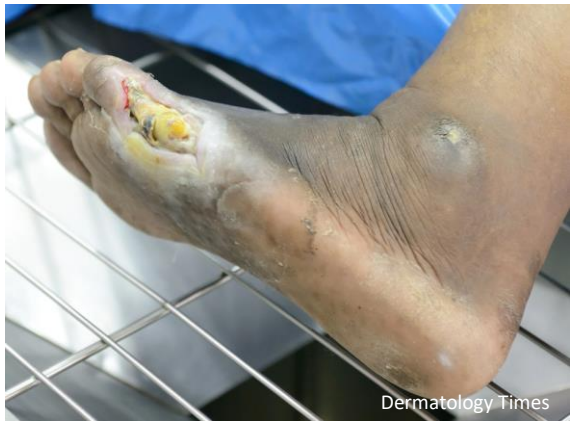
DIABETES UK
KNOW DIABETES. FIGHT DIABETES.

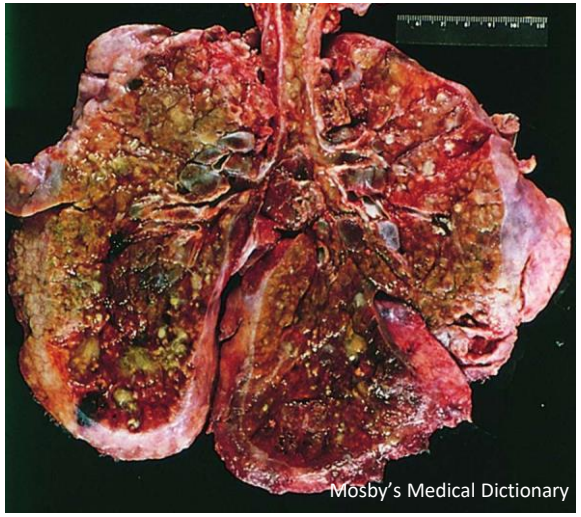
National Biofilms Innovation Centre

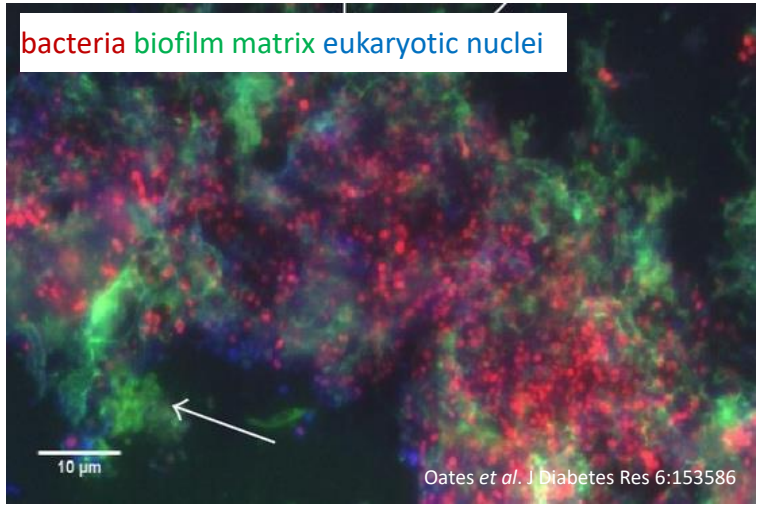
UKRI Biotechnology and Biological Sciences Research Council

Biofilm in cystic fibrosis (CF) lungs & diabetic foot infections (DFI)







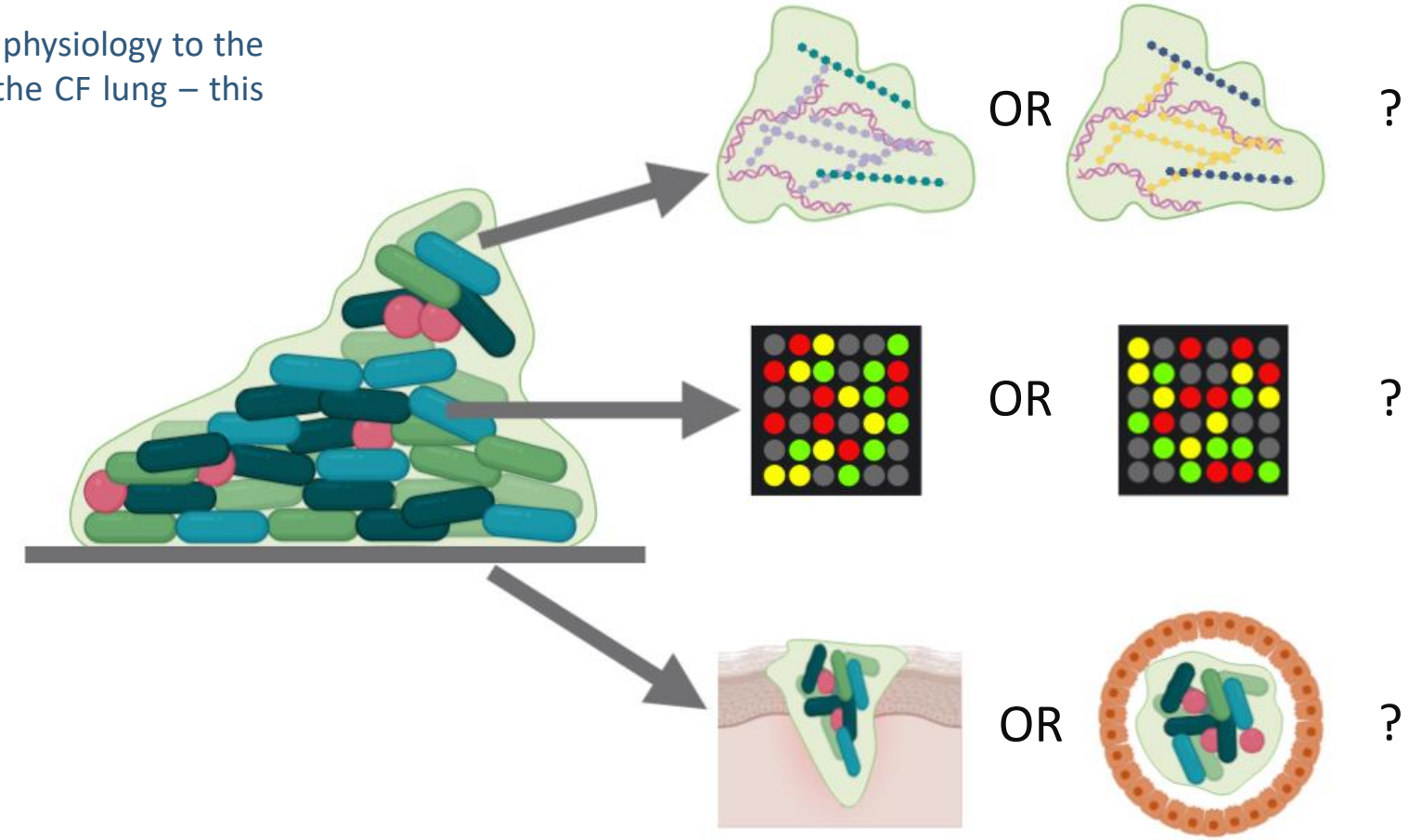


Biofilm in CF & DFI: similarities

- Normal defence / clearance mechanisms are compromised
- Site of infection is biochemically abnormal
- Infection lasts for months, year or even decades, despite antibiotic treatment
- Even if isolated bacteria from swabs etc. are susceptible in standard *in vitro* tests
- Some pathogens in common (ESKAPE), often biodiverse
- Huge health and economic burden
- New biofilm-busting therapies desperately needed!

