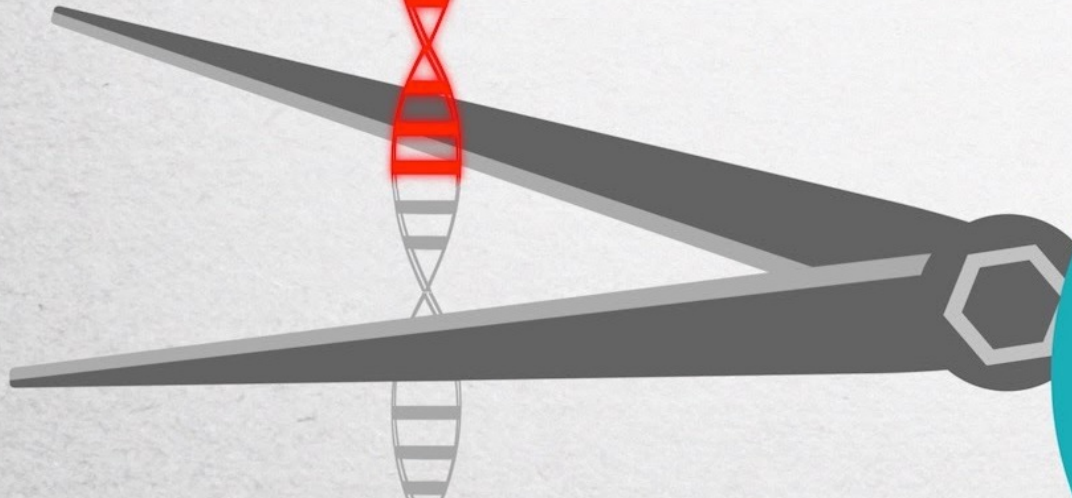
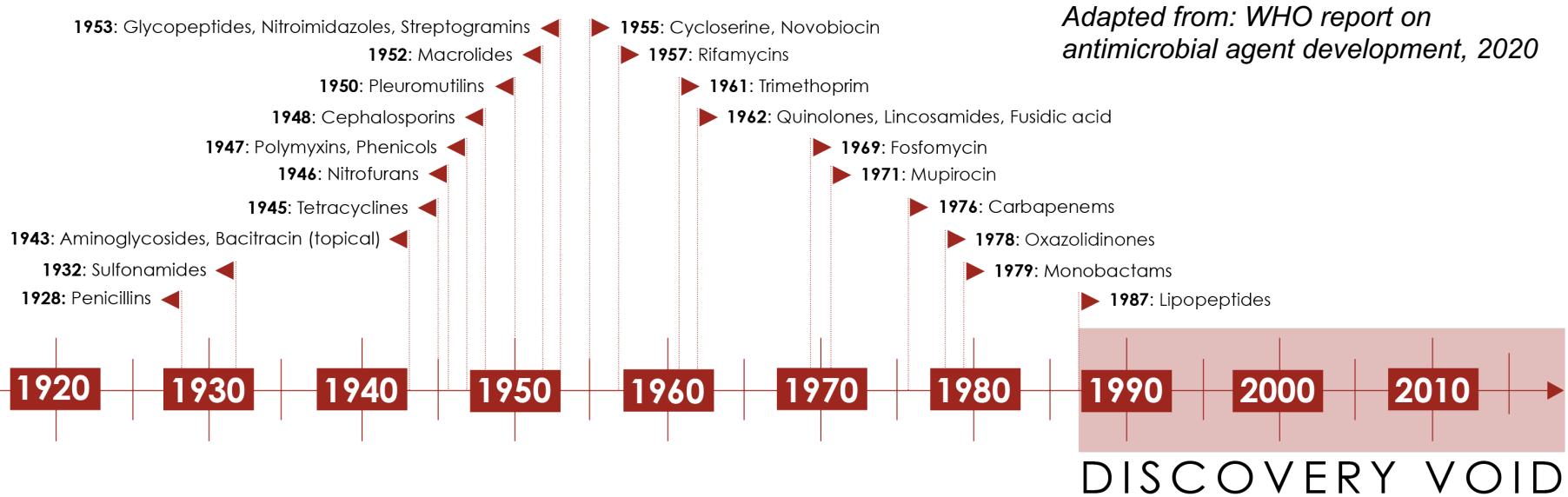


