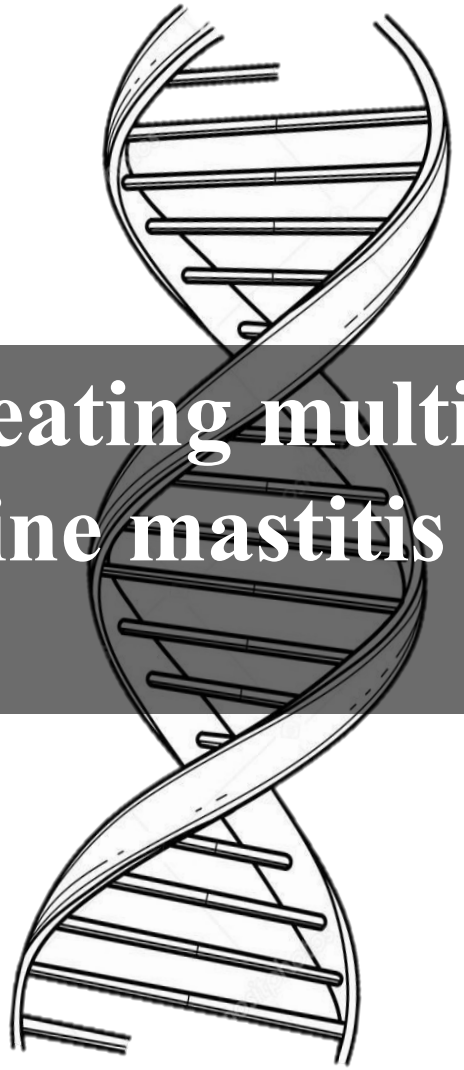
ANSC-691-001

Use of CRISPR-Cas9 based nanoparticles in treating multiple drug resistant *Klebsiella pneumoniae* in bovine mastitis



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ROADMAP

Introducti

Introducing the background

Strategies & delivery

Crispr-Cas9 strategies and delivery

Nanoparticle based CRISPR-Cas9

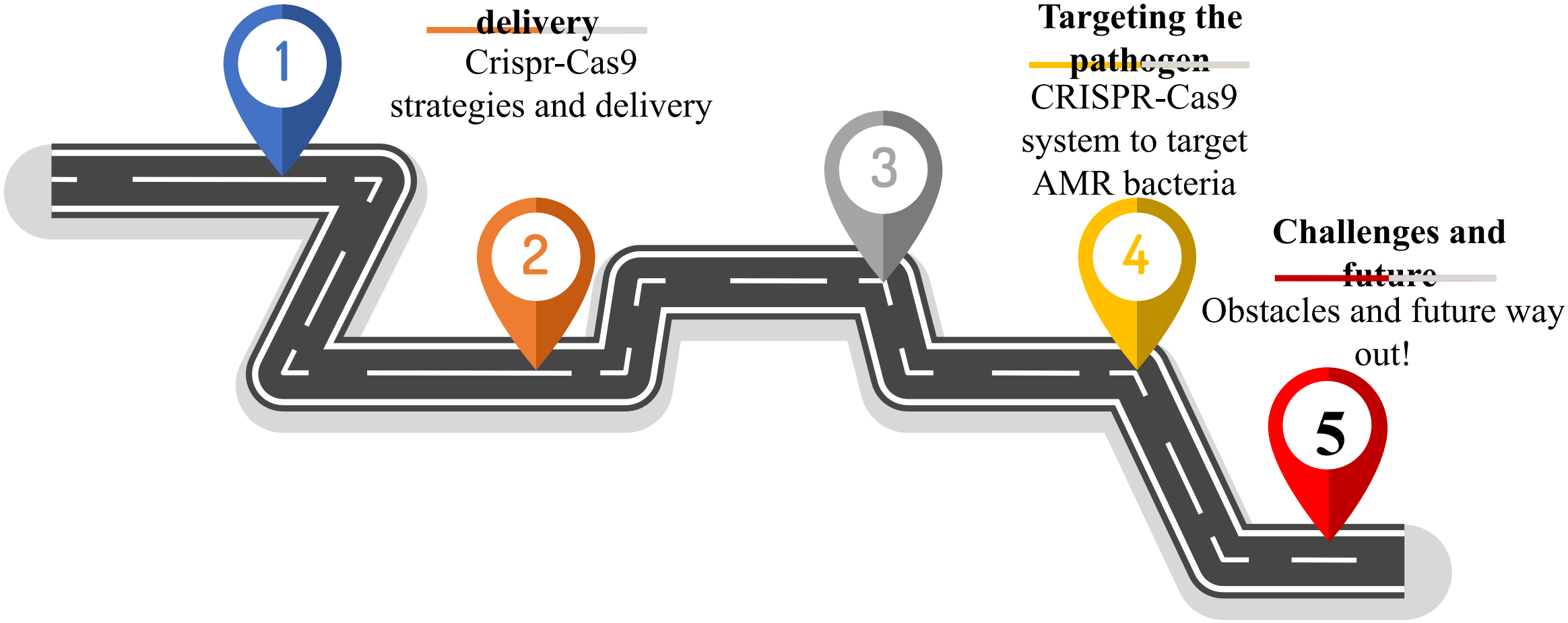
Insight!