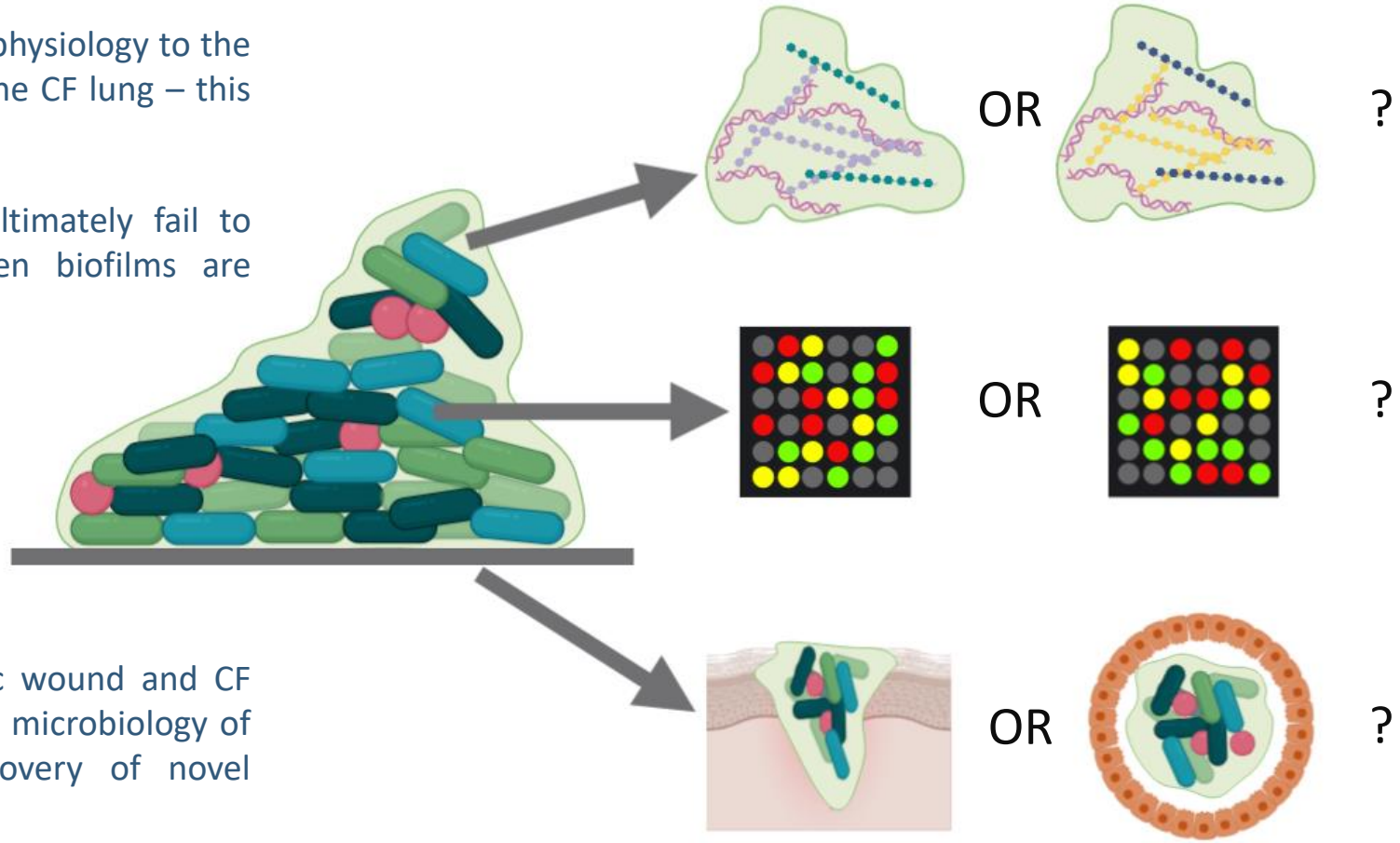
Biofilm in CF & DFI: differences

- Biofilm biology is highly context-specific
- Opportunistic pathogens flexibly adapt their physiology to the unique environments of a diabetic ulcer or the CF lung – this affects antibiotic senstivity

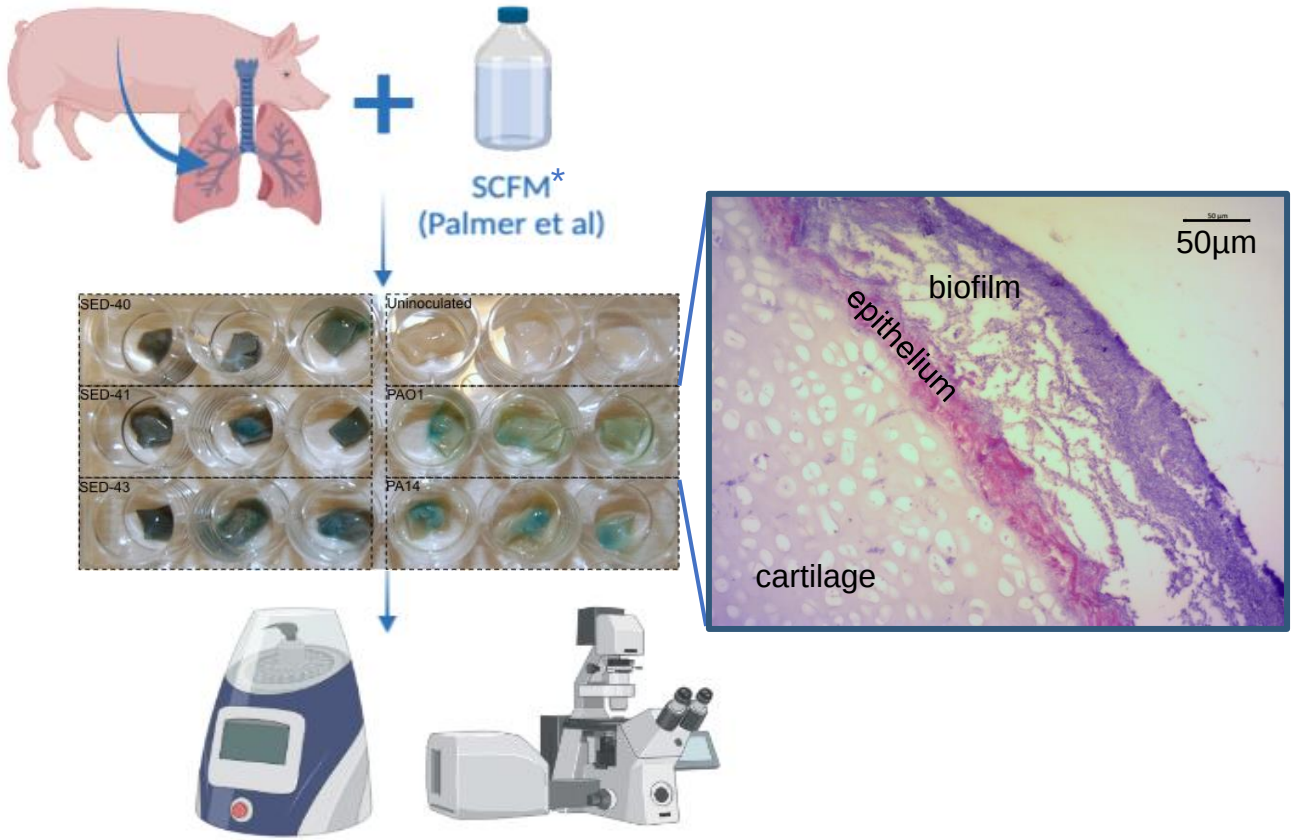


Biofilm in CF & DFI: differences

- Biofilm biology is highly context-specific
- Opportunistic pathogens flexibly adapt their physiology to the unique environments of a diabetic ulcer or the CF lung – this affects antibiotic senstivity
- Most candidate antibacterial compounds ultimately fail to translate to clincial use – especially when biofilms are considered
- We need context-specific biofilm models
  - For drug discovery
  - For better prescribing
  - For better understanding of AMR evolution
- My lab uses high-validity models of chronic wound and CF lung biofilm in our work on the fundamental microbiology of common biofilm pathogens and for discovery of novel therapeutics