# CRISPR-Cas9 as a tool to fight AMR: challenges and future prospects



Stineke van Houte  
University of Exeter  
ONE conference  
23 June 2022

# Emergence of new antibiotics on the market has dwindled



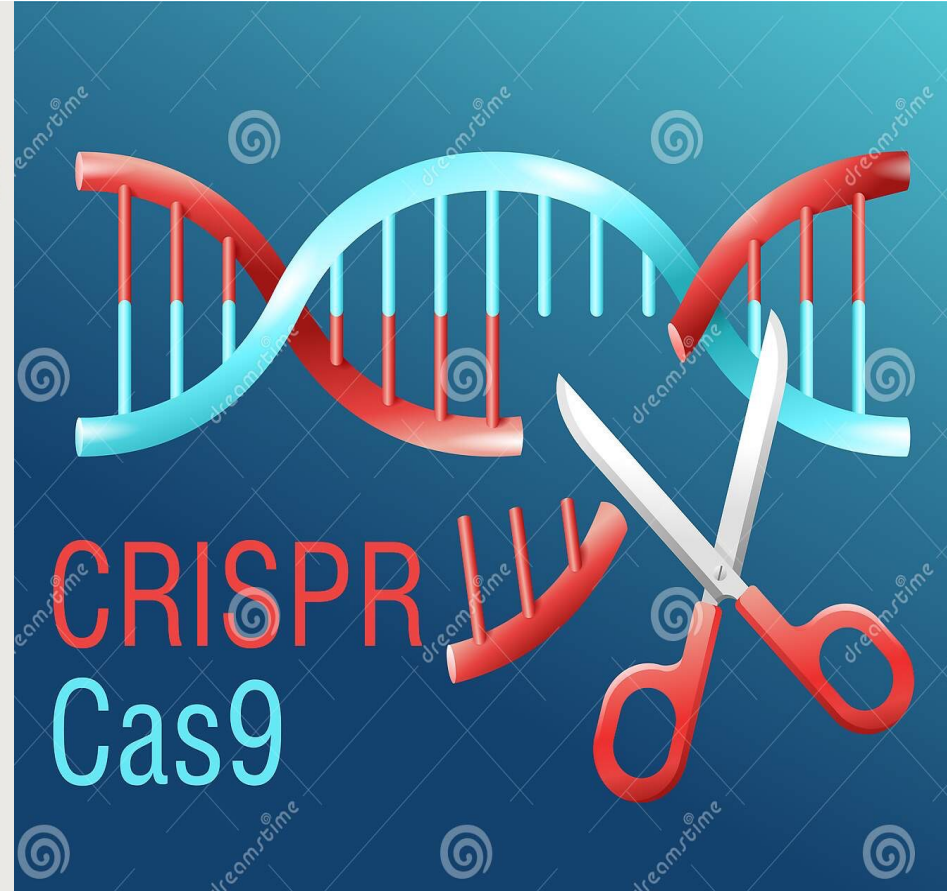
© ReAct Group 2015

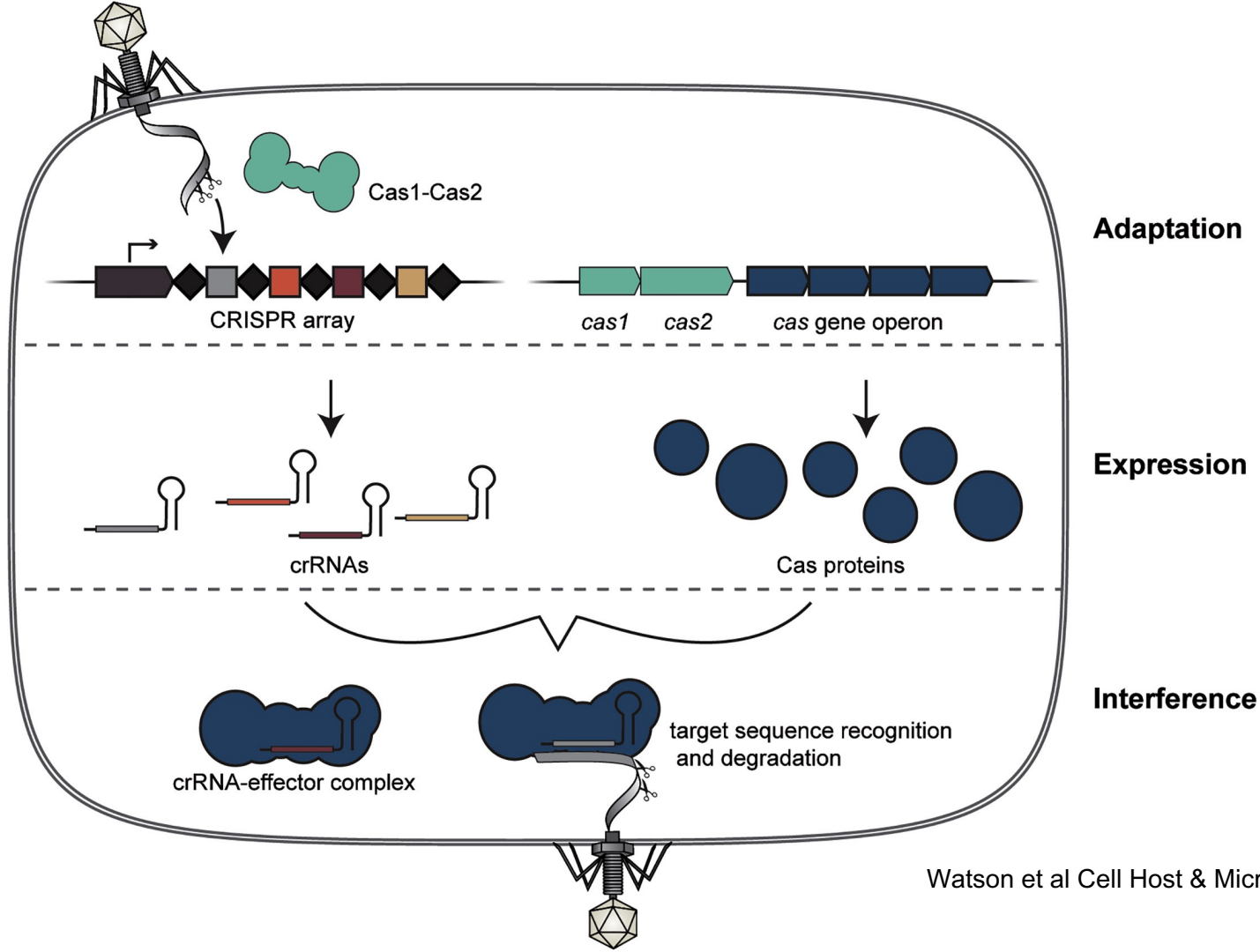
**WE NEED  
ALTERNATIVE STRATEGIES TO TARGET AMR**

# CRISPR-Cas as new tool to target AMR

## The Nobel Prize in Chemistry 2020

awarded "for the development of a  
method of genome editing"