Targeting the pathogen

CRISPR-Cas9 system to target AMR bacteria

Challenges and future

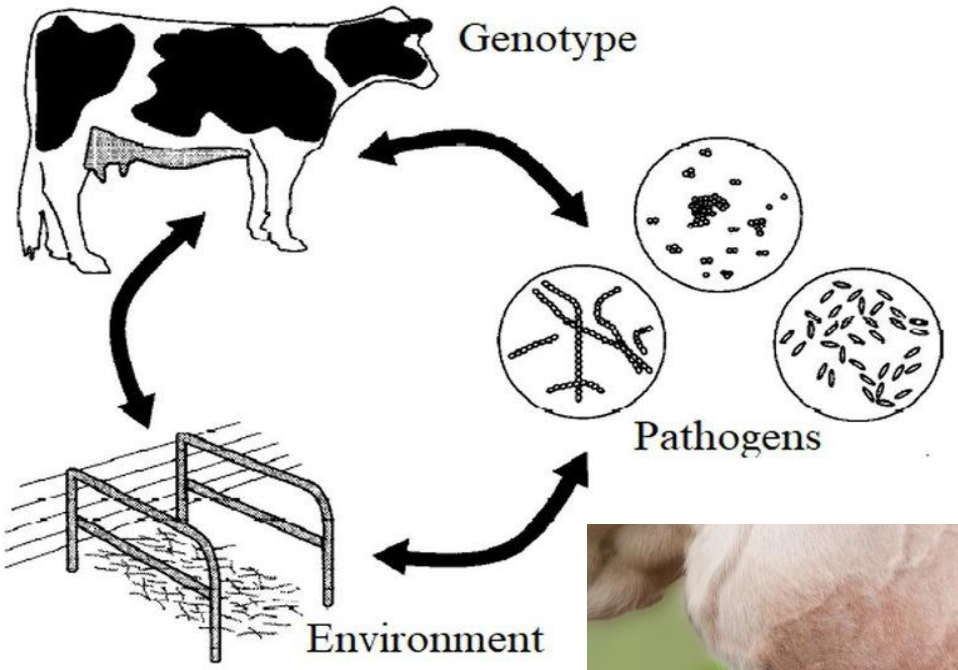
Obstacles and future way out!

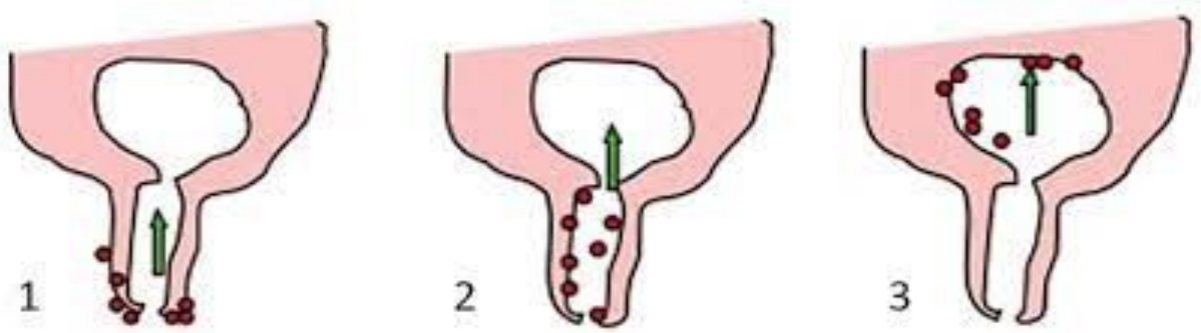


Background -
Bovine mastitis, *Klebsiella pneumoniae,* Antimicrobial resistance (AMR)

Introduction

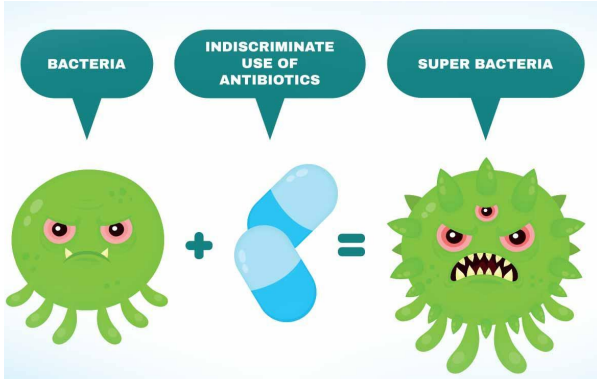
- *Klebsiella* pervasive in the natural environment, benignly colonizes the GIT of humans and animals
- *K. pneumoniae* □ threat due to rapid emergence of MDR
- Bovine mastitis costs the global dairy industry upto US$32 billion annually
- *K. pneumoniae* - environment derived bovine mastitis (Ohnishi *et al*., 2013)



