Plasmid curing as a strategy to combat antibiotic resistance

Joint Graduate Seminar
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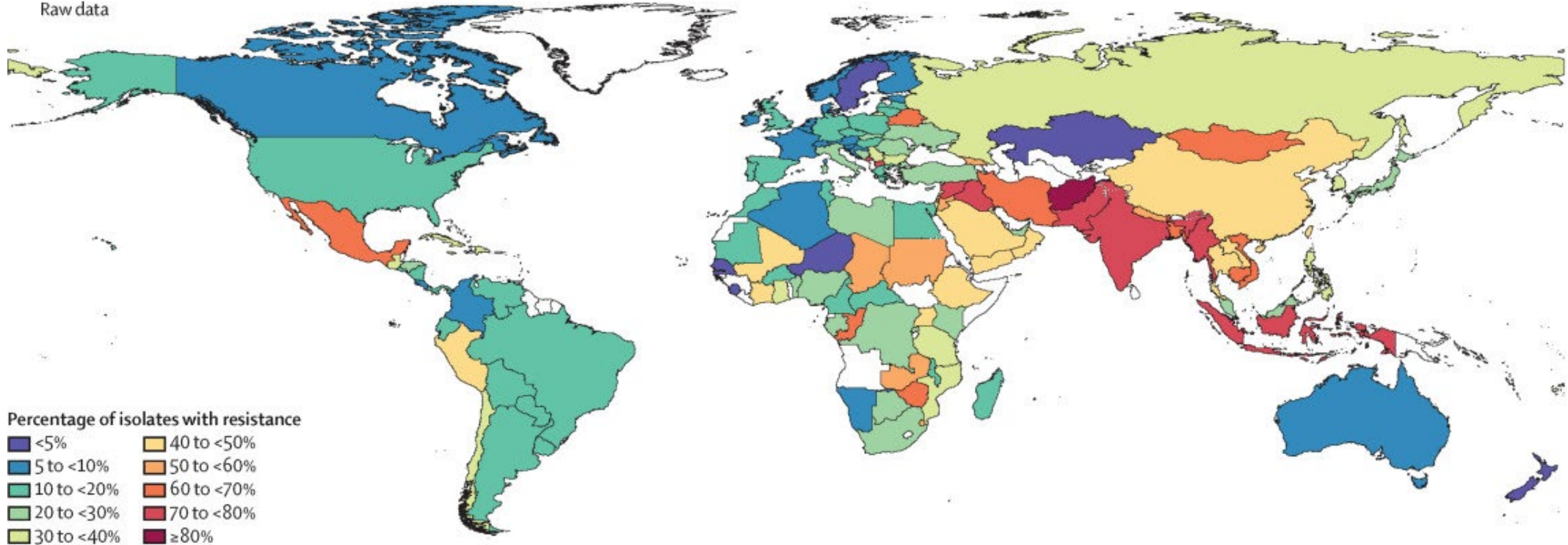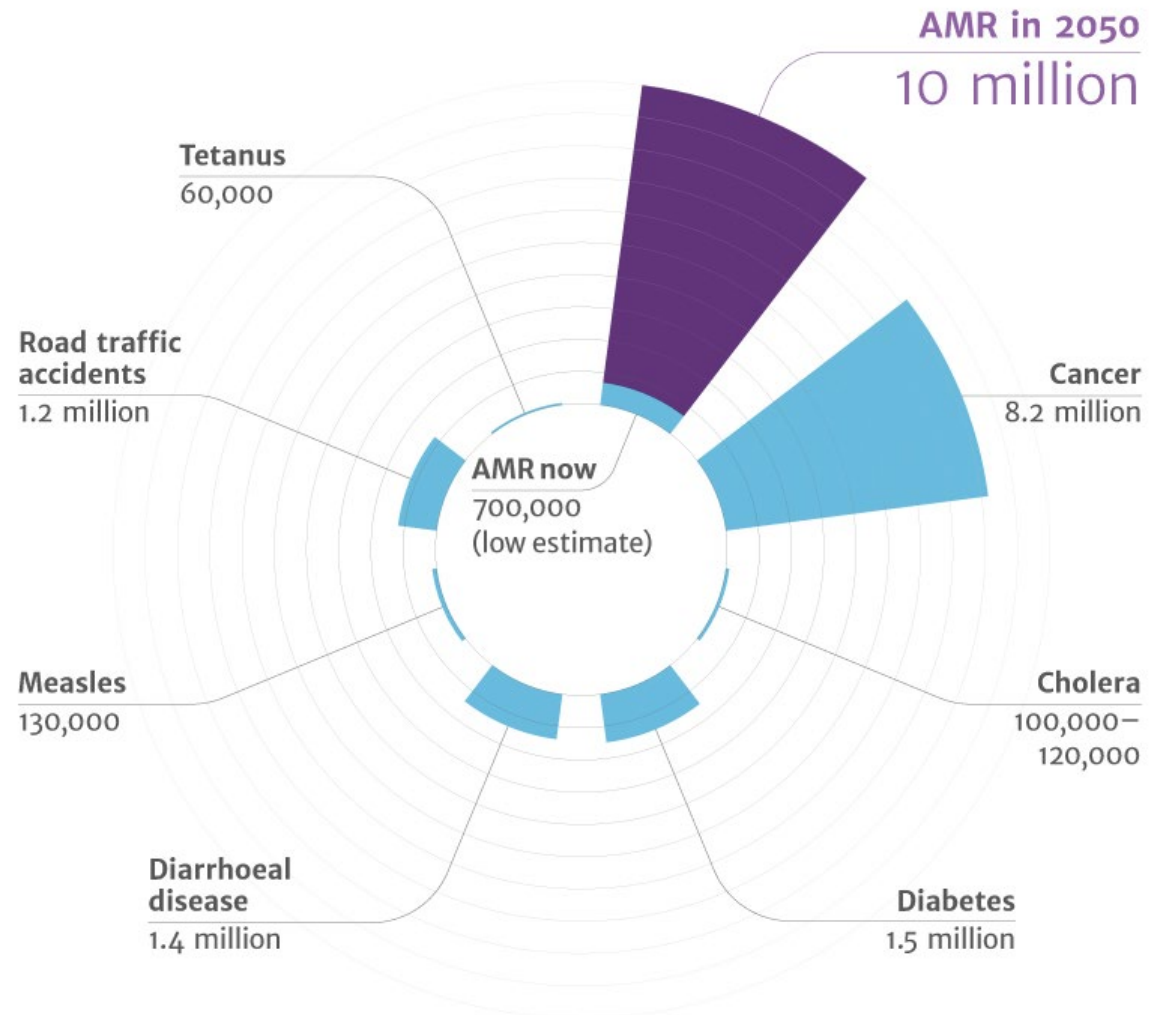
Outline

1.1 Threats of Antimicrobial Resistance (AMR)

Fig 1. Raw data for the percentage of pathogen isolates that are resistant by country and territory, 2019. By Antimicrobial Resistance Collaborators. *The Lancet*. 2022. (CC BY 4.0)

1.1 Threats of AMR

AMR in 2050
10 million

Tetanus
60,000

Road traffic accidents
1.2 million

AMR now
700,000
(low estimate)

Cancer
8.2 million

Measles
130,000

Cholera
100,000–
120,000

Diarrhoeal disease
1.4 million

Diabetes
1.5 million

- The World Health Organization (WHO) lists AMR among top 10 threats for global health.