Ex vivo CF lung model & synthetic chronic wound model



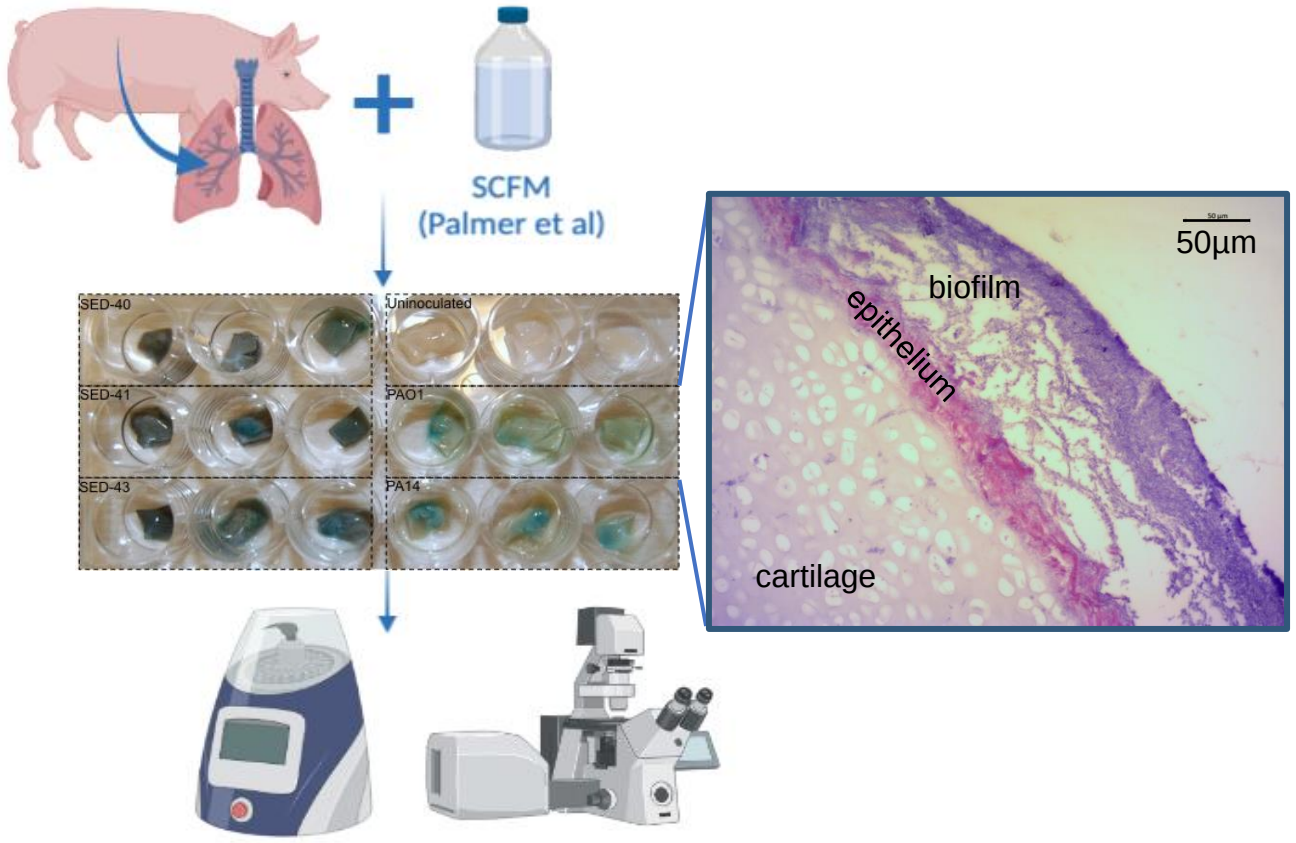
Recover biofilm cells for plating & other downstream assays; assay supernatant for secreted products etc.

In situ imaging of biofilm

More info:
freyaharrison.weebly.com/publications

*SCFM – Synthetic cystic fibrosis sputum

Ex vivo CF lung model & synthetic chronic wound model



Recover biofilm cells for plating & other downstream assays; assay supernatant for secreted products etc.

In situ imaging of biofilm

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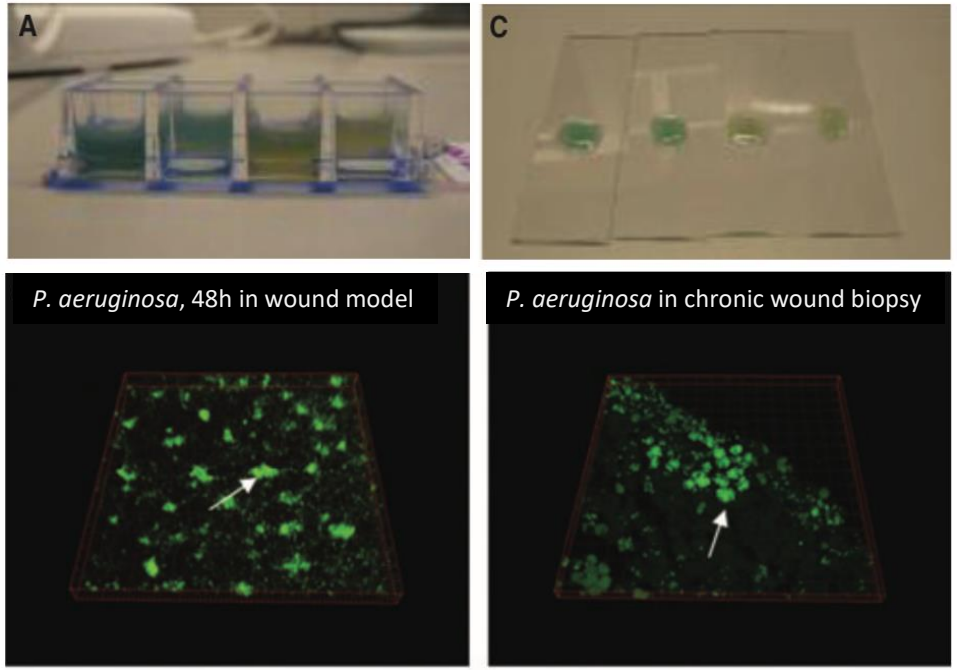
APMIS 118: 156–164

DOI 10.1111/j.1600-0463.2009.02580.x

An *in vitro* model of bacterial infections in wounds and other soft tissues

MARIA WERTHÉN,[1] LINA HENRIKSSON,[1] PETER ØSTRUP JENSEN,[2] CLAUS STERNBERG,[3] MICHAEL GIVSKOV[4] and THOMAS BJARNSHOLT[2,4]

Peptone water + fetal bovine serum + collagen



Why using a tailored biofilm model is important in drug/target discovery

- Avoid false positives: drugs or formulations that look efficacious *in vitro*, but fail *in vivo*
  - And use your model to find adjuvants that could overcome this (e.g. aid biofilm penetration)

- Avoid false negatives: drugs or formulations that fail *in vitro* testing, but prove efficacious *in vivo* in at least some contexts

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A tale of two models…

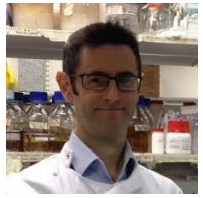
Story 1: Using the *ex vivo* lung model to better understand the physiology and AMR of *P. aeruginosa* biofilm in CF

Story 2: How we've used the CF and chronic wound models in our work on antibacterial natural product preparations (avoiding both false positives and false negatives!)