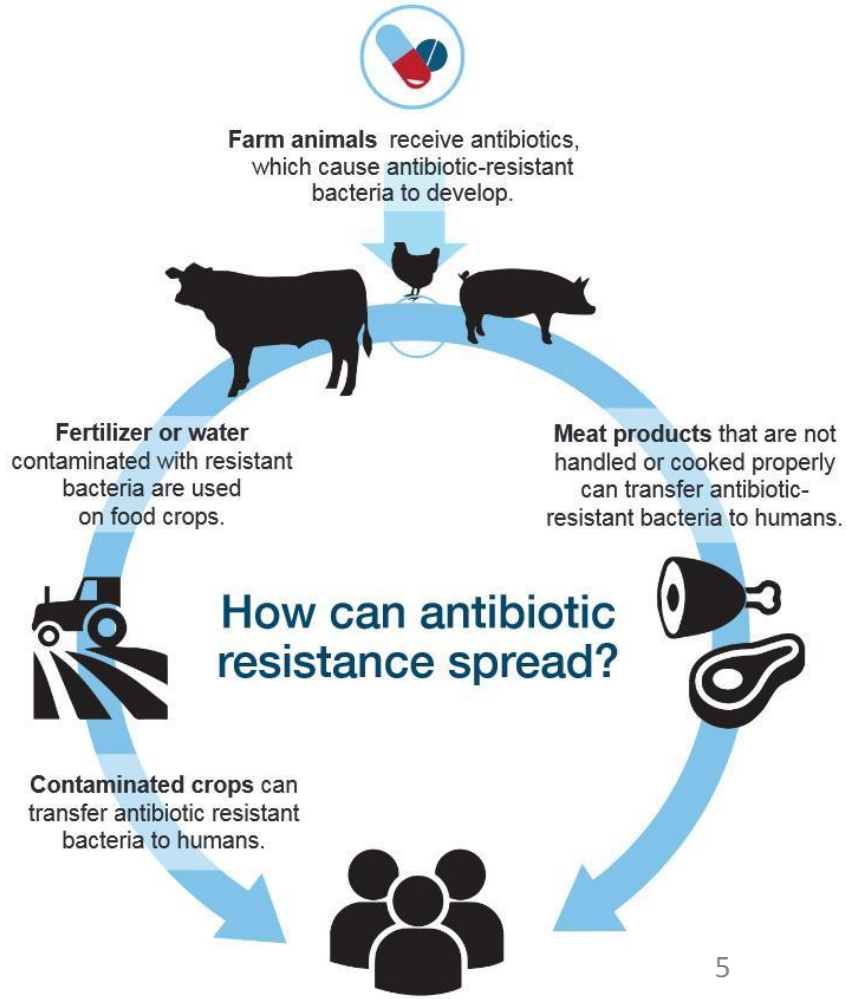


Excerpted from Rasheed *et al*., 2020 Excerpted from : Farmdairy.com

Klebsiella and AMR- Cause of concern



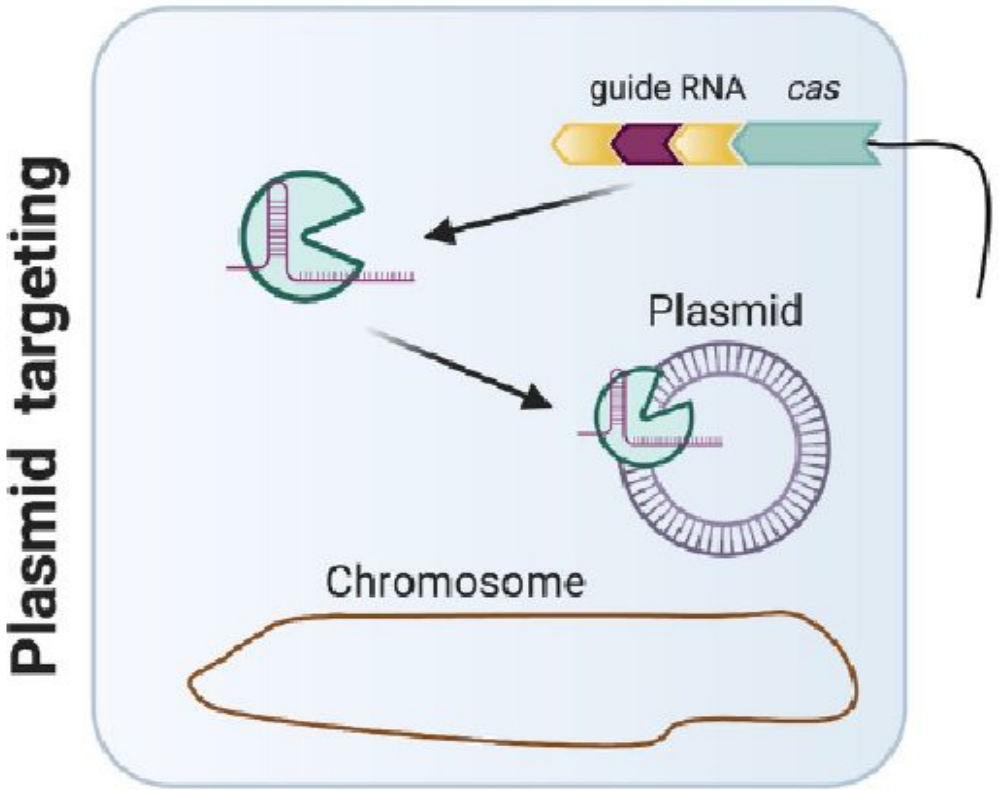
- *K. pneumoniae* can rapidly spread and transfer the AMR genes (Tzouvelekis *et al.*, 2012)
- *Klebsiella* - increasingly resistant to antibiotics, infection by these strains very challenging to treat.
- The enormous exploitation of antibiotics in the field - prevention and growth increased AMR
- "Alternatives to the antibiotics"