- Deaths directly attributed to AMR infections in 2019: 1. 27 million [1]

Fig 2. Predicted mortality from AMR compared to common causes of death today. By *Review on Antimicrobial Resistance* 2016 *(CC BY 4.0)*

[1] Antimicrobial Resistance Collaborators. The Lancet. 2022

1.2 Plasmids: Primary vehicle of Antibiotic Resistance Genes (ARGs)

- **Plasmid**:
- Small, extrachromosomal DNA molecule
- Can replicate independently

Fig 3. Genetic location of ARGs predicted from the nine Nanopore metagenomic datasets of environmental samples. By Che Y et al. Microbiome. 2019. (CC BY 4.0)
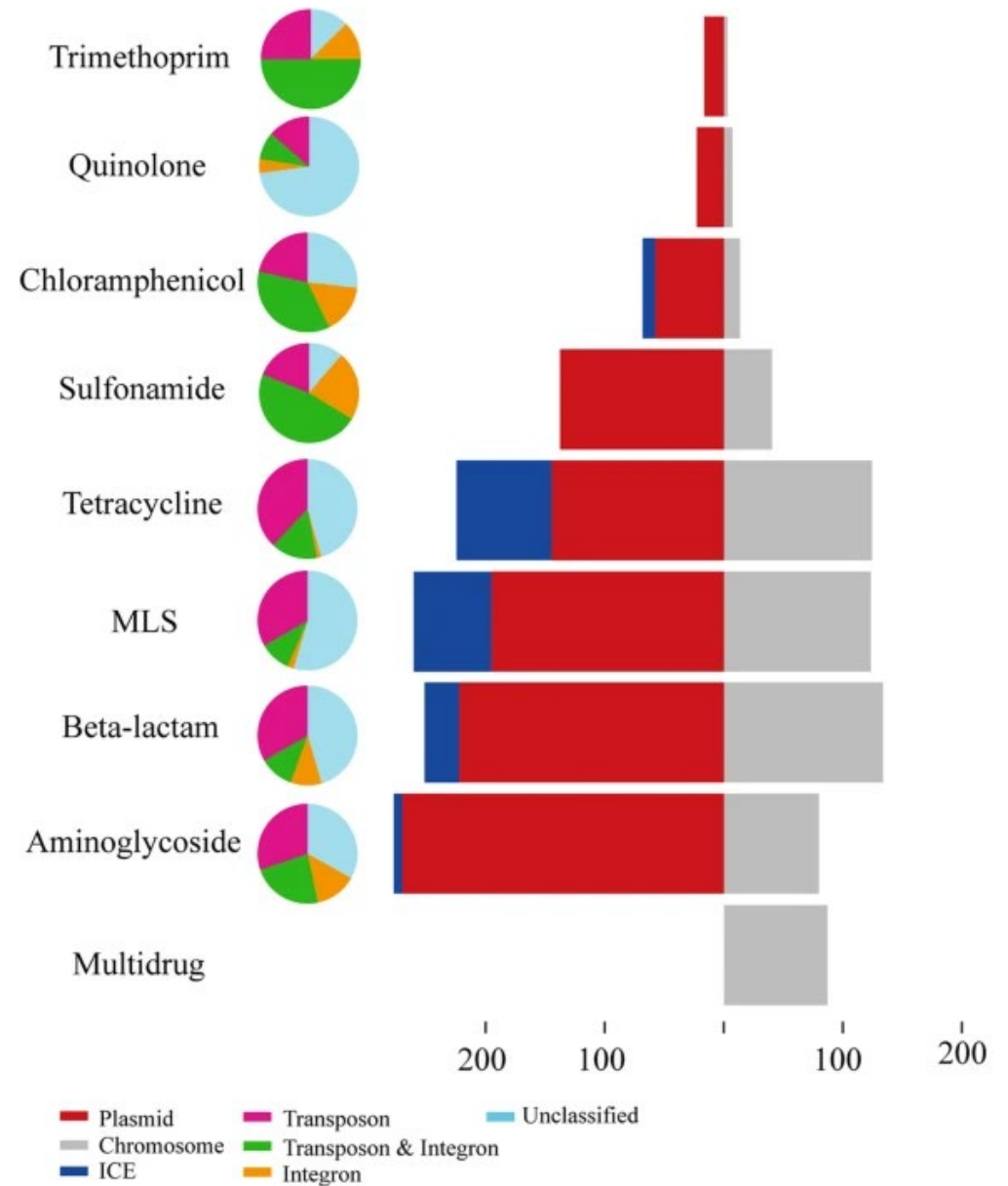
1.2 Plasmids: High Prevalence in Microbes and Wide Geographic Distribution

- **Highly prevalent across phyla** [2]:
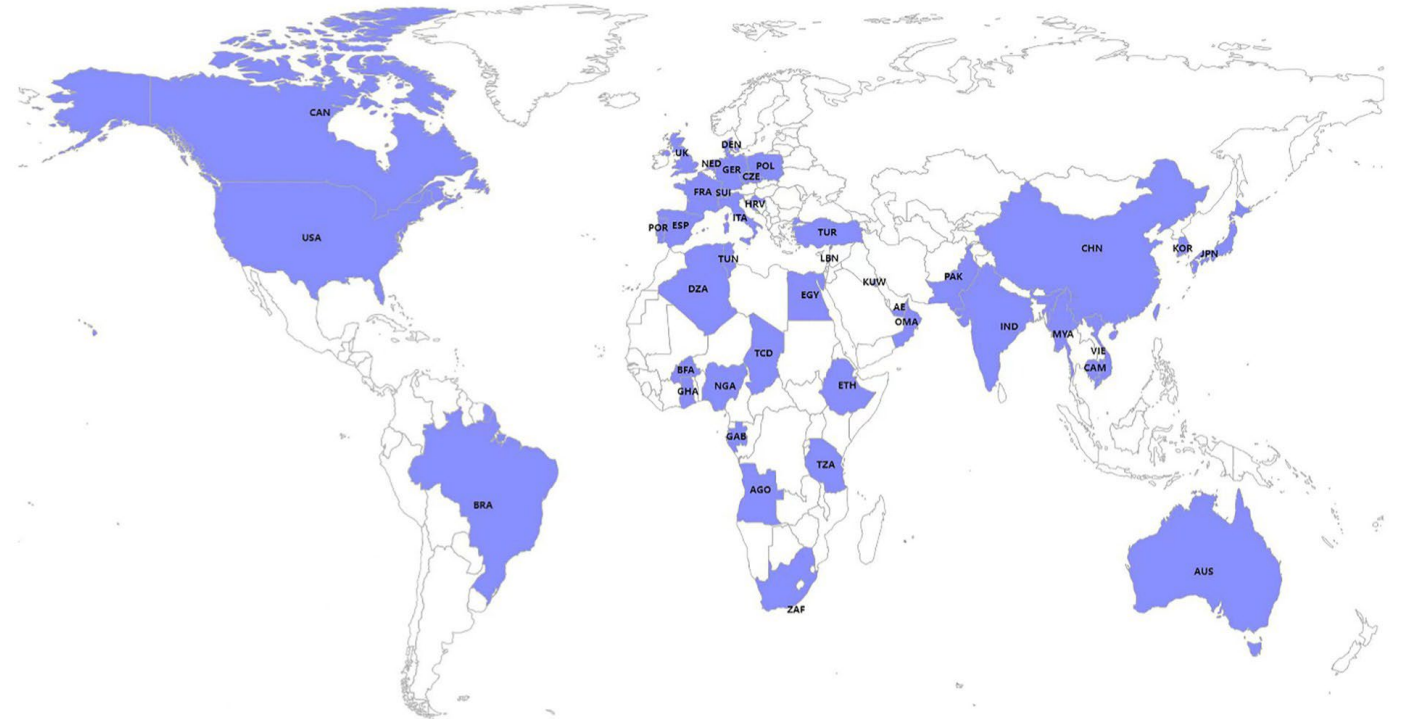
Chloroflexi: 40-49%