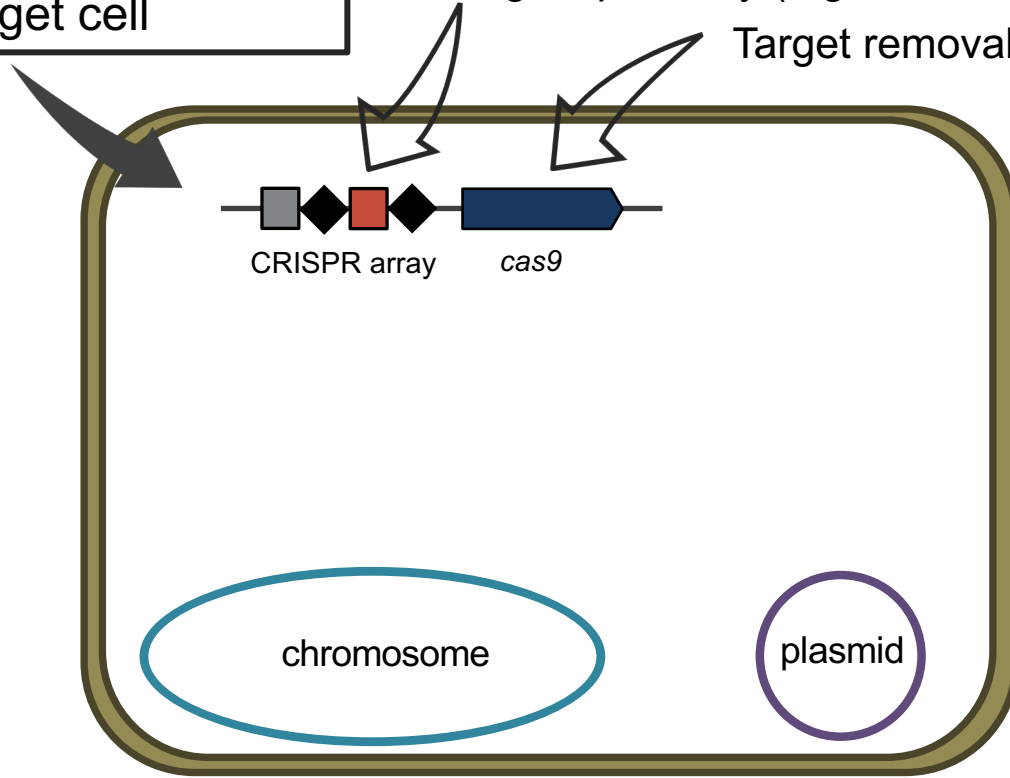
# How can we use CRISPR as an antimicrobial tool?

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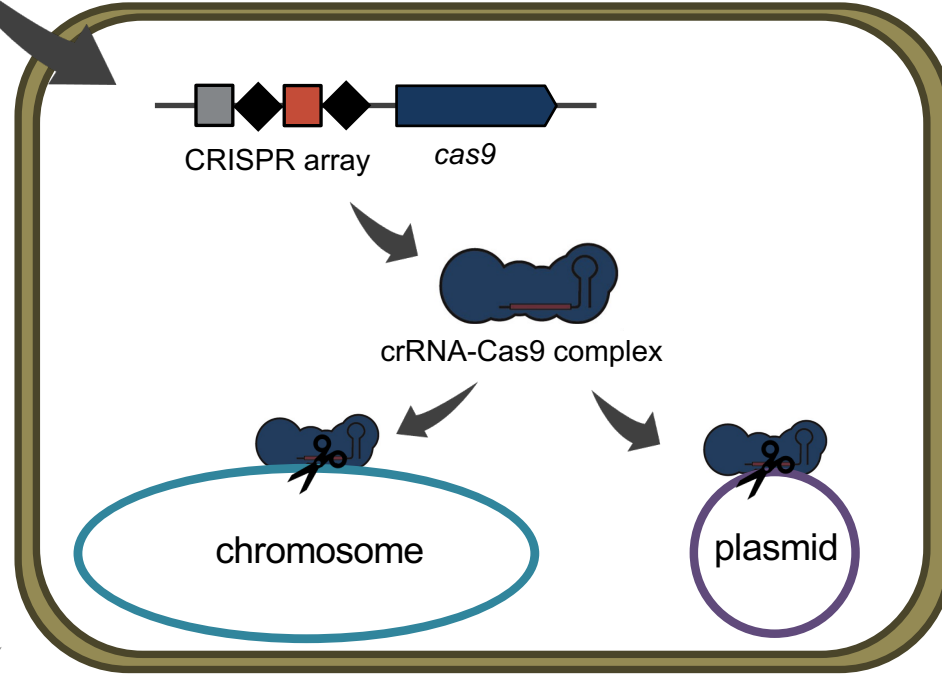
CRISPR-Cas delivered to  
target cell

Target specificity (e.g. AMR or virulence genes)