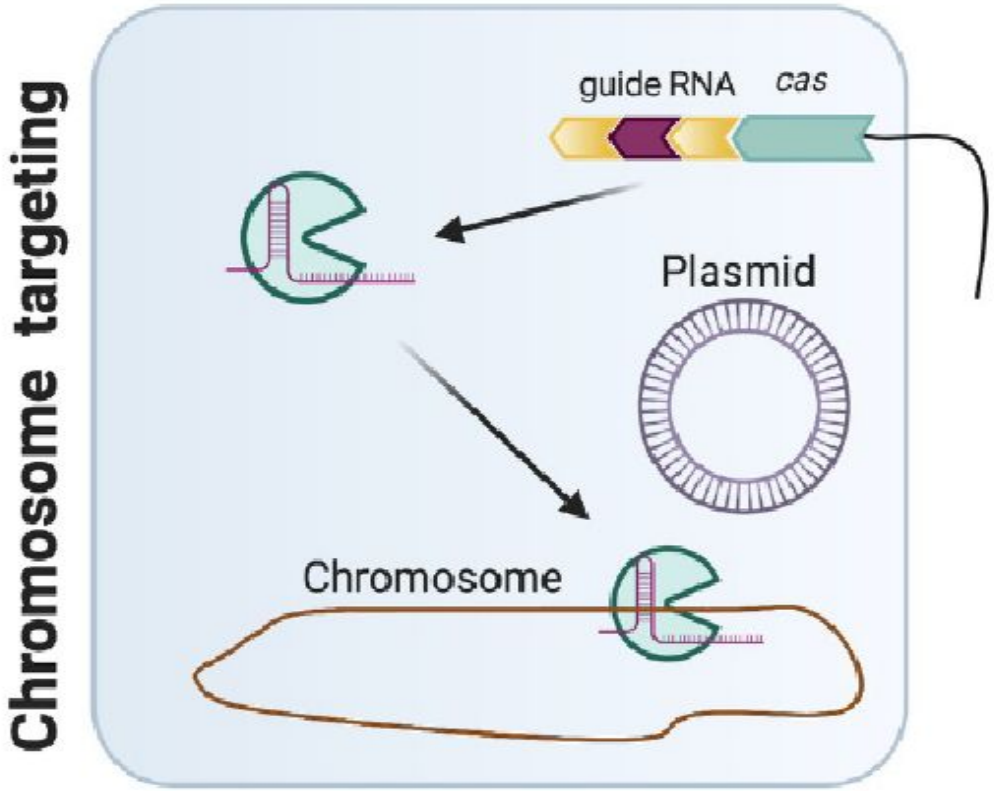


Excerpted from :medicine.net Excerpted from :Inspection.canada.c

Inhibition of AMR bacterial pathogens using CRISPR-Cas9

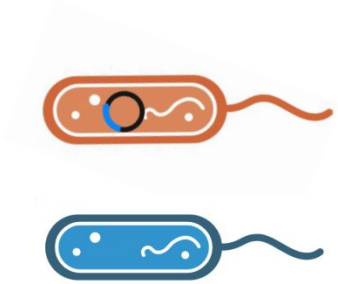
Strategies of CRISPR-Cas9 to target bacteria





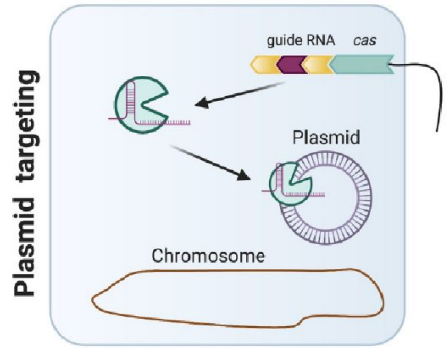
Palacios Araya *et al*., 2021

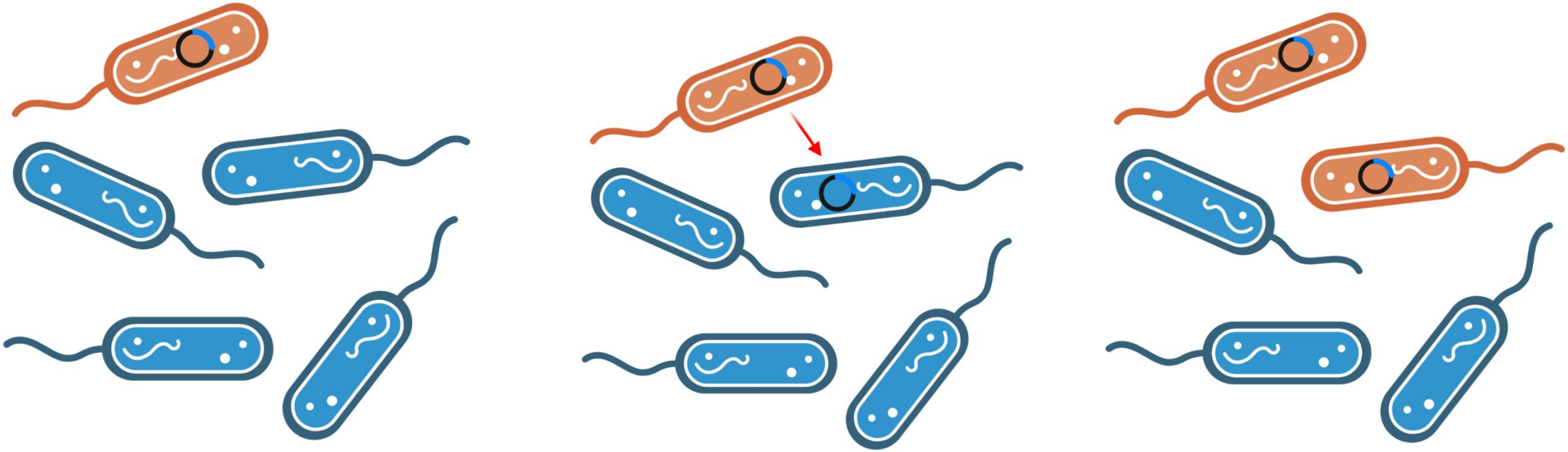
Plasmid targeting strategy



AMR pathogen (e.g. AMR *K. pneumoniae)*

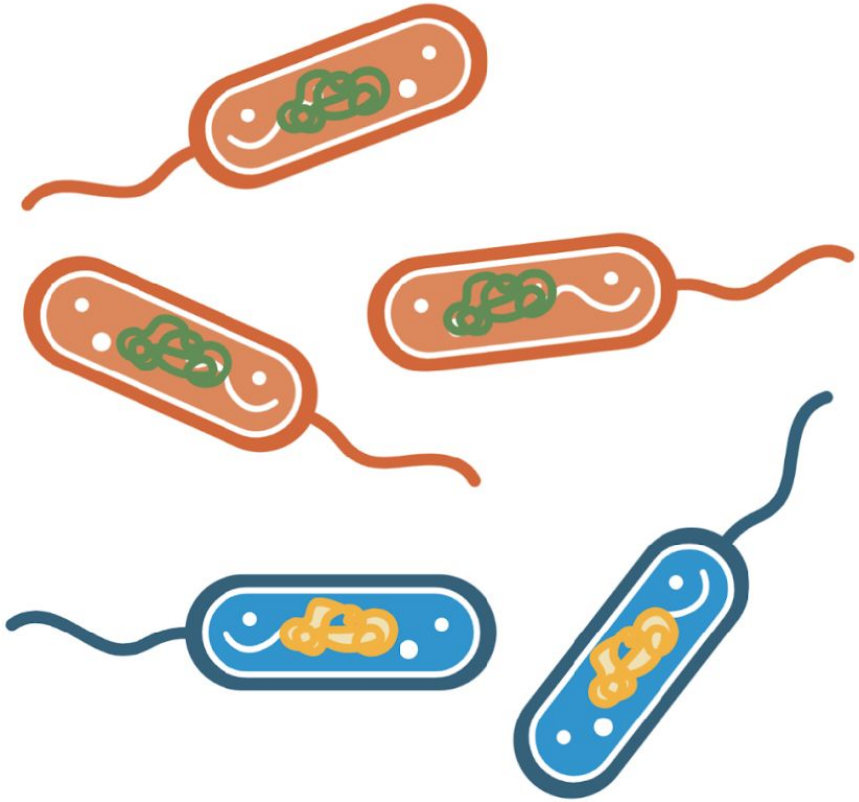
Non-AMR bacteria (e.g. *E. coli* K-12)