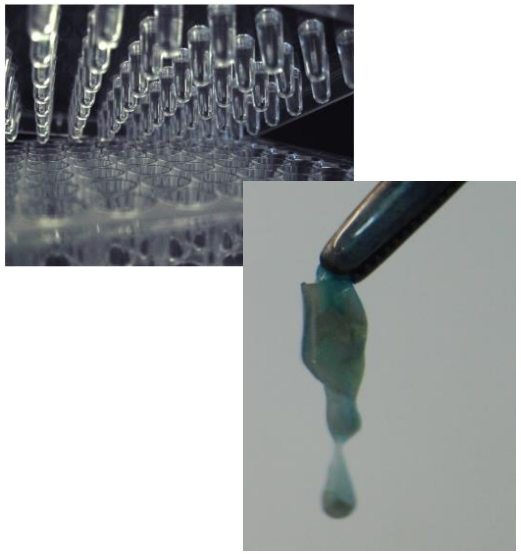
Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

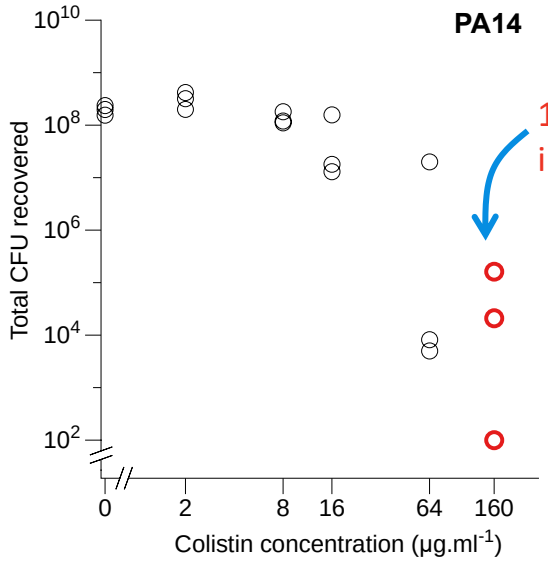
Probing efficacy of colistin for treating *P. aeruginosa* in the lung model

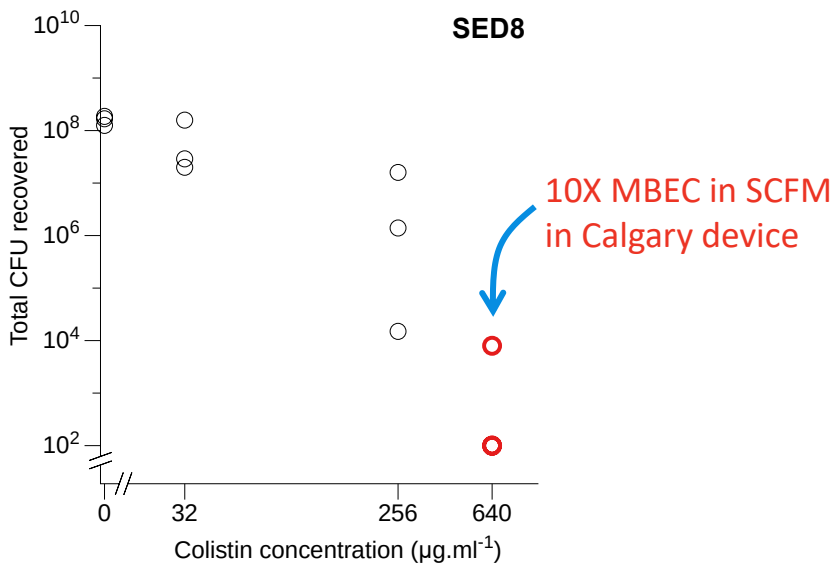
As you might expect, lung-grown biofilms could survive concentrations >> MBEC* as measured in a Calgary biofilm device using SCFM.




Dr Andrew Edwards, Akshay Sabnis (Imperial)
Sweeney (2020) *Microbiology* 166:1171







*MBEC – Minimal biofilm eradication concentration

Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

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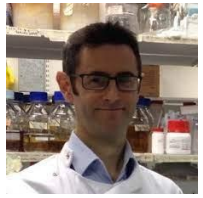


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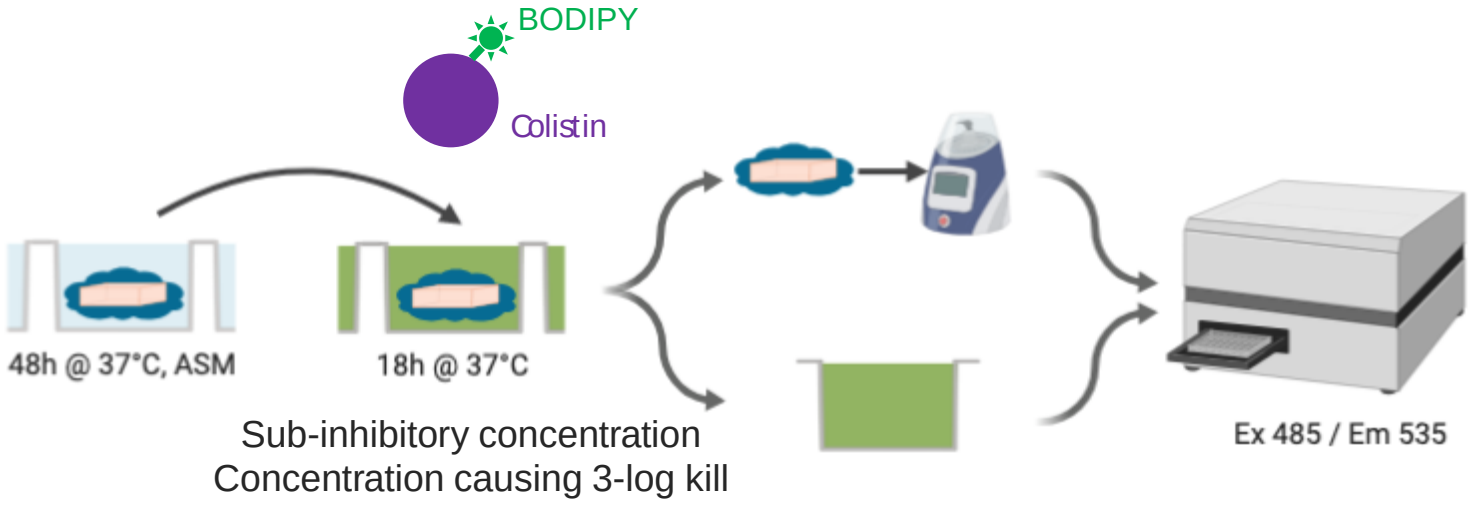
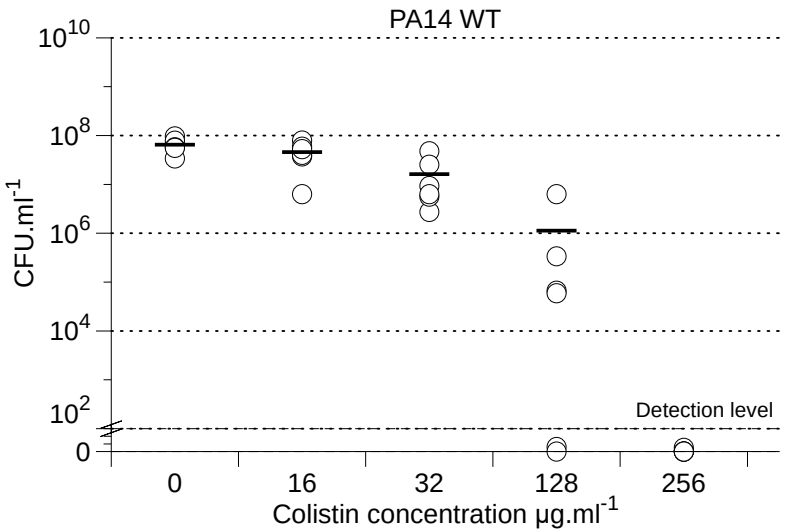