Target removal



CRISPR-Cas delivered to  
target cell

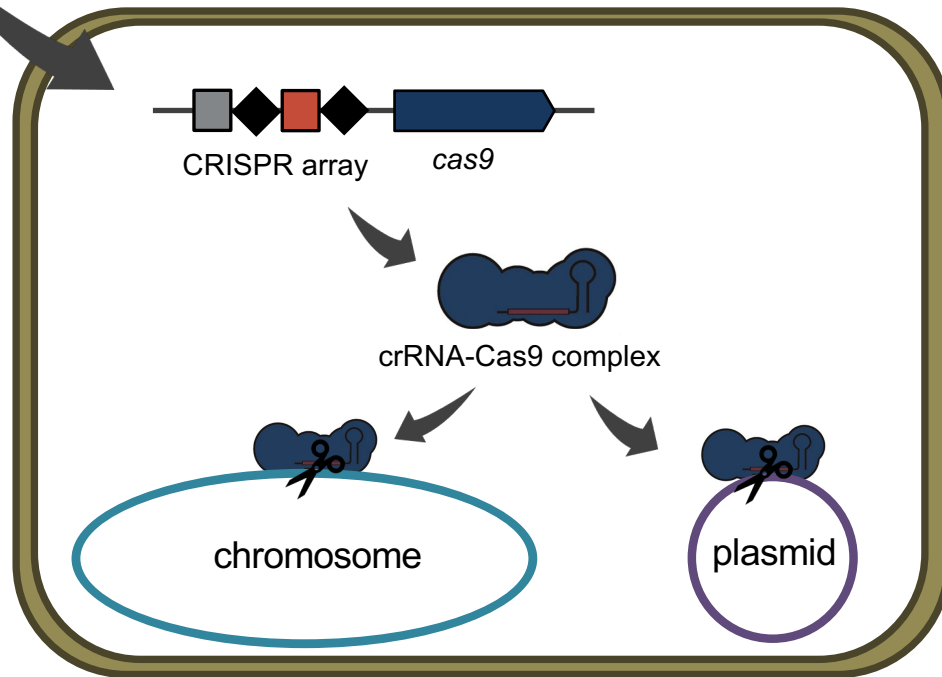


killing of drug-resistant bacterium

antibiotic resensitization

CRISPR-Cas delivered to  
target cell

?



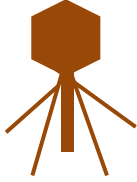
killing of drug-resistant bacterium

antibiotic resensitization

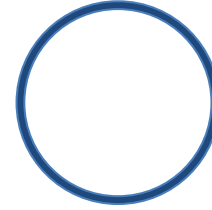


# Vectors for CRISPR-Cas9 delivery to target cell

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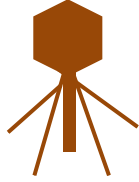
bacteriophages



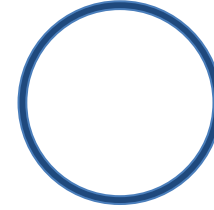
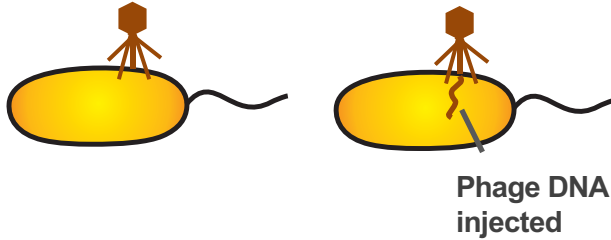
conjugative plasmids

# Vectors for CRISPR-Cas9 delivery to target cell

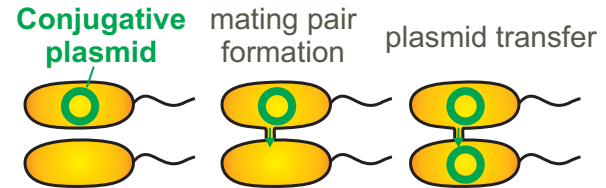
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bacteriophages

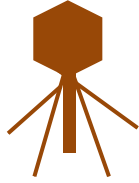


conjugative plasmids

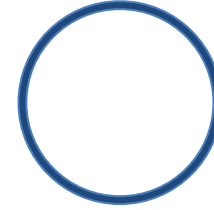
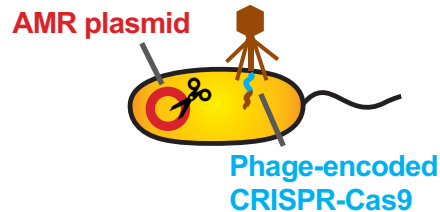
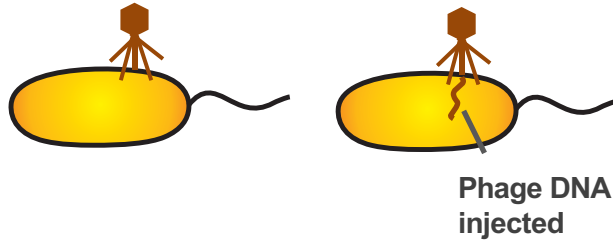


# Vectors for CRISPR-Cas9 delivery to target cell

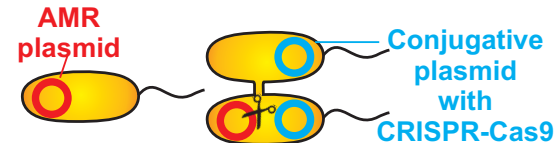
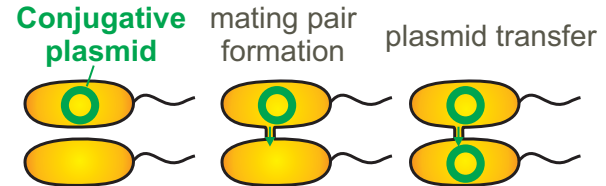
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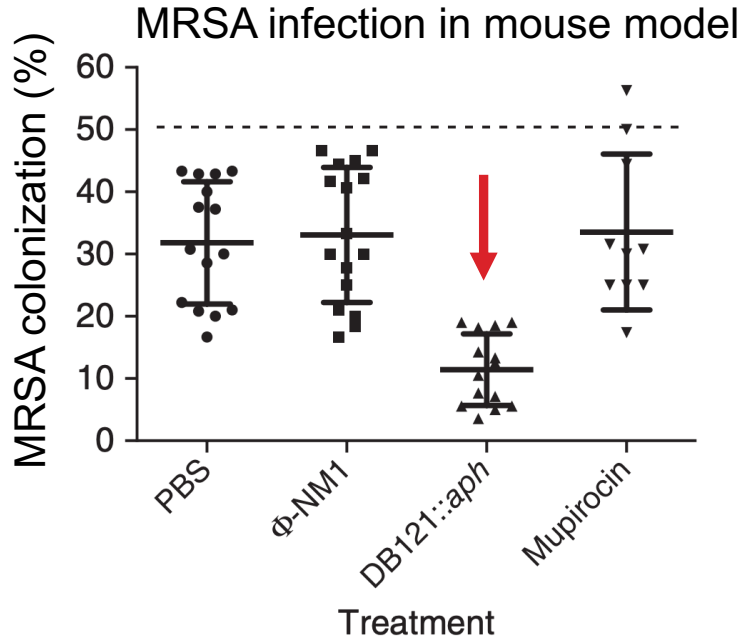
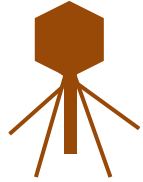
bacteriophages