- Acquired AMR genes in plasmids as targets
  - Preservation of viability of bacteria

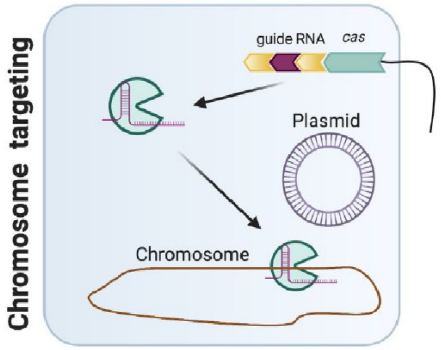
Chromosome targeting strategy



- Intrinsic AMR genes in chromosome as targets

Pathogen (e.g. *K. pneumoniae)*

Commensal bacteria (e.g. *E. coli* K-12)



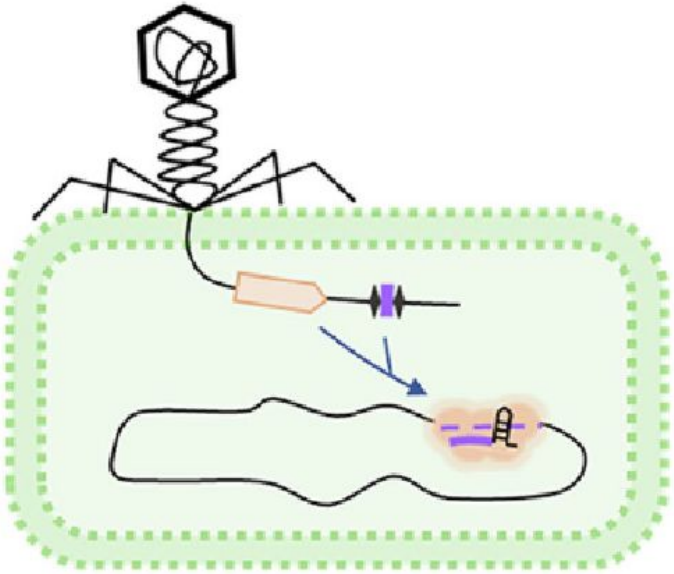
ATCGGGCATTACTT
TAGCCCGTAATGAA

ATCGGGCATT
TAGCCCGTAA

TACCGAA
TGAATGGCTT

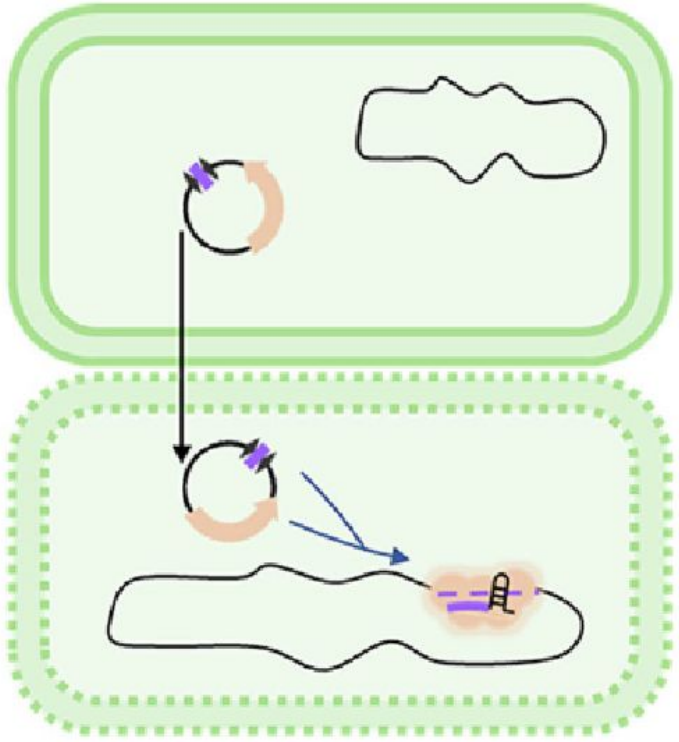
Bacteria cannot repair DNA strand with different ends