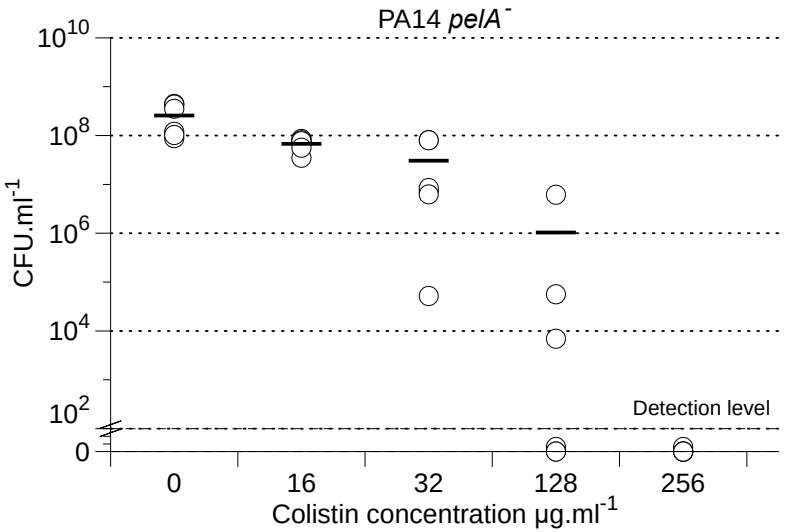
Only 12-19% of the supplied dose can enter the biofilm matrix!

Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

But reduced penetration is not the full story…

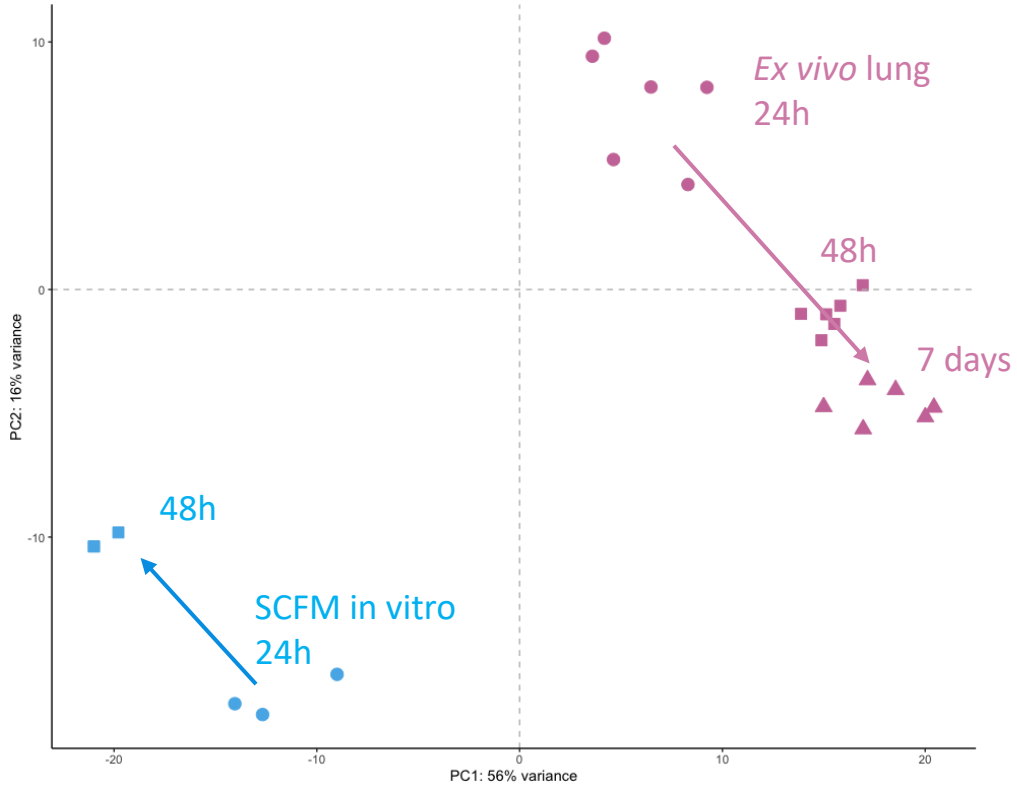
Jenny Littler
Unpublished data





Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

Transcriptome in the model: RNAseq of 5,829 genes in *P. aeruginosa* PA14 over 7 days of infection



Niamh Harrington & Jenny Littler
Harrington (2021) BioRχiv doi: 10.1101/2021.07.23.453509

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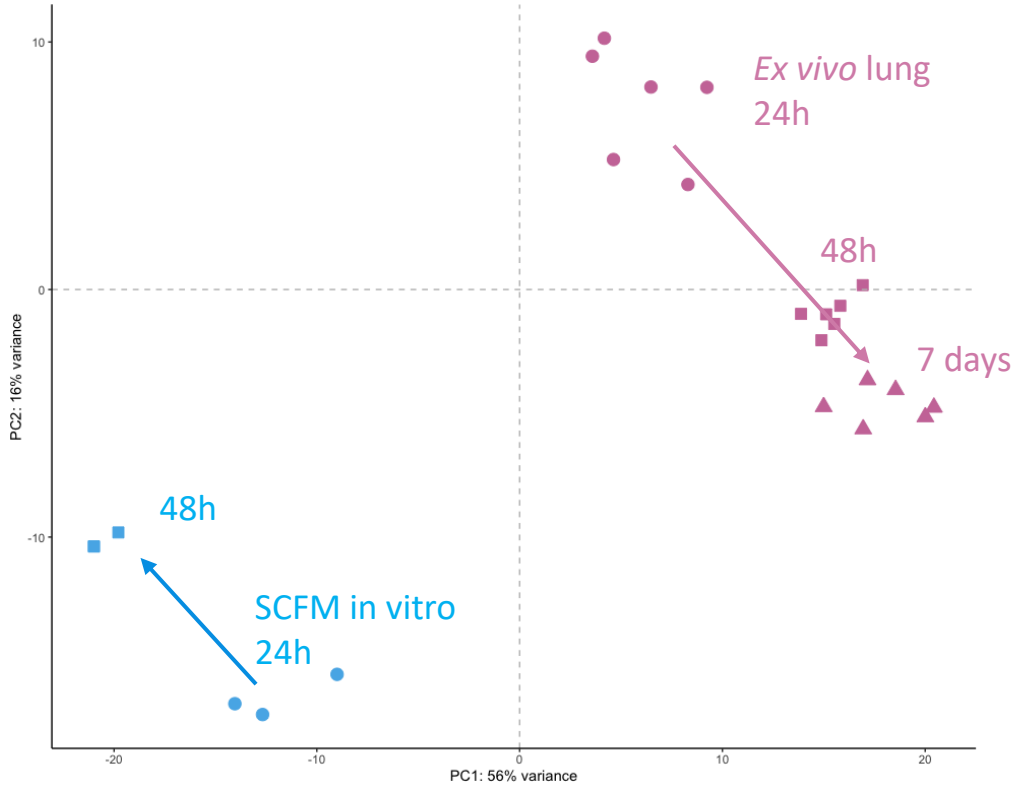
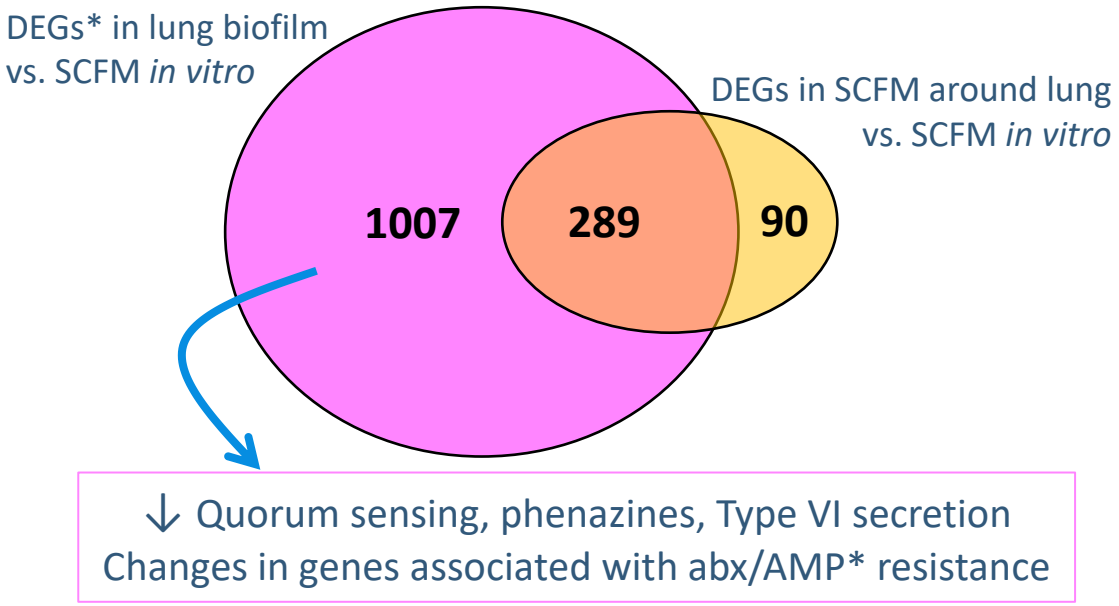