Proteobacteria: 60-79%

Eurycharchaeaota: 80-89%

Bacteroidetes: >= 90%

Firmicutes: >= 90%

…

[2] Rodríguez-Beltrán J *et al. Nat Rev Microbiol. 2021.*

Fig 4. Distribution of isolates harboring IncX3 plasmids. By Gro X *et al. Front. Microbiol. 2022.* (CC BY 4.0)

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2. To Control AMR Using Plasmid Curing

Plasmid curing:

(1) remove the vehicle of ARGs from a population

(2) keep the bacterial community intact

Potential application:

(1) Patients prior to surgery

(2) International travellers
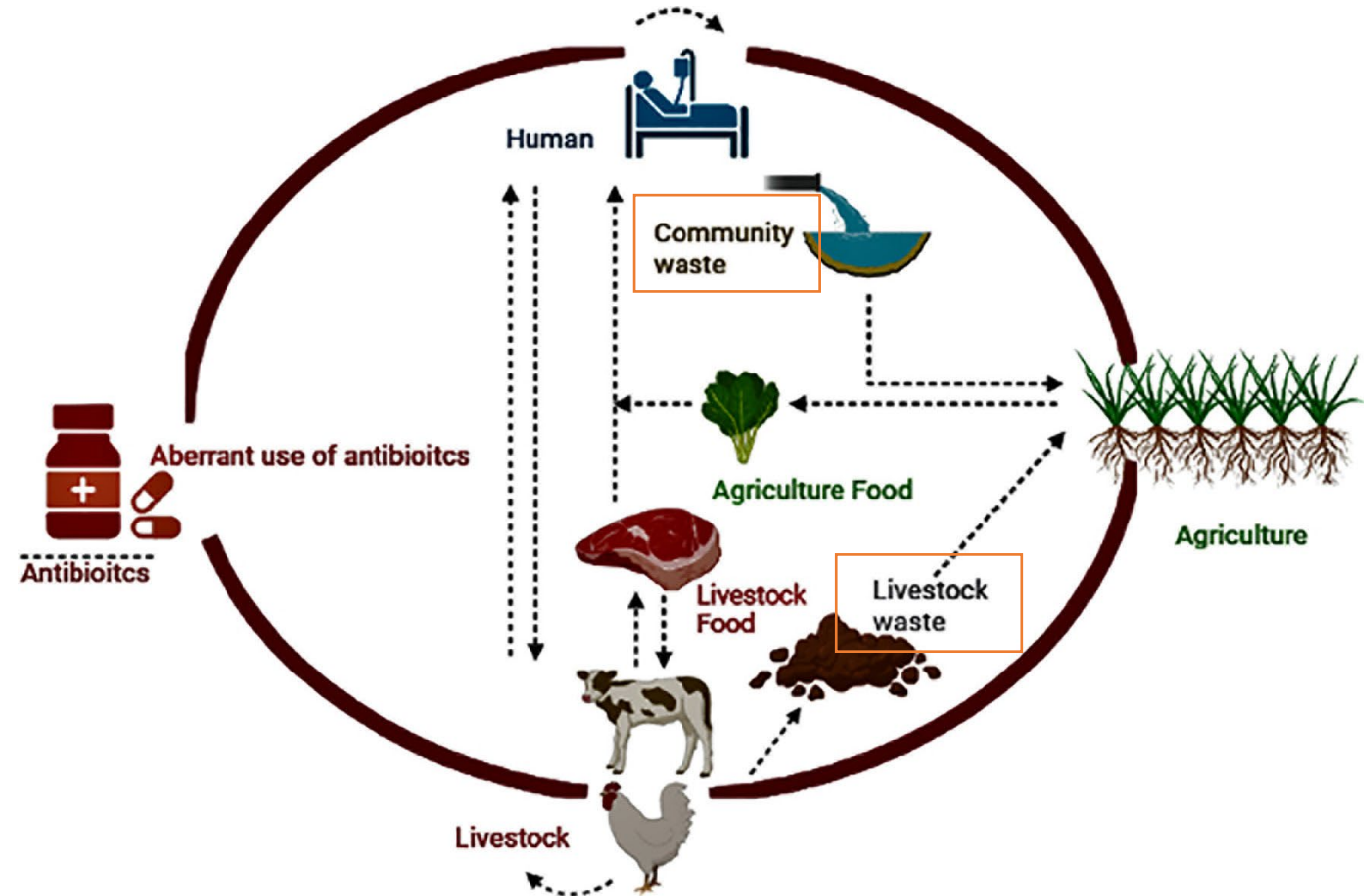
(3) In perspective of "One Health": sewage, animal waste …

Fig 5. Potential One Health drivers associated with AMR. By Aslam B et al. Front. Cell. Infect. Microbiol. 2021. (CC BY 4.0)

2. To Control AMR Using Plasmid Curing

Table 1 Various strategies and representative agents of plasmid curing

Strategies	Curing agent	Species	Plasmid cured	Reference	Limitations
Detergents	Bile salts (10-15%)	*Salmonella enterica* serovar Typhimurium	Virulence plasmid pSLT	García-Quintanilla M *et al.* 2006	Too high concentration required to treat human and animals
Natural products	Plumbagin	*K. pneumoniae*	Drug-resistant plasmids	Patwardhan RB *et al.* 2015	Low efficiency
Conjugation inhibitors	TraE inhibitor	*Brucella abortus*	pKM101	Paschos A *et al.* 2011	Needed to determine the *in vivo* safety and efficacy
Phage therapies	Phage PRD1	*Escherichia coli*, *Salmonella* spp.	Plasmids RP4 and RN3	Jalasvuori M *et al.* 2011	(1) Unclear regulatory pathways (2) Bacterial resistance
Incompatibility -based curing	pCURE plasmids	*E. coli*	IncF and IncP$_{-1\alpha}$ plasmids	Hale L *et al.* 2010	(1) Limited delivery (2) May acquire ARGs
CRISPR-Cas	CRISPR/Cas9 -plasmid	*E. coli*	ESBL plasmids	Kim JS *et al.* 2016	Bacterial resistance