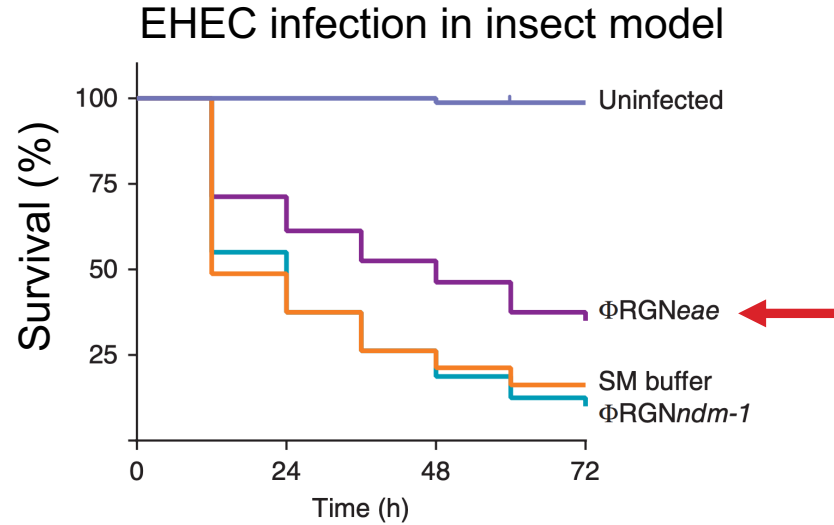
conjugative plasmids



# Bacteriophages for CRISPR-Cas delivery to remove AMR



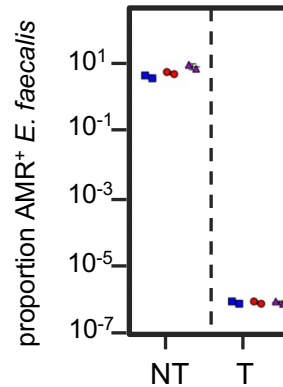
Bikard *et al*, Nature Biotechnology 2014



Citorik *et al*, Nature Biotechnology 2014

# Conjugative plasmids for CRISPR-Cas delivery to remove AMR

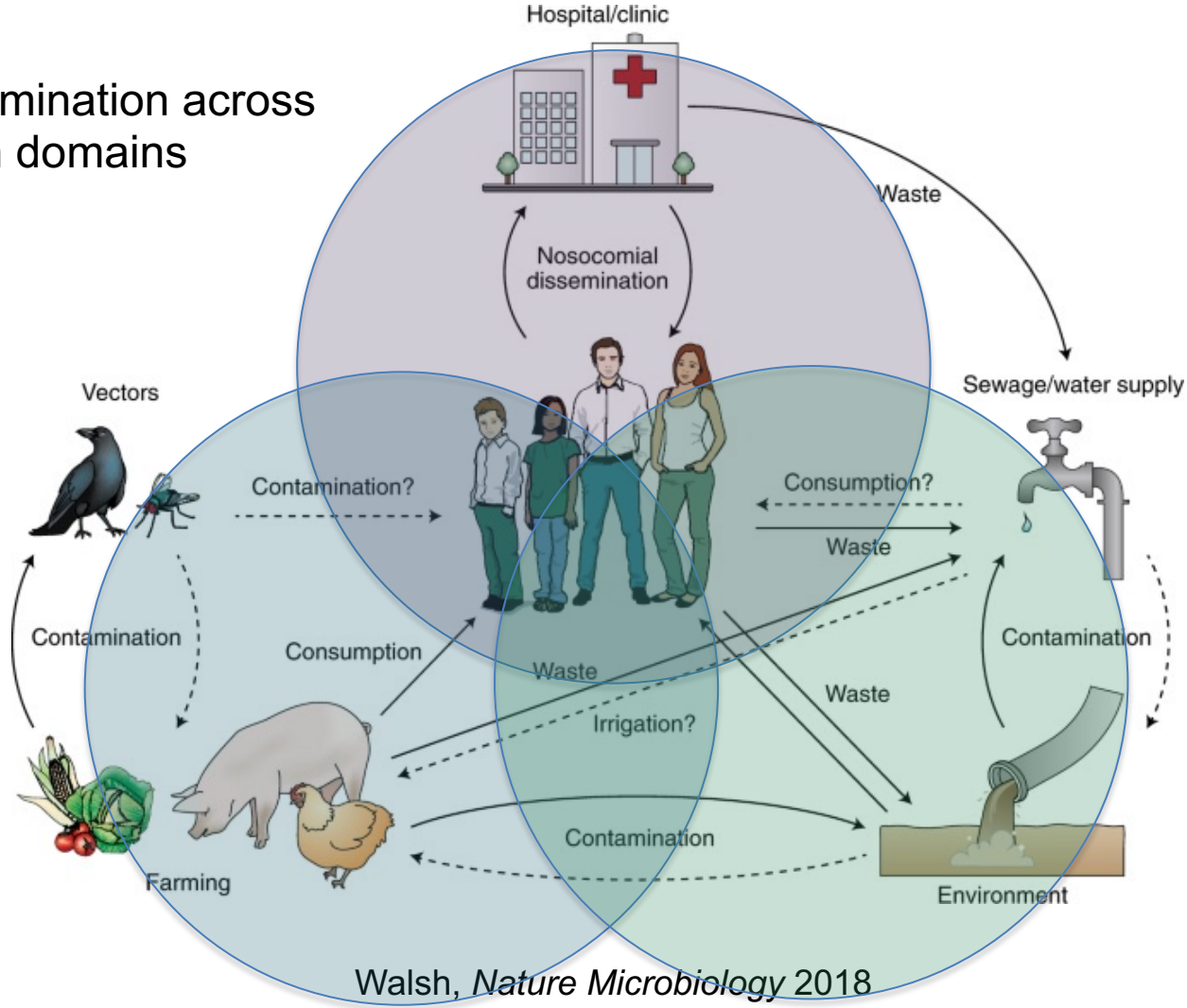
Drug-resistant *Enterococcus faecalis* – *in vitro*