A snapshot at 48h

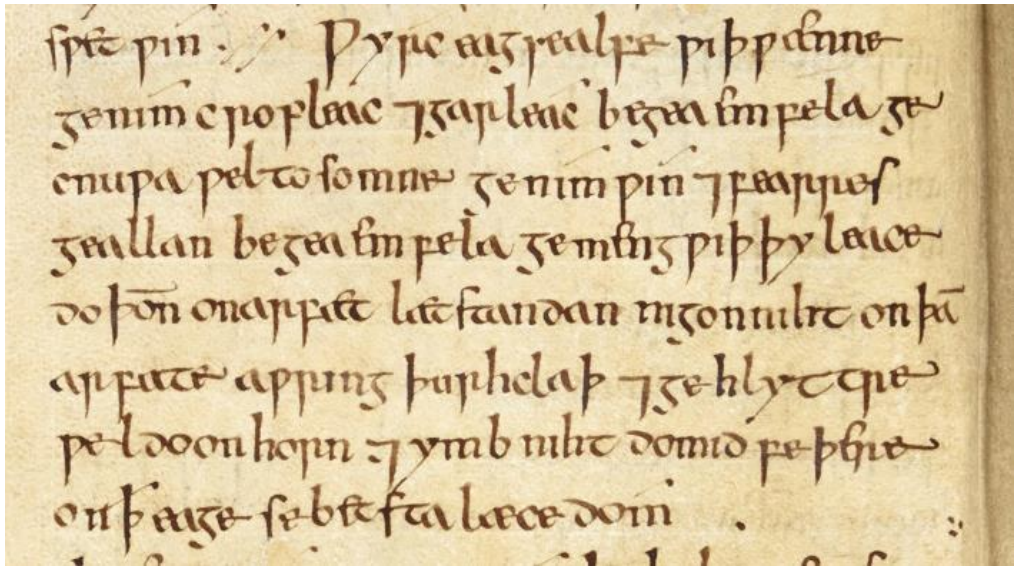


*DEGs – Differentially expressed genes, abx – antibiotics, AMP – Antimicrobial peptides

Niamh Harrington & Jenny Littler
Harrington (2021) BioRχiv doi: 10.1101/2021.07.23.453509

Use the right biofilm model for drug discovery, and use it early in testing

One of our candidate ancientbiotics: ‘Bald’s eyesalve’



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Steve Diggle (GA Tech), Christina Lee (Nottingham)

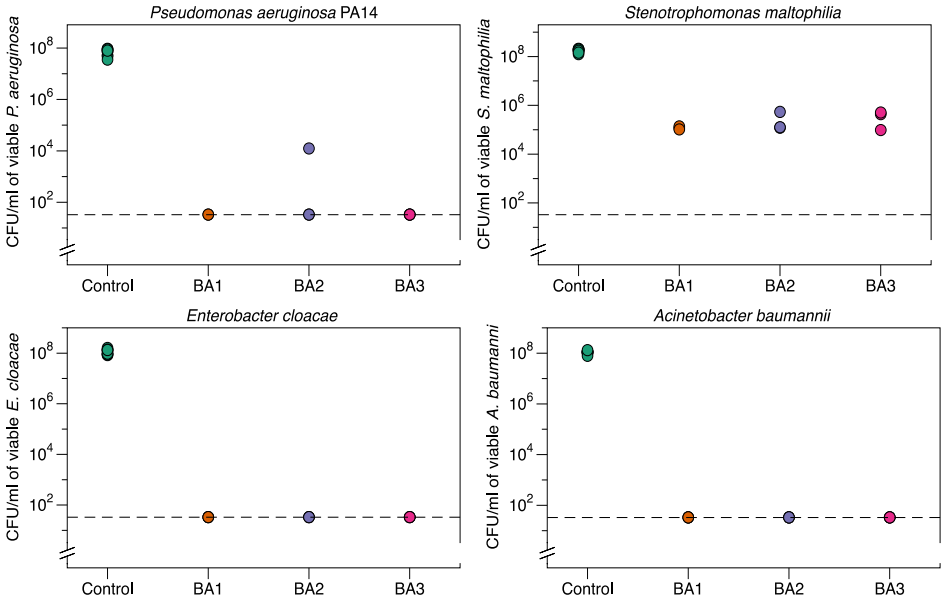
Use the right biofilm model for drug discovery, and use it early in testing

Planktonic killing of soft tissue pathogens in synthetic wound fluid (SWF)

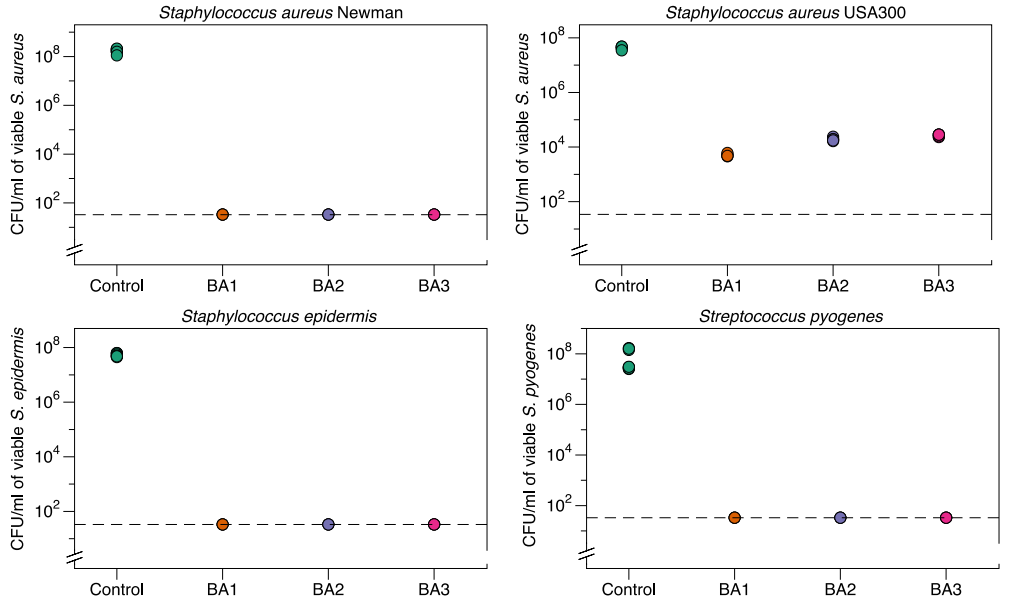
Peptone water

Fetal bovine serum

Gram-negatives



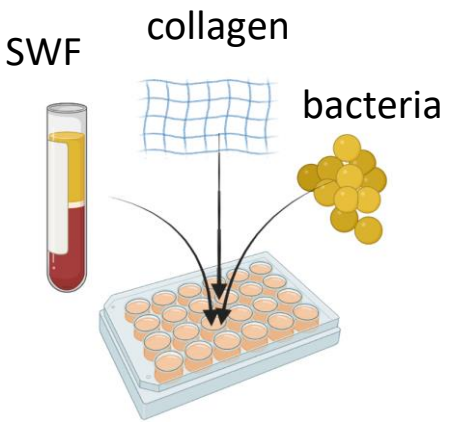
Gram-positives



Dr Blessing Anonye (UCLan)