3. A Promising Strategy: CRISPR-Cas Systems
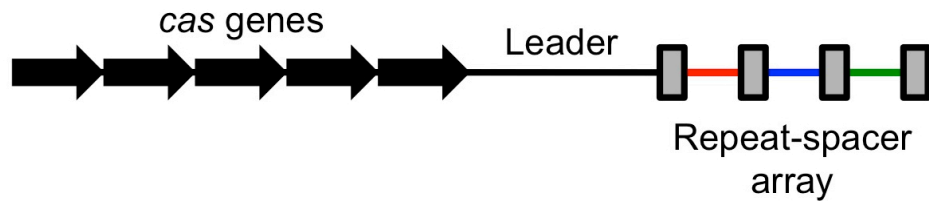
- **CRISPR-Cas systems:**
- CRISPR: Clustered Regularly Interspersed Short Palindromic Repeats
- Induce a specific lethal cut in the targets

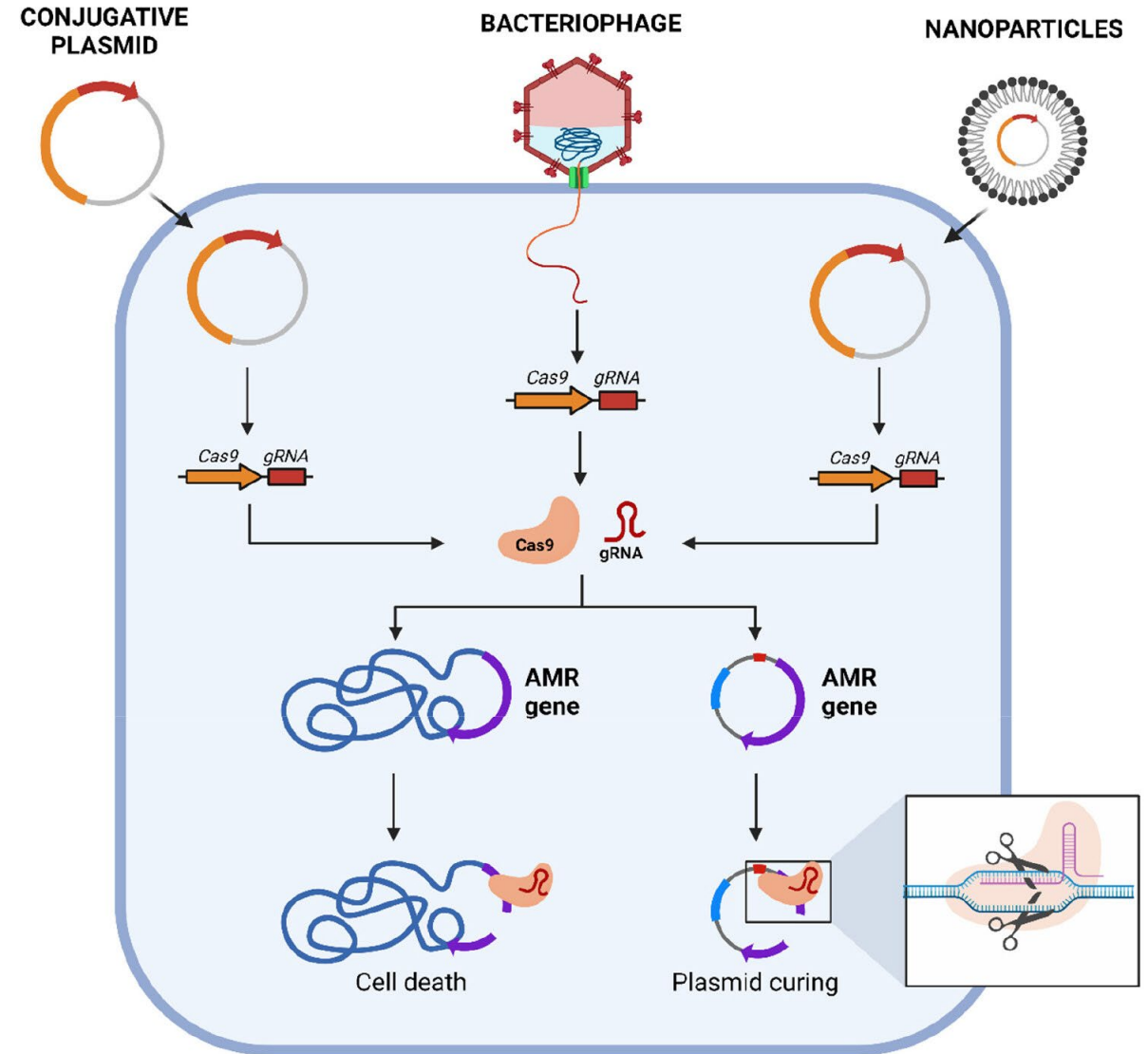**Spacer:** sequence regions between each repeat region, acquired from the target sequences

Fig 6 a Simplified diagram of a CRISPR locus From Wikipedia (CC BY-SA 4.0). b CRISPR-Cas system antimicrobials: mechanisms of action and delivery. By Mayorga A *et al. ACS Infectious Diseases*. 2023. (CC BY 4.0)

4. Case Study: Curing IncF plasmids in Multidrug Resistant (MDR) *Klebsiella pneumoniae*

Study 1 [2]

381 high risk clinical strains

Type I-E CRISPR-Cas systems (-)
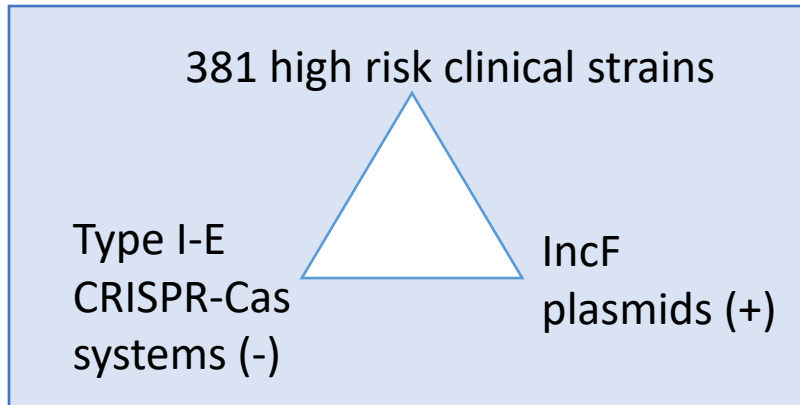
IncF plasmids (+)

415 distinct spacers

Study 2 [3]

