

**A Good Virus: Investigating Increased Antibiotic  
Sensitivity in *Pseudomonas chlororaphis* 14B11  
Induced by PC2 Bacteriophage Resistance**

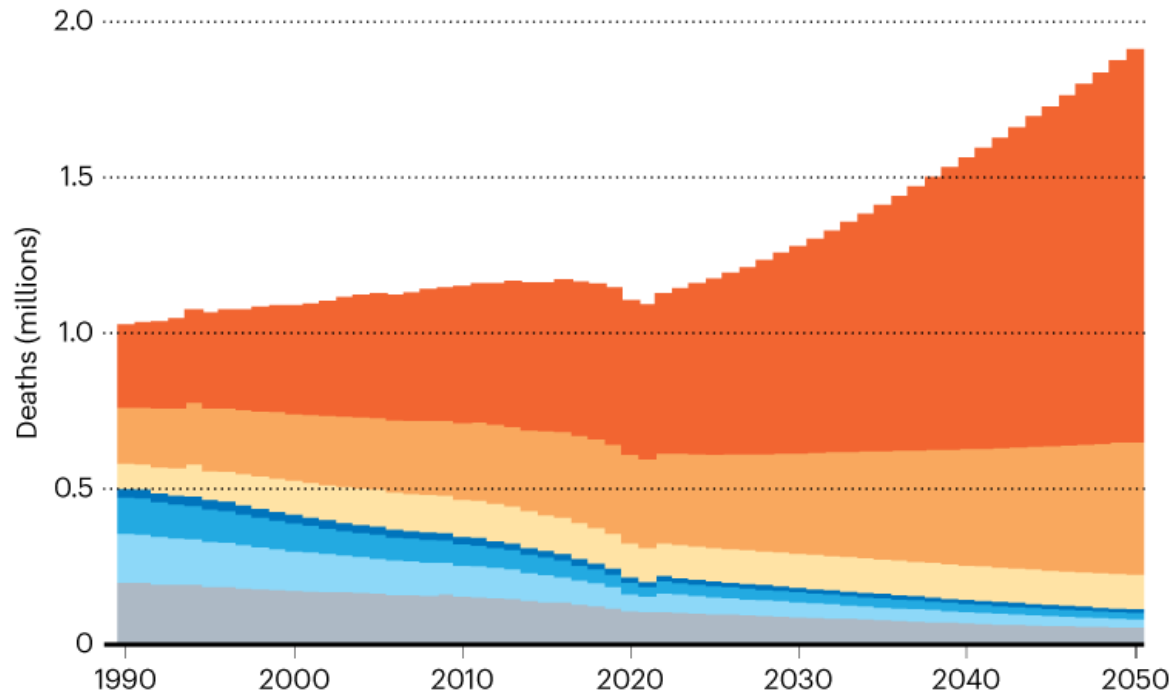
Lauren St. Germaine

# Overuse and Misuse of Antibiotics Results in Antibiotic Resistance

## RESISTANCE CRISIS

By 2050, antimicrobial resistance could be responsible for 1.91 million deaths per year. Mortality is projected to rise by around 70% among people aged 70 and older, but will continue to fall in young children and babies.

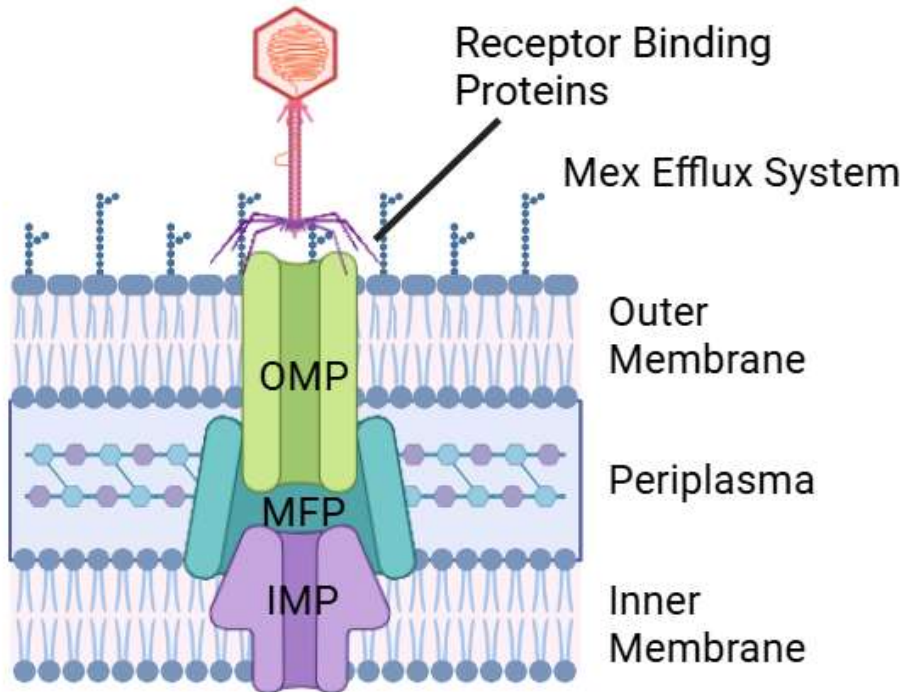
■ Neonatal ■ Postneonatal ■ 1-4 years ■ 5-14 years  
■ 15-49 years ■ 50-69 years ■ ≥70 years