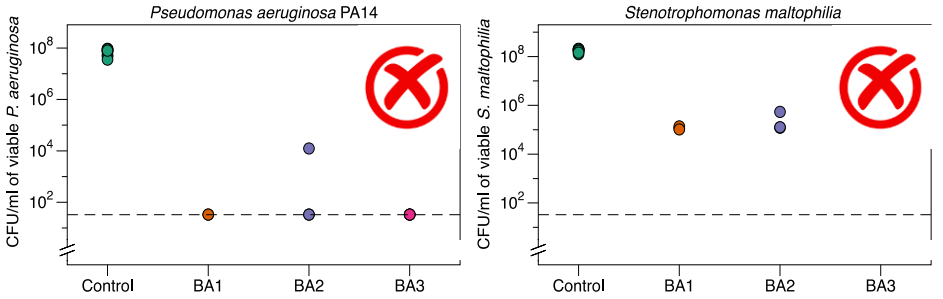
Furner-Pardoe et al. 2020. *Scientific Reports* 10:12687

Use the right biofilm model for drug discovery, and use it early in testing



Do we see killing of biofilms in synthetic chronic wound?

Gram-negatives



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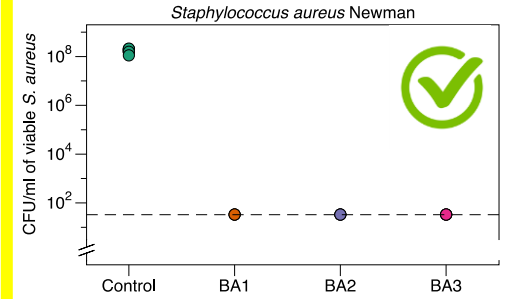
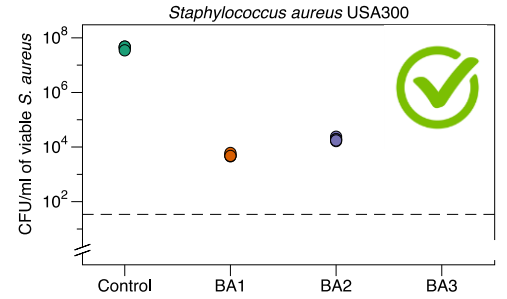
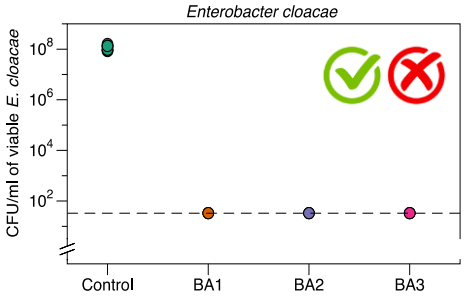
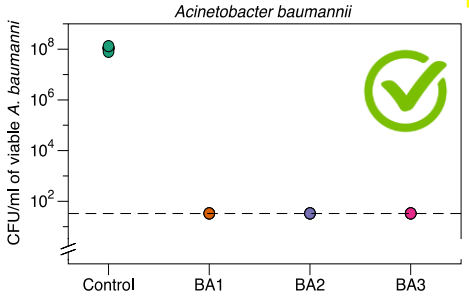
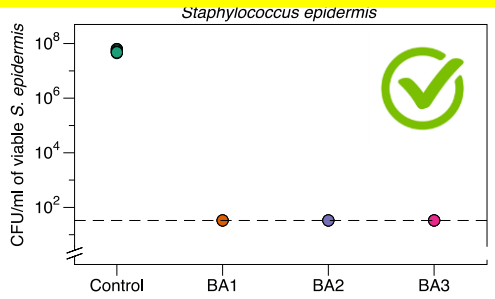
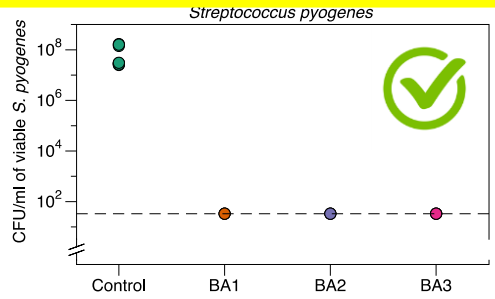










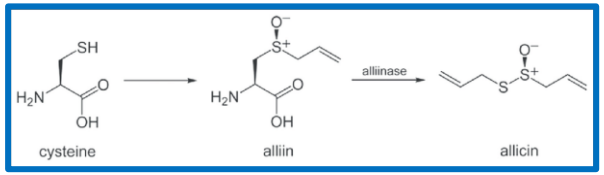


Dr Blessing Anonye (UCLan)

Furner-Pardoe et al. 2020. *Sci. Rep.* 10:12687

Use the right biofilm model for drug discovery, and use it early in testing

The synthetic chronic wound biofilm model revealed the need for >1 active molecule!



- Explains most bactericidal activity in planktonic culture
- Is not a good drug candidate

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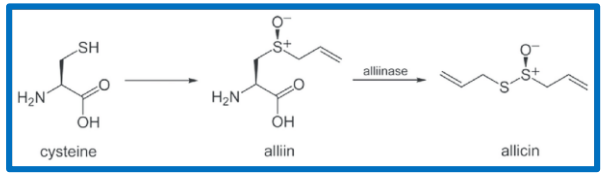
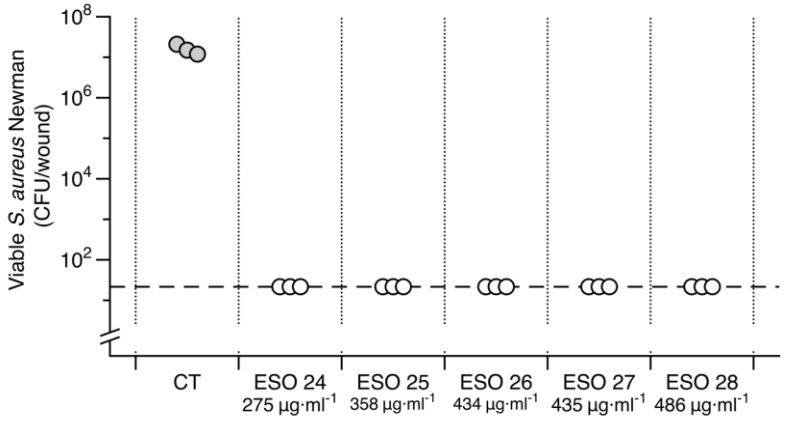
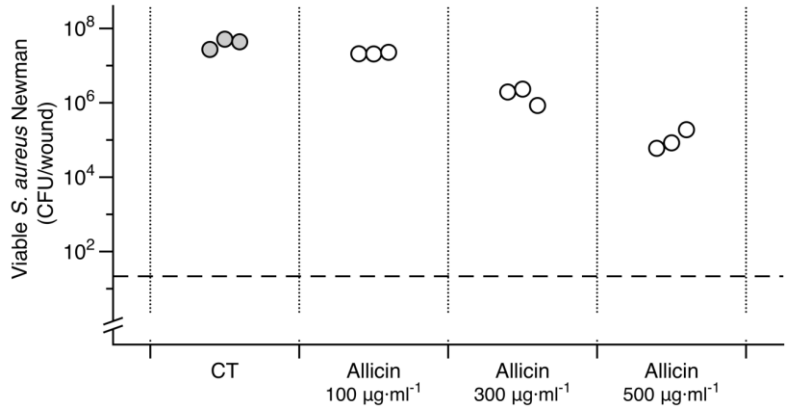
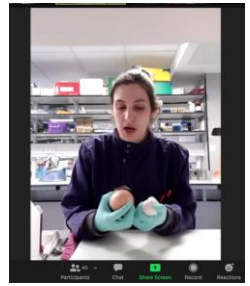