Delivery of CRISPR-Cas9 system



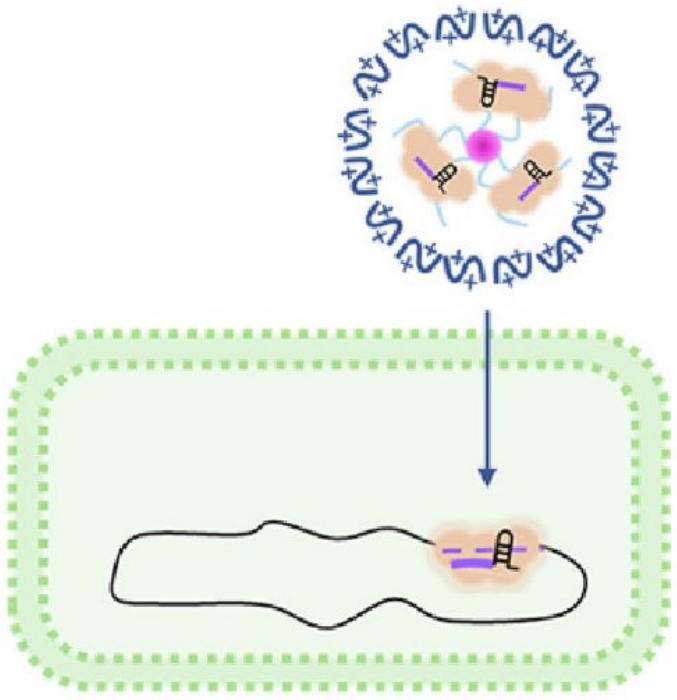
Phage-based delivery

- High specificity
- Less genome capacity



Conjugative delivery

- No specific receptors
- Low delivery efficiency



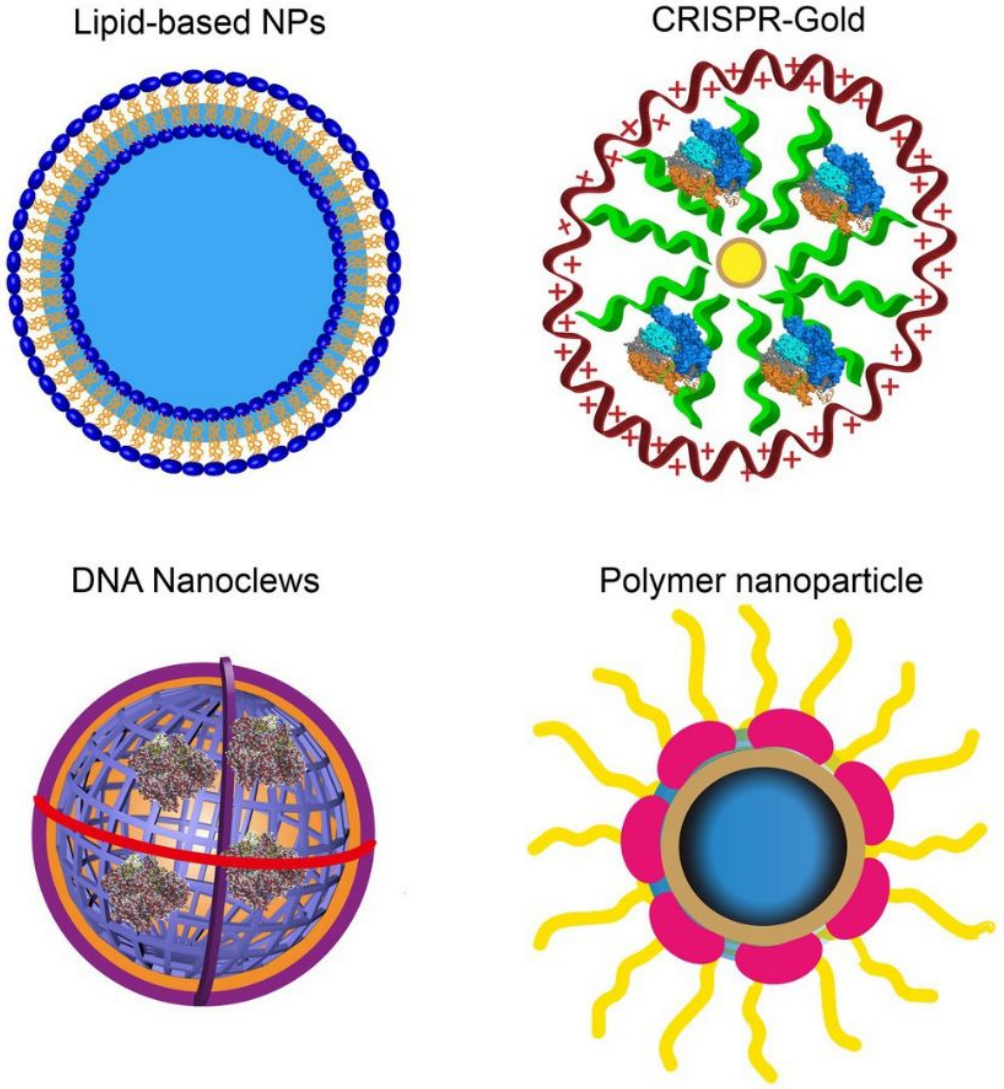
Nanoparticle-based delivery

- High specificity
- **Direct delivery into the targeted bacteria**

Duan *et al.*, 2021

Nanoparticle based CRISPR-Cas9

- ✔ Delivery of Cas effectors and crRNA molecules into the target bacterial cells
- ✔ Biodegradable
- ✔ High efficiency of delivery
- ✔ Low toxicity