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# Bacteriophages Are Viruses That Infect Bacterial Cells and are Used in Phage Therapy

## Bacteriophage Binding to Cell Receptors



## Phage Replication

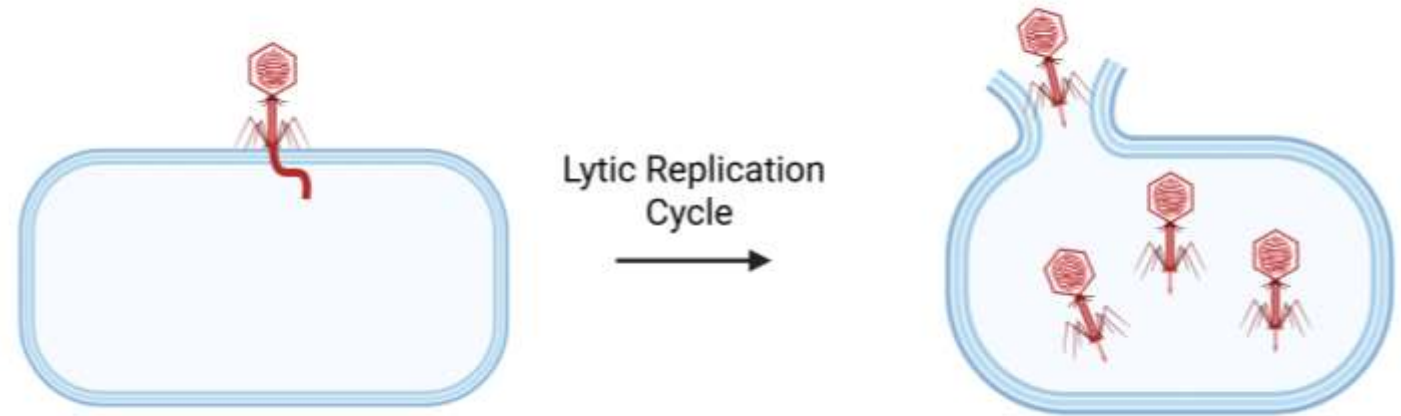


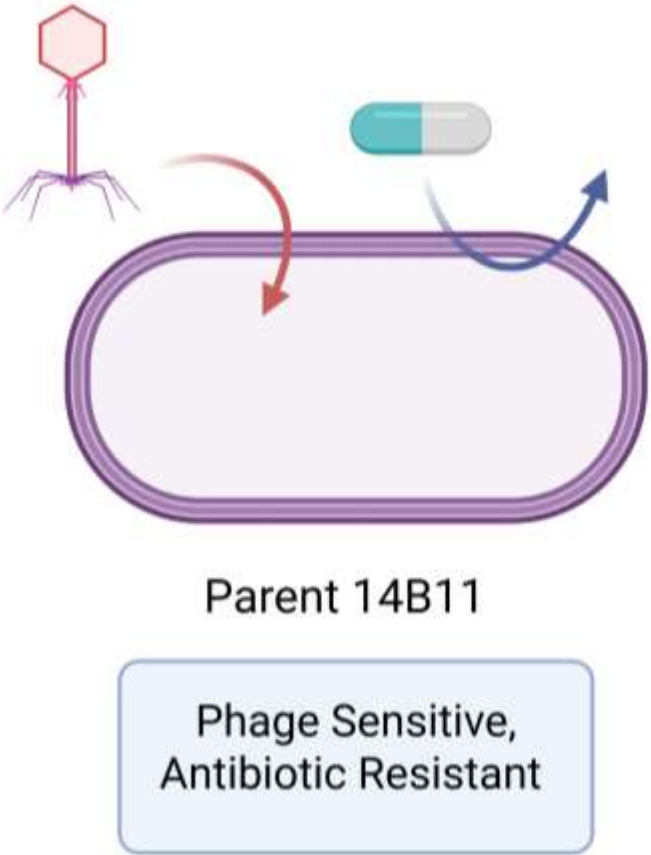
Image made with BioRender

Christaki, E.; Marcou, M.; Tofarides, A. Antimicrobial Resistance in Bacteria: Mechanisms, Evolution, and Persistence. *J Mol Evol* 2020

Chan, B. K.; Sistro, M.; Wertz, J. E.; Kortright, K. E.; Narayan, D.; Turner, P. E. Phage Selection Restores Antibiotic Sensitivity in MDR *Pseudomonas Aeruginosa*. *Sci Rep* 2016

Lorusso, A. B.; Carrara, J. A.; Barroso, C. D. N.; Tuon, F. F.; Faoro, H. Role of Efflux Pumps on Antimicrobial Resistance in *Pseudomonas Aeruginosa*. *Int J Mol Sci* 2022