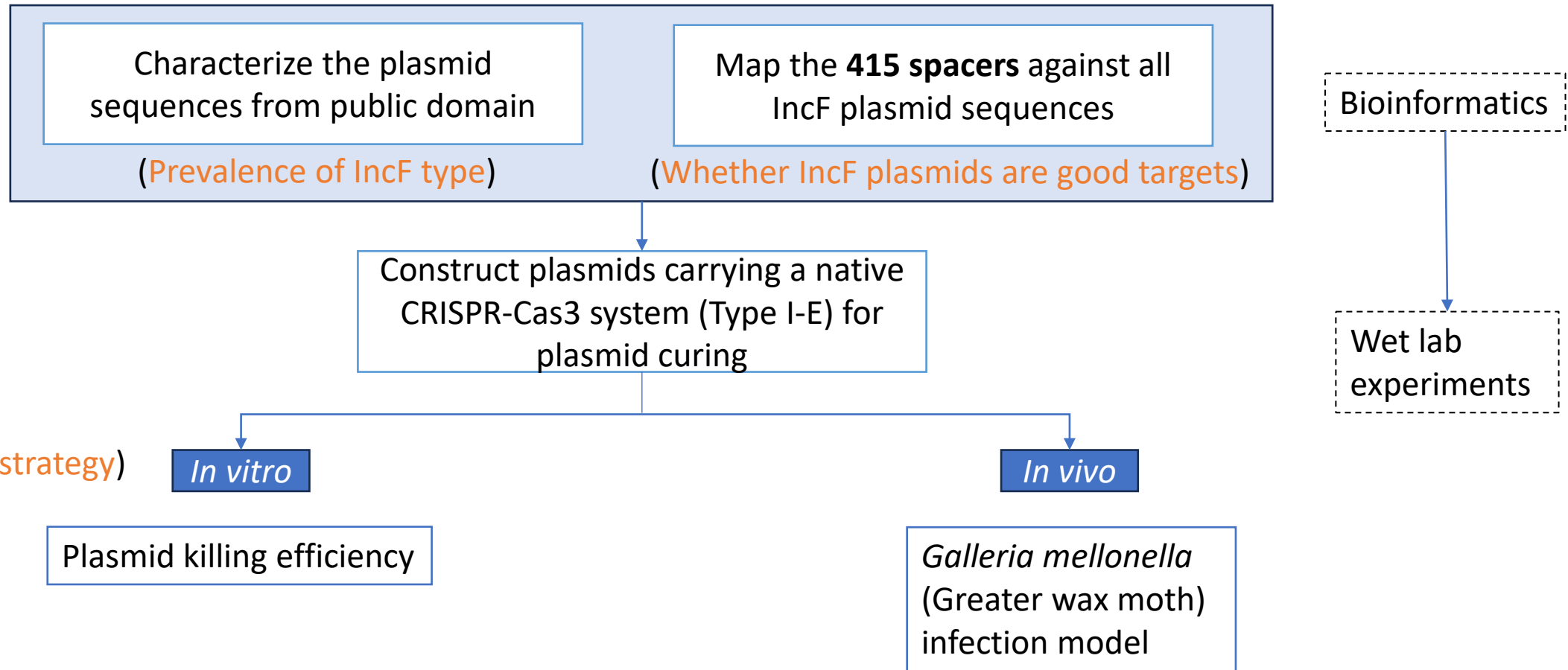(1) What's the prevalence of the IncF type in the AMR plasmids?

(2) Are IncF plasmids good targets for native CRISPR-Cas systems?

(3) How efficient is the CRISPR-Cas strategy in curing IncF plasmids ?

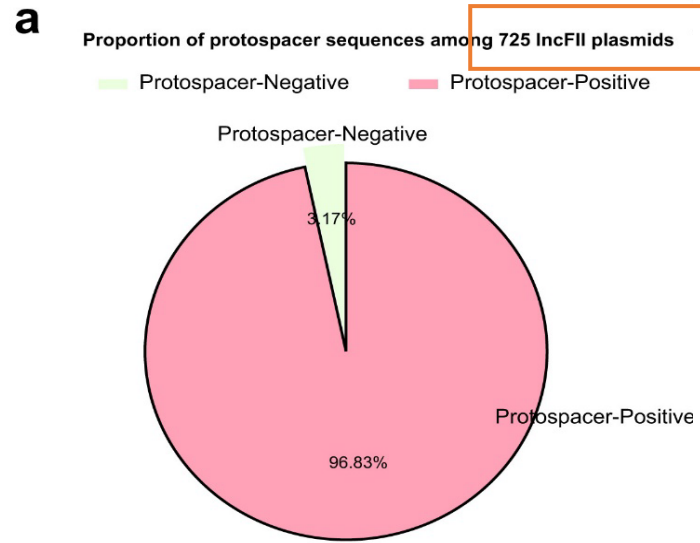[2] Tang Y *et al*. Journal of Antimicrobial Chemotherapy. 2020
[3] Zhou Y *et al*. EBioMedicine. 2023

4. Case Study: Curing IncF plasmids in Multidrug Resistant (MDR) *Klebsiella pneumoniae*

Characterize the plasmid sequences from public domain

(Prevalence of IncF type)

Map the **415 spacers** against all IncF plasmid sequences

(Whether IncF plasmids are good targets)

Bioinformatics

Construct plasmids carrying a native CRISPR-Cas3 system (Type I-E) for plasmid curing

Wet lab experiments

(Efficiency of the strategy)

In vitro

In vivo

Plasmid killing efficiency

*Galleria mellonella* (Greater wax moth) infection model

Prevalence of IncFII plasmids

- Determined by PlasmidFinder tool (sequence identity > 90%, coverage > 90%)

- 3117 plasmids from 932 completely sequenced *K. pneumoniae* in public domain
  - **725** IncFII plasmids (23.3%, 725/3117)

- 1439 AMR plasmids
  - 554 AMR IncFII plasmids (38.5%, 554/1439)
  - 68 AMR IncX plasmids (4.7%, 68/1439)

a Proportion of protospacer sequences among 725 IncFII plasmids

- Protospacer-Negative
- Protospacer-Positive

Protospacer-Negative 3.17%

Protospacer-Positive 96.83%

b Distribution of various protospacers among 725 IncFII plasmids

All 415 spacers were mapped against the 725 IncFII plasmids to retrieve protospacers

Protospacer: sequence region in the target DNA/RNA molecule, complementary to the spacer
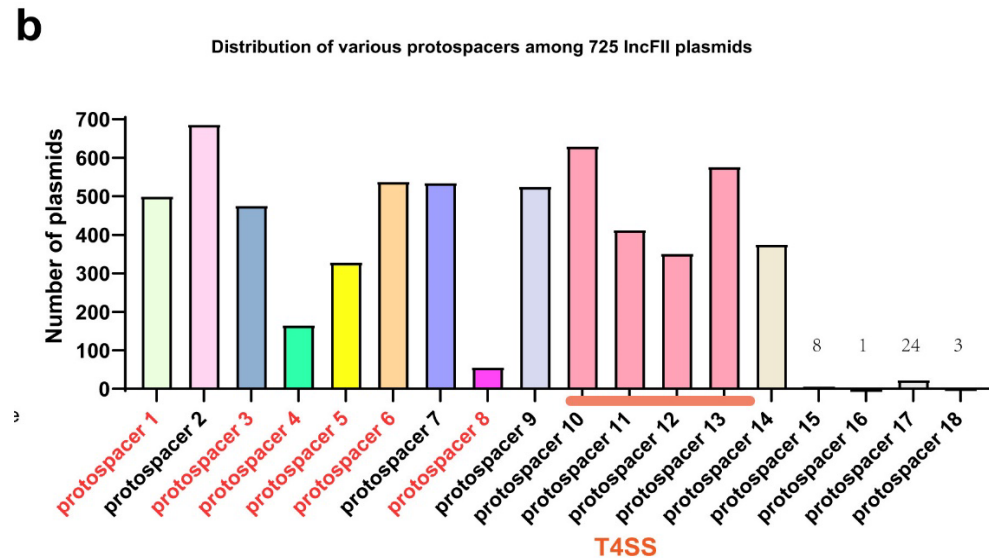
A combination of the five most abundant protospacers could cover > 70% of IncFII plasmids