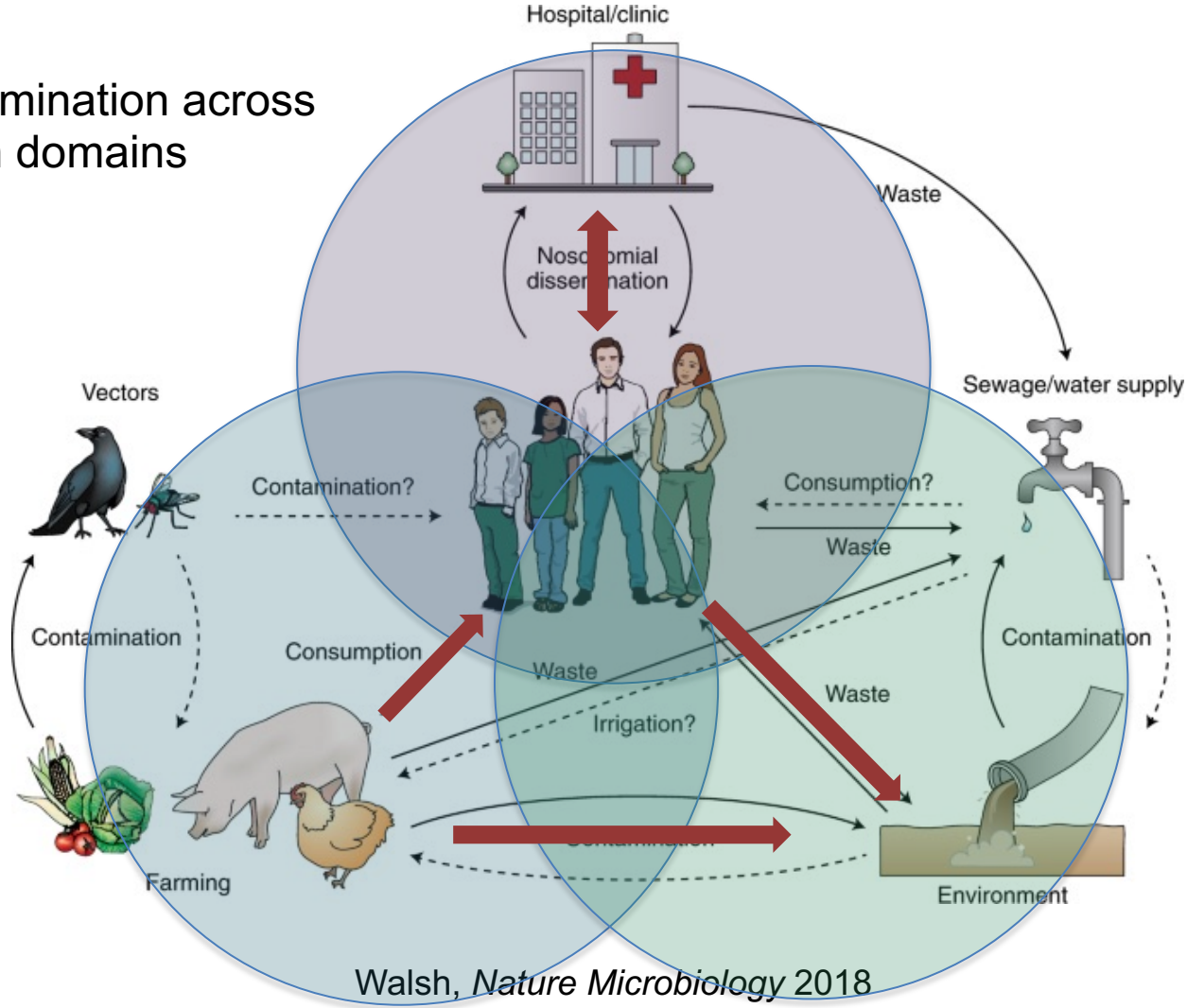
NT = non-targeting crRNA (control)