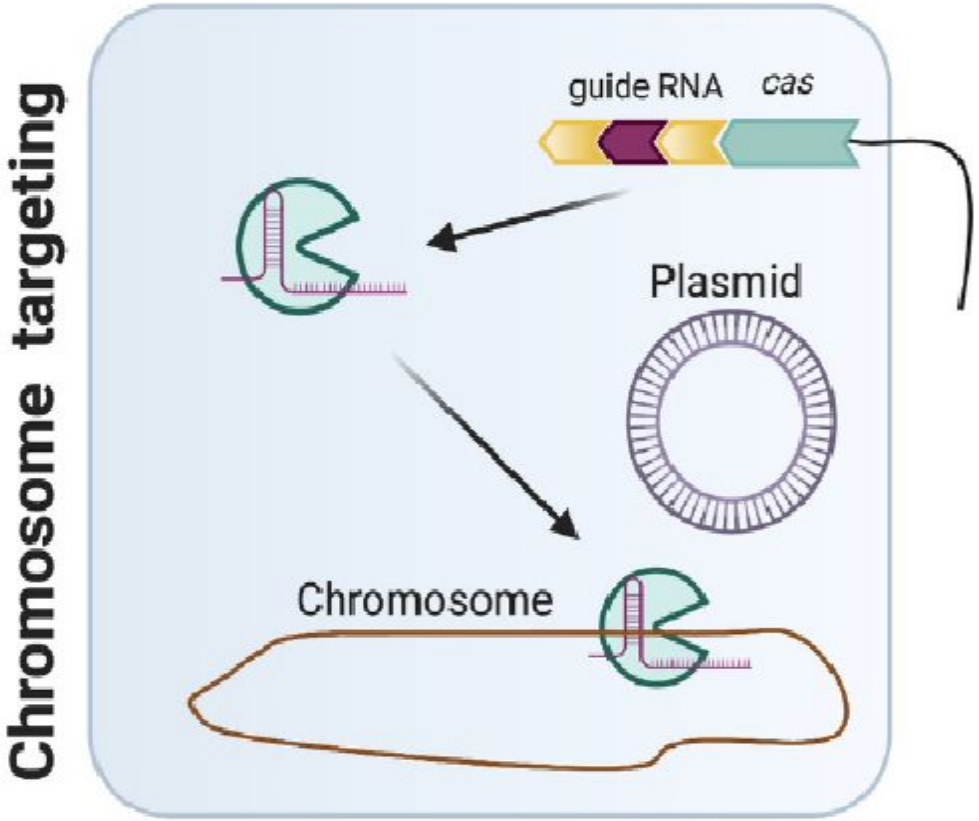
Duan *et al.*, 2021

CRISPR-Cas9 system to target AMR bacterial pathogens

- Multiple studies about CRISPR-Cas9 system targeting ESKAPE group

- Methicillin resistant *Staphylococcus aureus* (MRSA) inhibition

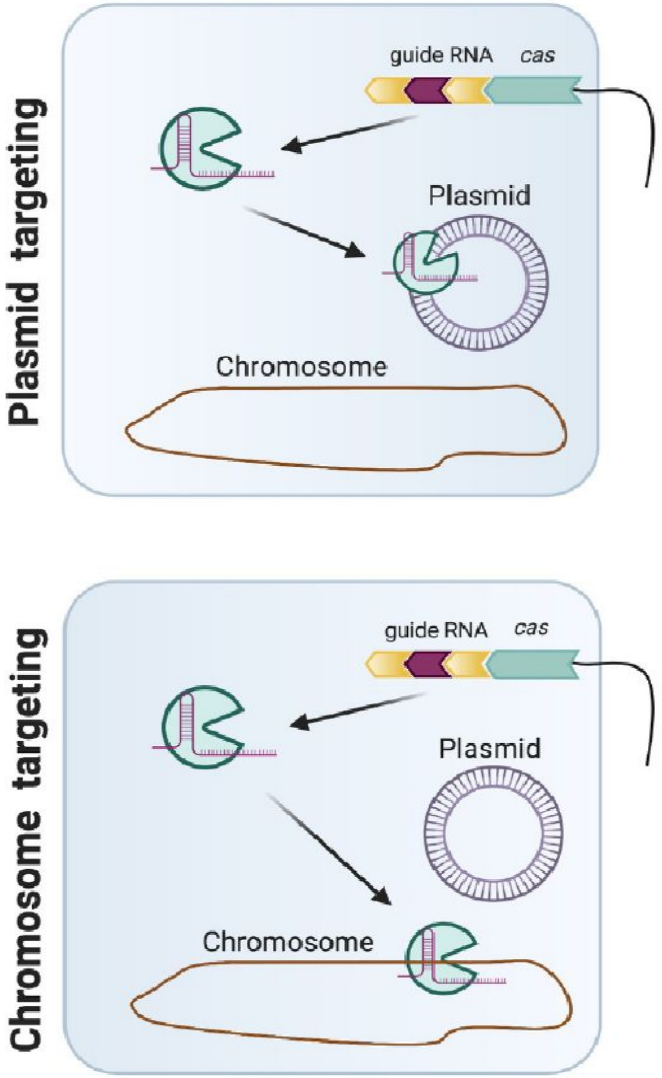
  - Cationic polymer-based nanosized CRISPR complex could kill MRSA by targeting *mec*A (Kang *et al.*, 2017).