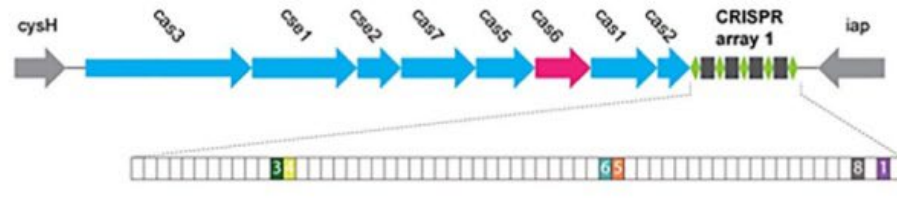Fig 7a-b. Characteristics of protospacers on IncFII plasmids. By Zhou Y *et al. EBioMedicine*. 2023 (CC-BY-NC-ND 4.0)
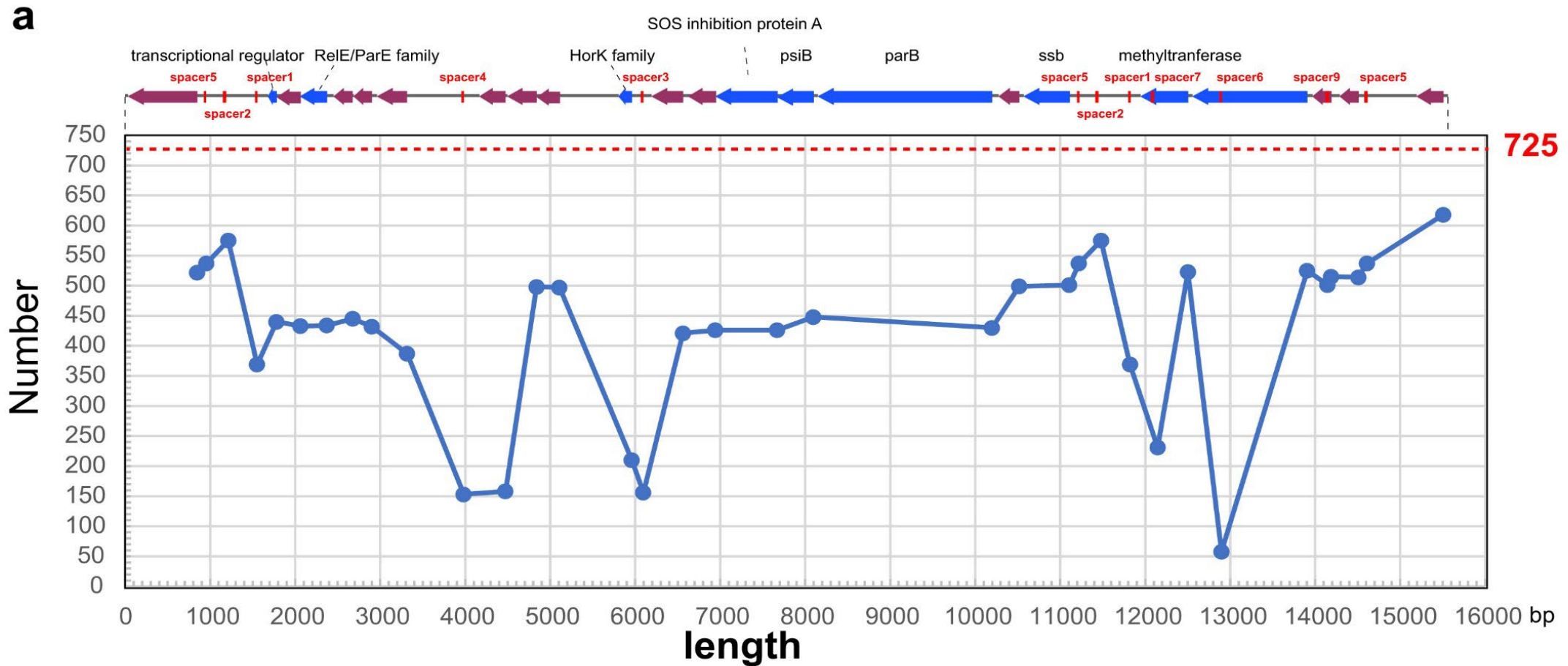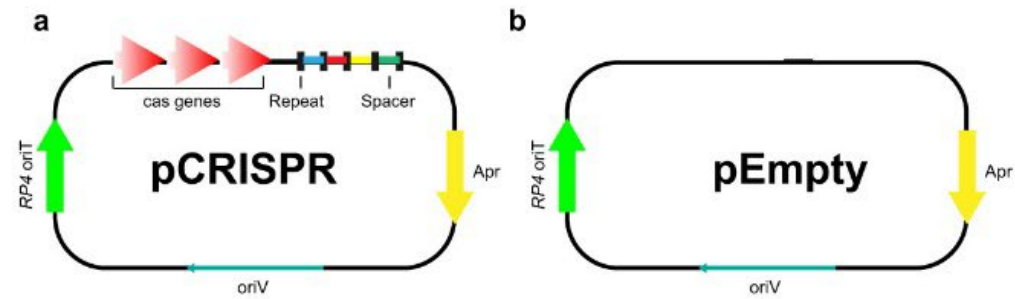
Reference plasmid:
IncFII-**p187-2** (in the presence of bla_{KPC}) obtained from a high-risk *K. pneumoniae* ST11 strain

Fig 8. Protospacers targeting the IncFII plasmids. By Zhou Y *et al. EBioMedicine*. 2023 (CC-BY-NC-ND 4.0)

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CRISPR-Cas3 (Type I-E) system:
Amplified from *K. pneumoniae* KP8 (CP025636.1)
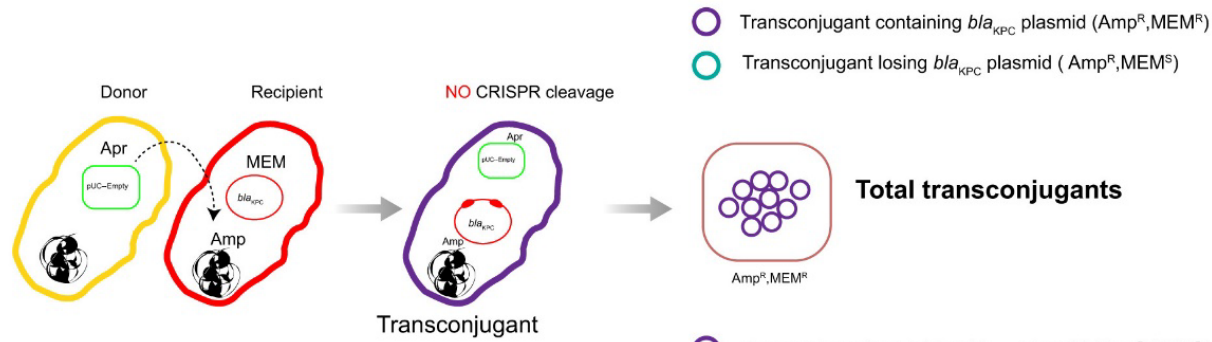Carrying six matched spacers – spacer3,4,6,5,8,1