T = targeting crRNA

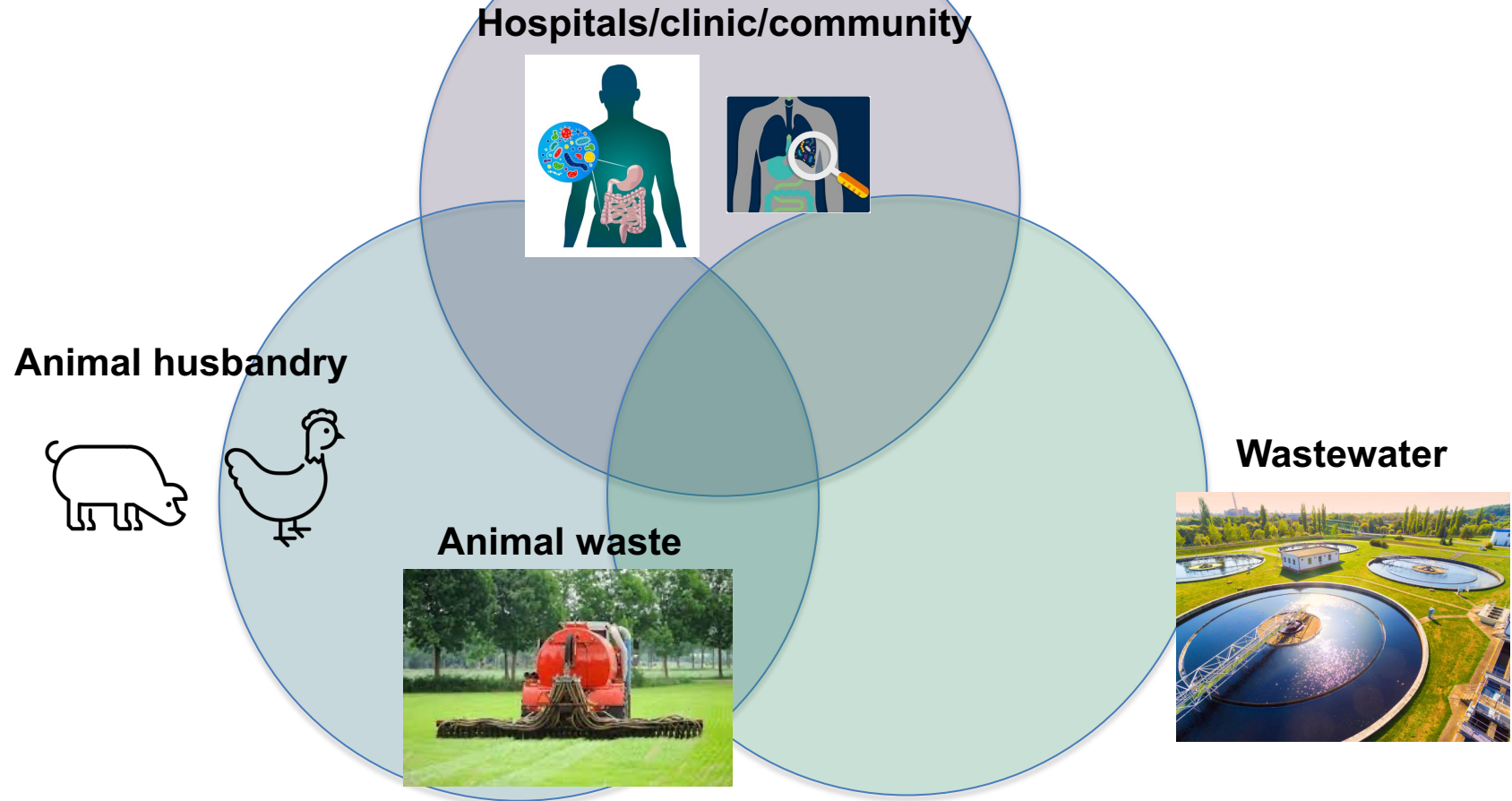
# AMR contamination across One Health domains



# AMR contamination across One Health domains



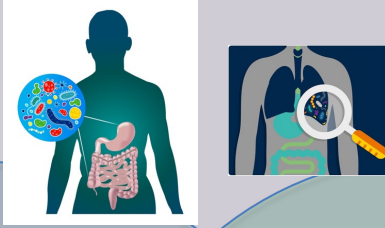
# Opportunities for CRISPR-Cas-based AMR removal across One Health



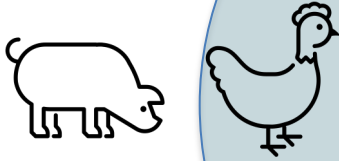


# Opportunities for CRISPR-Cas-based AMR removal across One Health

**Hospitals/clinic/community**



**Animal husbandry**



- *Salmonella*
- *Pathogenic E. coli* (APEC/ETEC)
- *Streptococcus suis*

**Animal waste**

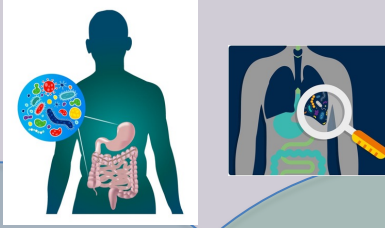


**Wastewater**

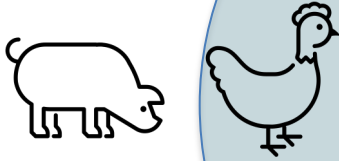


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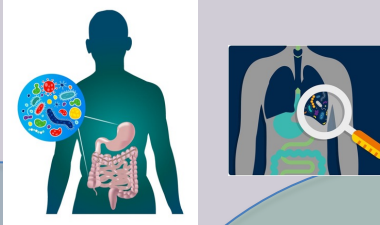


**Wastewater**



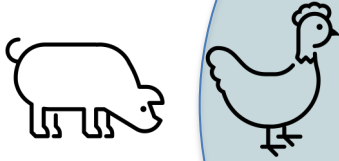
# Opportunities for CRISPR-Cas-based AMR removal across One Health

## Hospitals/clinic/community



- *Clostridium difficile*
- *Pathogenic E. coli*
- *Enterococcus faecalis / faecium*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*

## Animal husbandry



- *Salmonella*
- *Pathogenic E. coli* (APEC/ETEC)
- *Streptococcus suis*

## Animal waste

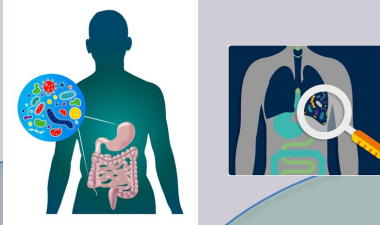


## Wastewater



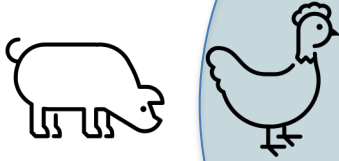
# Opportunities for CRISPR-Cas-based AMR removal across One Health