- Explains most bactericidal activity in planktonic culture
- Is not a good drug candidate

But allicin <u>cannot</u> explain activity of Bald's eyesalve in SCW biofilm…







Jessica Furner-Pardoe

Furner-Pardoe 2020 *Sci. Rep.* 10:12687

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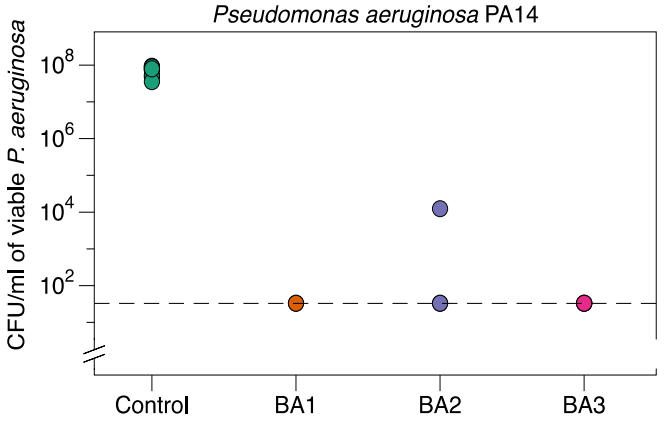
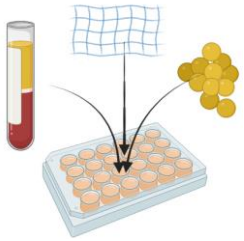
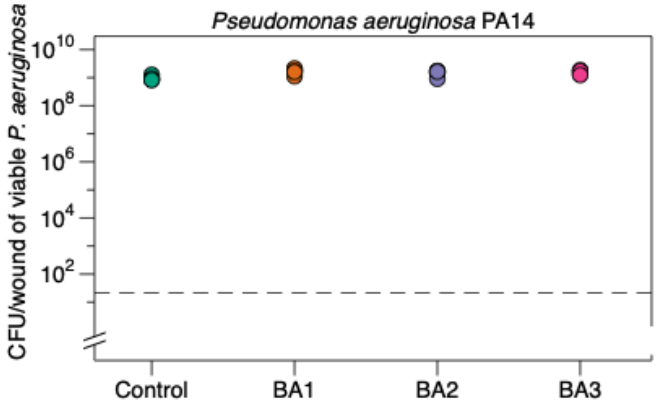
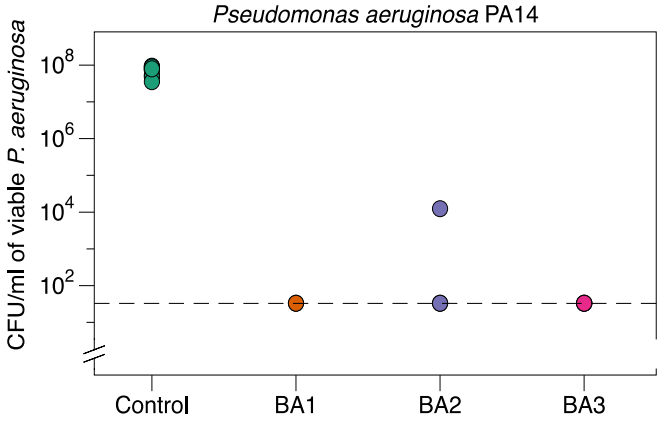
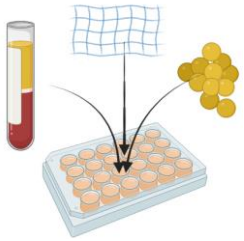


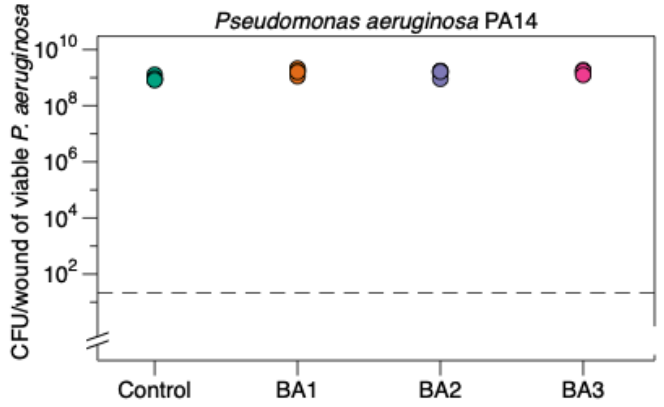


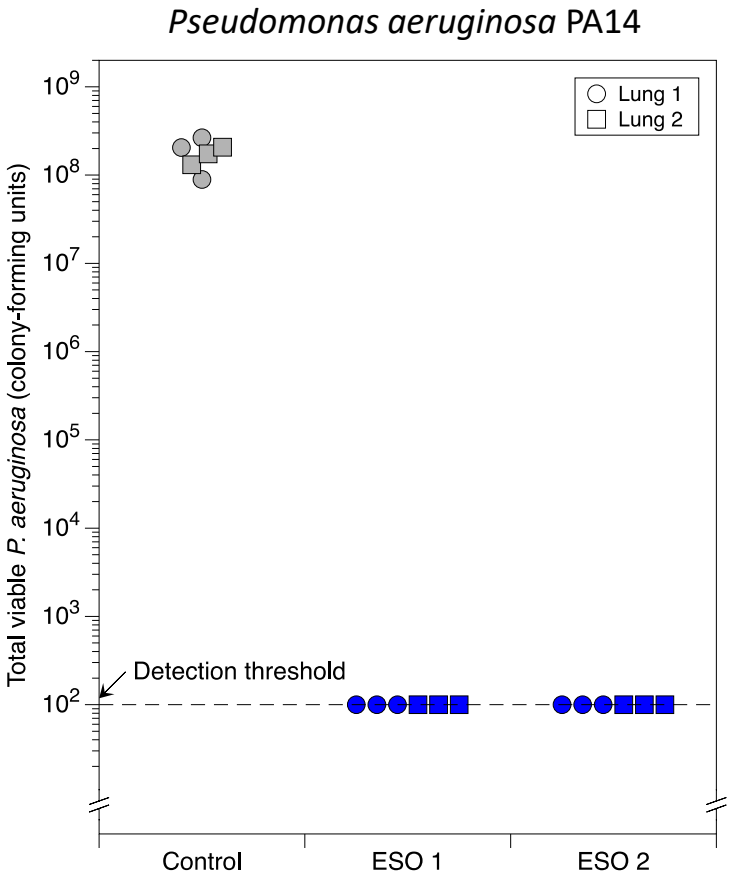


Use the right biofilm model for drug discovery, and use it early in testing









Use the right biofilm model for drug discovery, and use it early in testing

What we, as microbiologists, agree are priorities in antibacterial R&D

- Chronic biofilm infections – extensive & unpredicatble AMR
- Better diagnostic / R&D testing of agents to treat these
- Novel agents to treat biofilm infection

How we're trying to address these

- Developing and using high-validity *ex vivo* and *in vitro* models of biofilm infection
- Context specific – match physicochemical environment of pathogens (CF, wounds)
- Aid in drug/adjuvant discovery
- Evolution of resistance in different infection models

Today’s speakers

Biofilms: What are they and why do we care?

Mark Webber
Group Leader
Quadram Institute (UK)

Freya Harrison
Principal Investigator
University of Warwick (UK)

Moderator:
Laura Piddock
Scientific Director
GARDP

Thank you for joining us