Structure of plasmids:
- *oriV*: high-copy pBR322 origin of replication, responsible for Cas-operon overexpression
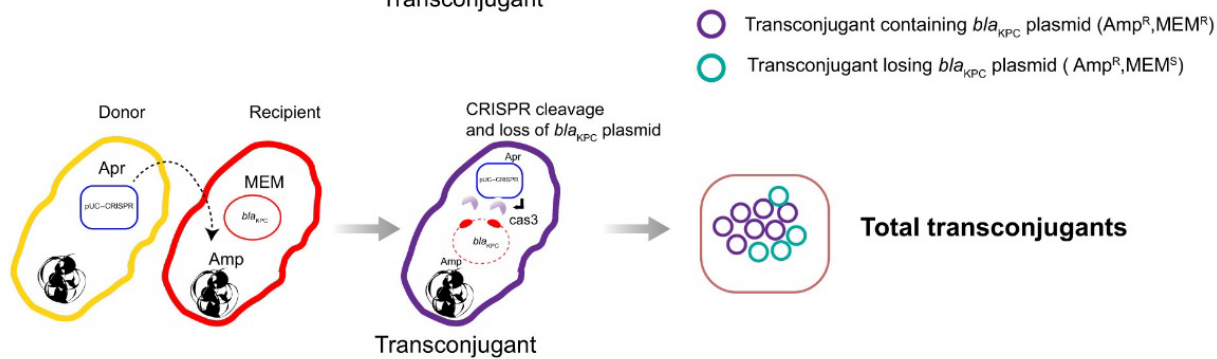- *oriT*$_{RP4}$: contributed to broad-host RP4 conjugation

Fig 9a. Conjugative delivery of endogenous CRISPR-Cas3 platform. By Zhou Y *et al. EBioMedicine.* 2023 (CC-BY-NC-ND 4.0)

b

i

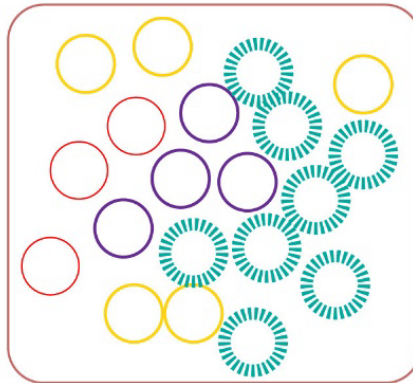**ii**

iii

Donor: *E. coli* S17-1

Recipients:
JS187: *K. pneumoniae* ST11 with an IncFII$_K$ type *bla*$_{KPC-2}$ plasmid

HD5914: *K. pneumoniae* ST751 with an IncFII (pHN7A8) type *bla*$_{KPC-2}$ plasmid
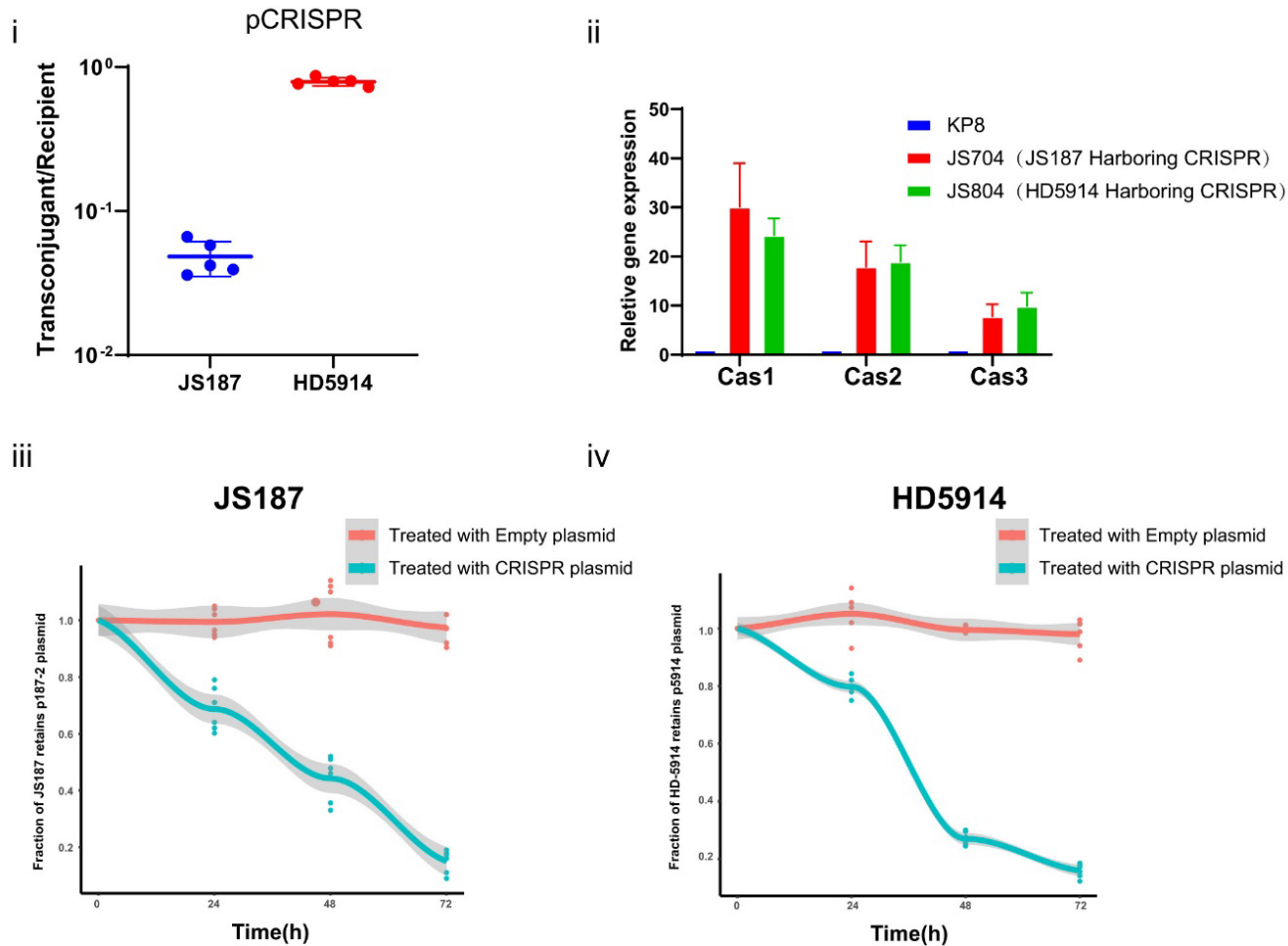
Negative control: pEmpty

Markers:
- Apr (apramycin) – donor
- Amp (ampicillin) – *K. pneumoniae*
- MEM (meropenem) – IncFII *bla*$_{KPC-2}$

Fig 9b. Conjugative delivery of endogenous CRISPR-Cas3 platform. By Zhou Y *et al. EBioMedicine*. 2023 (CC-BY-NC-ND 4.0)

c

(i) Conjugation frequency:
No. total transconjugants (Apr^R, Amp^R)/total recipient cells (Amp^R)