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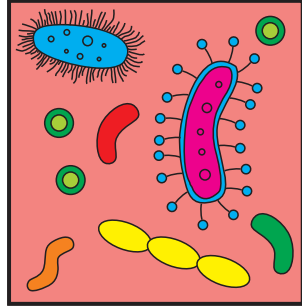
## Animal waste



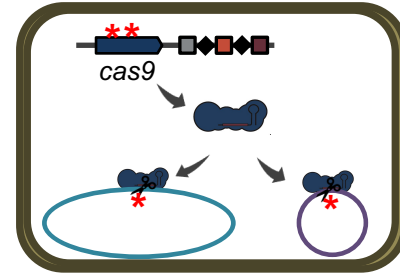
## Wastewater



# Challenges for CRISPR-Cas-based AMR removal across One Health



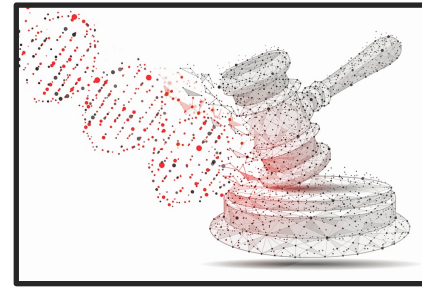
microbial community complexity



CRISPR targeting evasion



treatment delivery



legislative issues

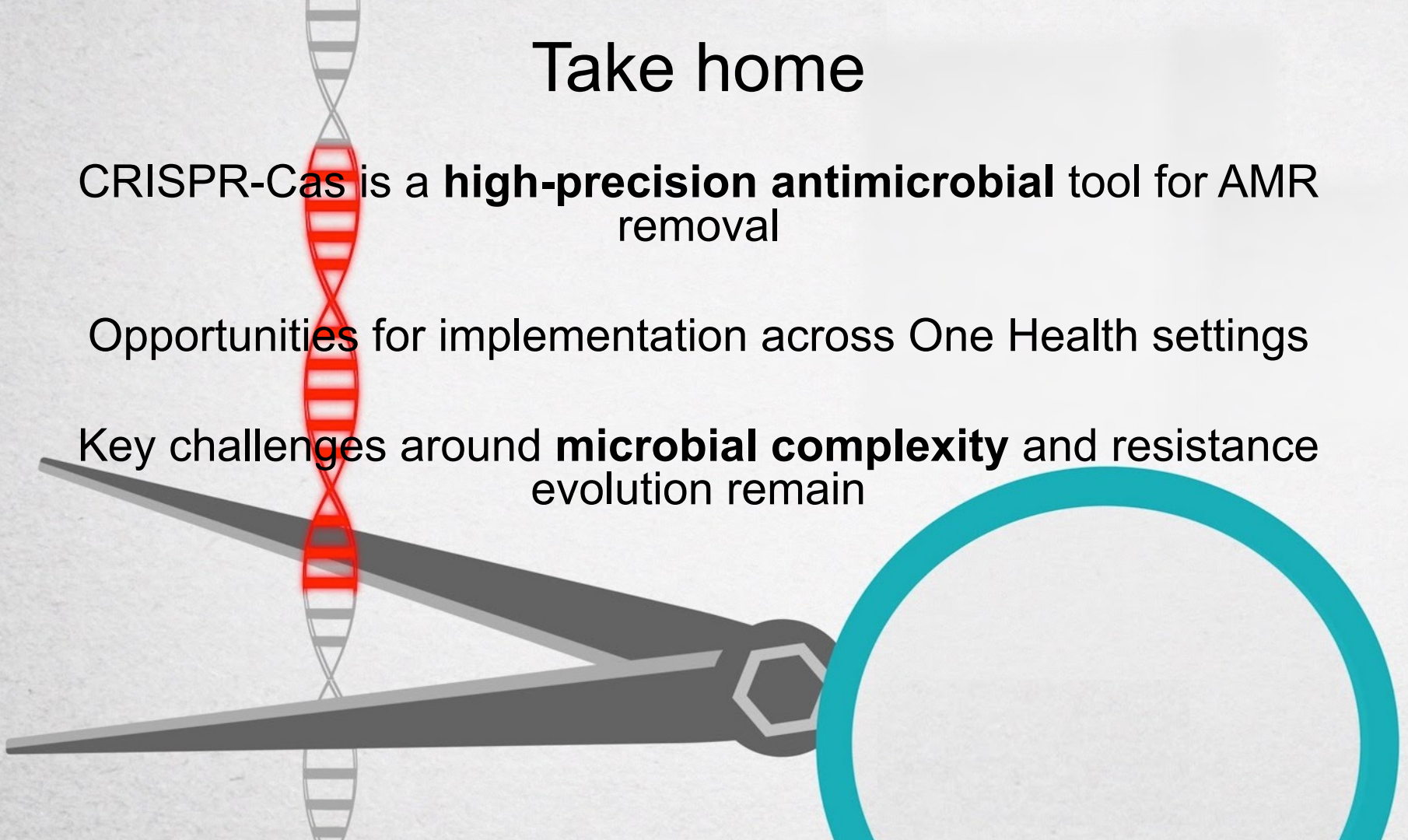


# Take home

CRISPR-Cas is a **high-precision antimicrobial** tool for AMR removal

Opportunities for implementation across One Health settings

Key challenges around **microbial complexity** and resistance evolution remain



# THANKS!

## University of Exeter

Jenny Broniewski  
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Mariann Landsberger  
Ellie Pursey  
Sean Meaden  
Clare Rollie  
Edze Westra  
Bridget Watson  
Jessica Forsyth  
Ben Raymond  
Will Gaze  
Anne Leonard  
Uli Klümper



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Fernanda Paganelli

## CNRS Montpellier

Sylvain Gandon  
Helene Chabas