Palacios Araya *et al.*, 2021

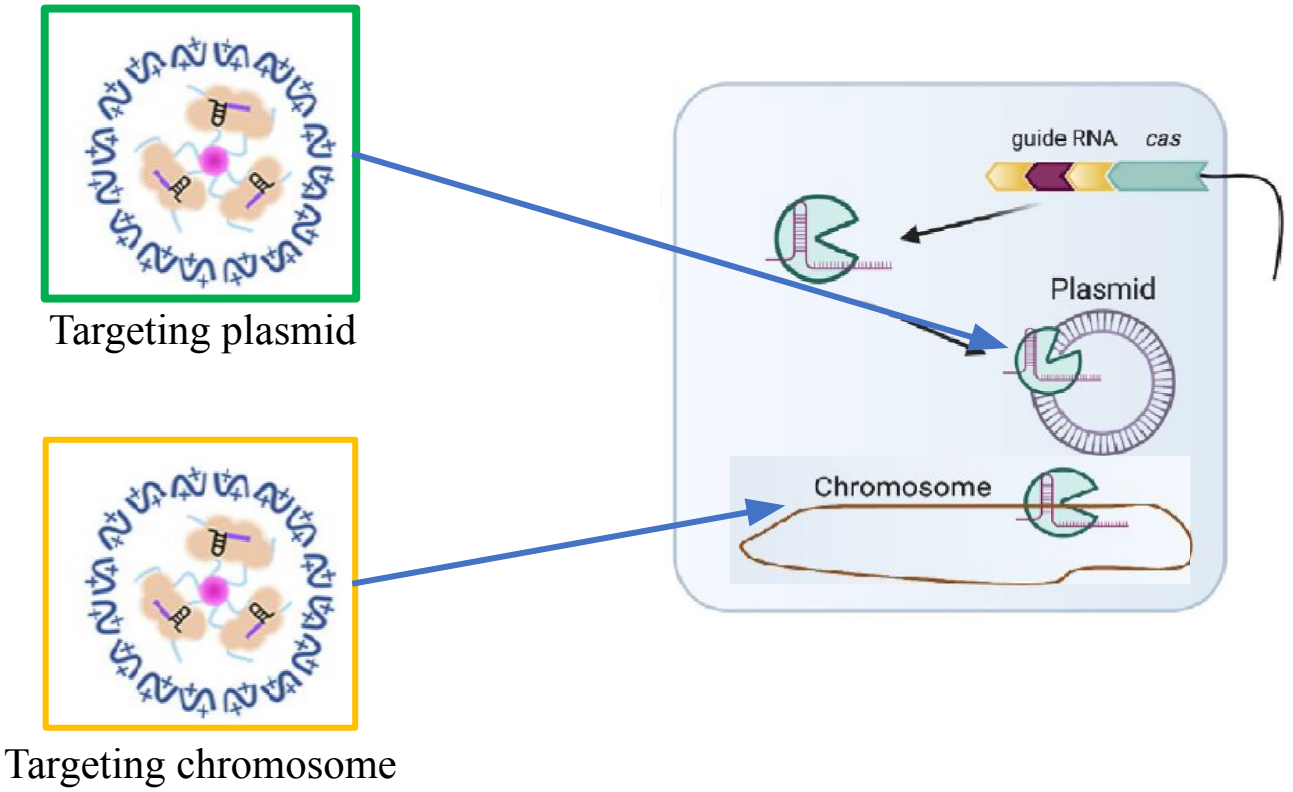
What is the best target for AMR *K. pneumoniae* inhibition?

- Studies on CRISPR-Cas9 targeting *K. pneumoniae*
  - Mostly targeting acquired resistance genes in plasmids
- One study targeted the intrinsic resistance gene in chromosome in the pathogen ($bla_{\text{SHV-1}}$)
- Targeting AMR gene in chromosome or plasmids?
- **Applying both strategies**
- **Prophylactic**



Palacios Araya *et al*., 2021

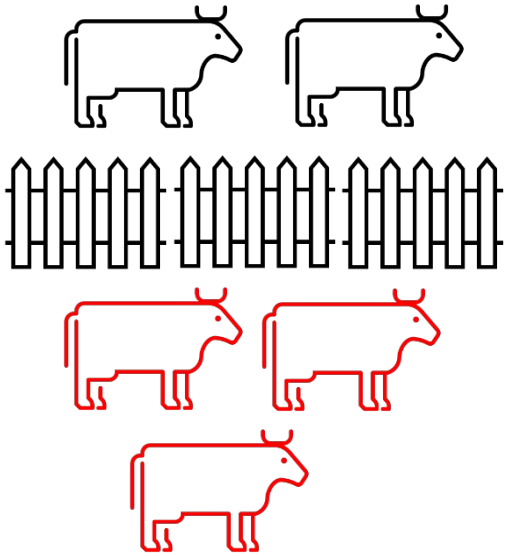
Kill two birds with one stone



- **Targeting both chromosome and plasmid – kill the *K. pneumoniae* and stop the spread of AMR genes**

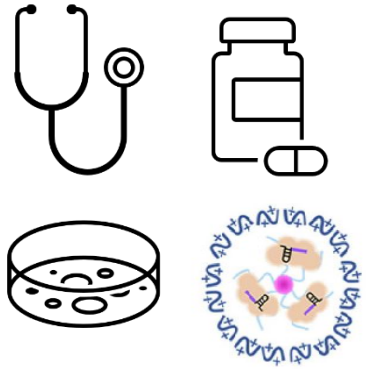
Prophylactic treatment

Culling

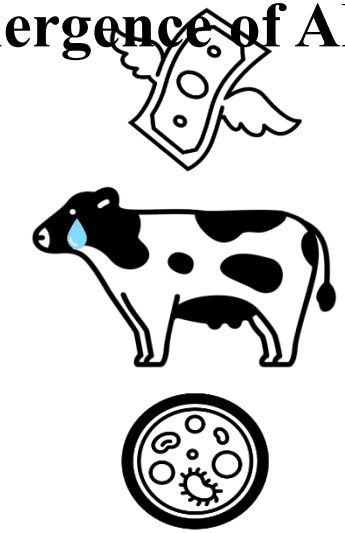


Loss of milk

Diagnosis and treatment



Financial loss, decreased animal welfare and emergence of AMR



- **Prophylactic treatment would secure the sustainability**

Challenges and future perspectives


Best target genes in K. pneumoniae
Encapsulation
Safety and delivery efficiency
Best delivery method into dairy udder
Policies on nanoparticles in dairy milk
Perspective of consumers and farmers
CHALLENGES

Future perspectives

- More CRISPR-Cas types could be explored for versatile antimicrobial applications

- Development of "smart" antibiotics using CRISPR-Cas approaches
  - ✔ Differentiation between beneficial and pathogenic microorganisms
  - ✔ Prevention of the spread of AMR genes
  - ✔ Elimination of MDR pathogens

- Modifying the nanoparticles with cell penetrating peptides, - enhance internalization
  - ✔ Chronic infections can be resolved by targeted co-delivery of **drug and gene** to host cells, since nanoparticles can be tailored in multiple ways

THANK YOU!!!