(ii) The expression of CRISPR in *K. pneumoniae* was determined by real-time PCR

(iii-iv) The plasmid killing efficiency:
No. cells (MEM^R, Amp^R)/No. cells (Amp^R)

Fig 9c. Conjugative delivery of endogenous CRISPR-Cas3 platform. By Zhou Y *et al. EBioMedicine*. 2023 (CC-BY-NC-ND 4.0)
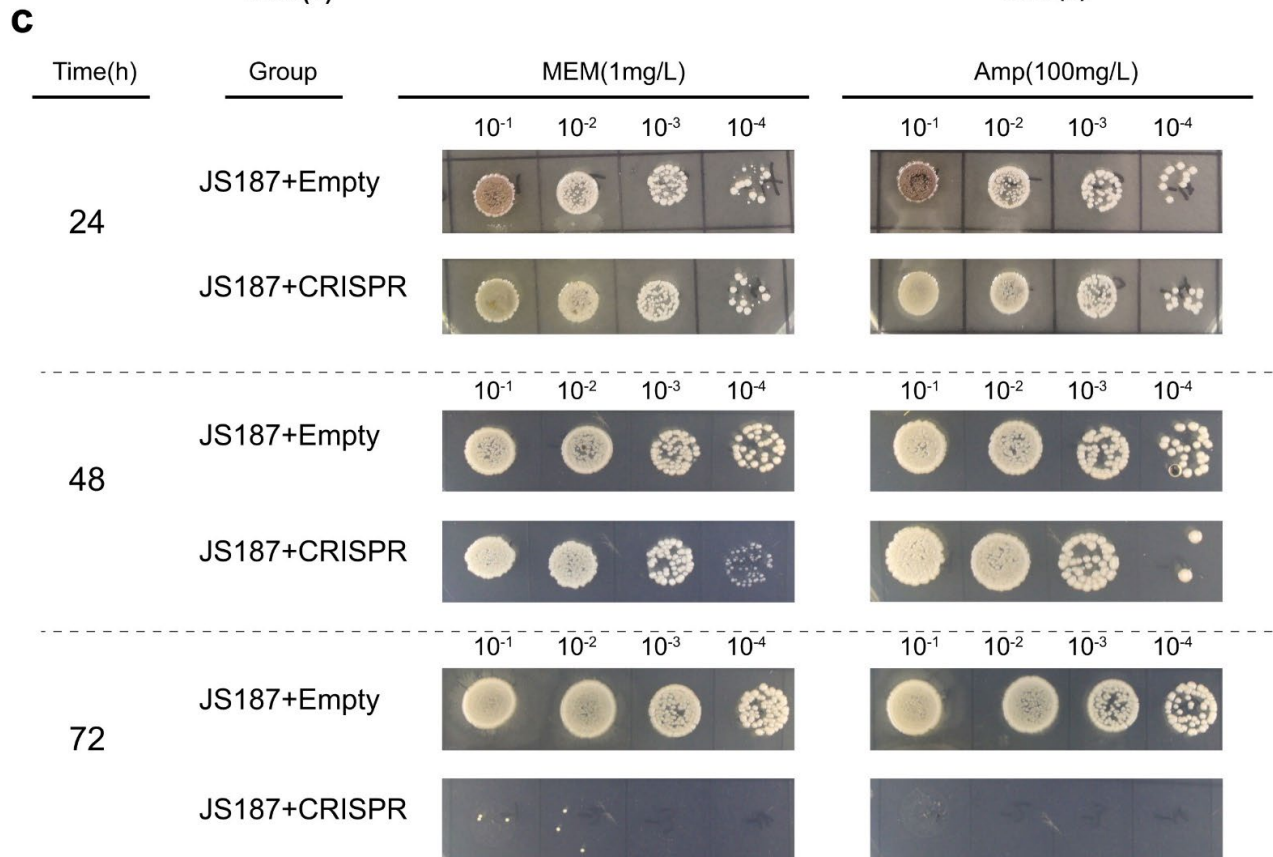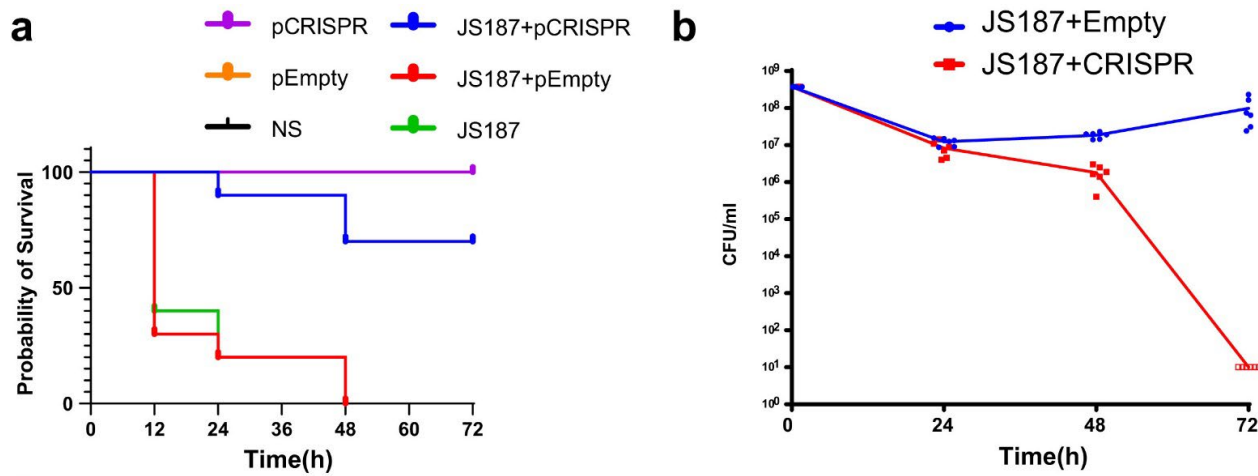
G. mellonella infection model (Greater wax moth)

JS187: *K. pneumoniae* harbouring IncFII-p187-2 plasmid
NS: Normal saline

a. Survival rate in 72h
b. Bacterial burden
c. Plasmid killing effect

- Decreased mortality and bacterial burden → reduction of virulence

- Decreased CFU → elimination of plasmids

Fig 10a-c. IncFII plasmid curing by CRISPR-Cas3 *in vivo*. By Zhou Y *et al. EBioMedicine.* 2023 (CC-BY-NC-ND 4.0)

5. Challenges in the Strategy of CRISPR-Cas systems

(1) Delivery via conjugative plasmids:
    a. conjugation frequency varies in different populations
    b. the risk of acquiring ARGs into the plasmid vector system
    c. possible loss of the CRISPR plasmid due to fitness effect or competition
between plasmids

(2) Evaluation in simulator of gut microbial systems, organoid systems, or more relevant animal models

(3) The risk of bacterial resistance to CRISPR-Cas systems

6. Take Home Messages

(1) Plasmids play a critical role in global AMR; plasmid curing is meaningful in both human medicine and in the perspective of One Health

(2) CRISPR-Cas systems stand out as a promising strategy of plasmid curing

(3) A case study demonstrated that CRISPR-Cas3 systems were highly efficient in killing MDR plasmids from *K. pneumoniae*

(4) Still, the technique has its limitations and is in development

Thank you for your time!