# Phage Selective Pressure May Induce Phage Resistance and Increased Antibiotic Sensitivity



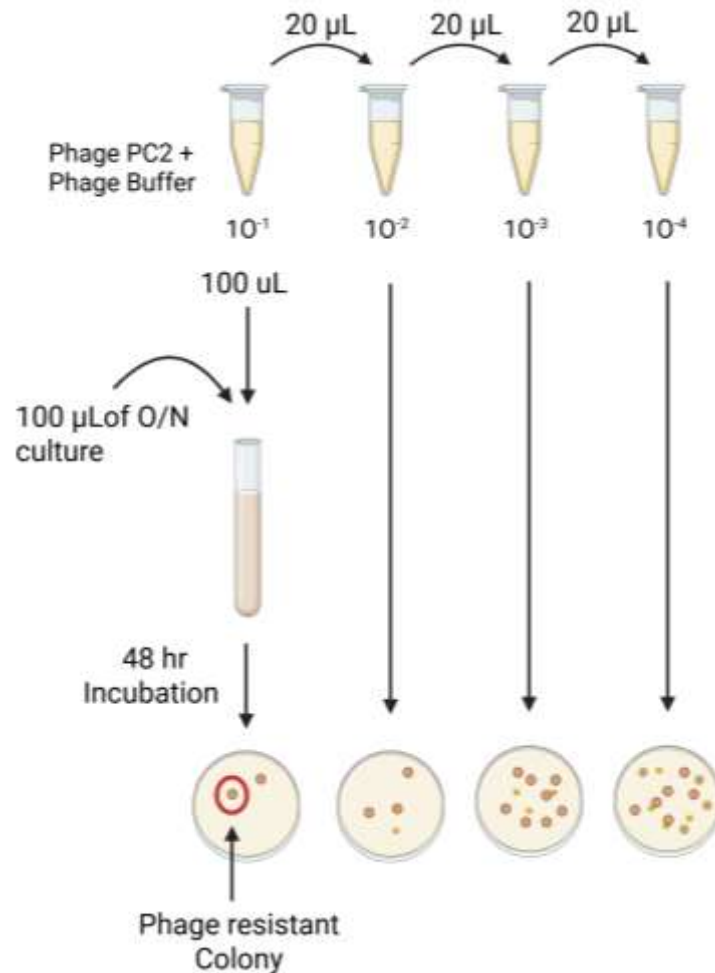
Phage PC2  
Antibiotic

Image made with BioRender

Image adapted from: Burmeister, A. R.; Turner, P. E. Trading-off and Trading-up in the World of Bacteria–Phage Evolution. *Current Biology* **2020**, 30 (19), R1120–R1124. <https://doi.org/10.1016/j.cub.2020.07.036>.

# Does *P. chlororaphis* 14B11 Evolve Resistance to Phage PC2?

## Isolation of Phage-resistant Colonies



Spot Test Assay Reveals All Isolated *P. chlororaphis* Colonies are Phage-Resistant

# Does Phage Resistance Result in an Increased Antibiotic Sensitivity to ampicillin (AMP)?

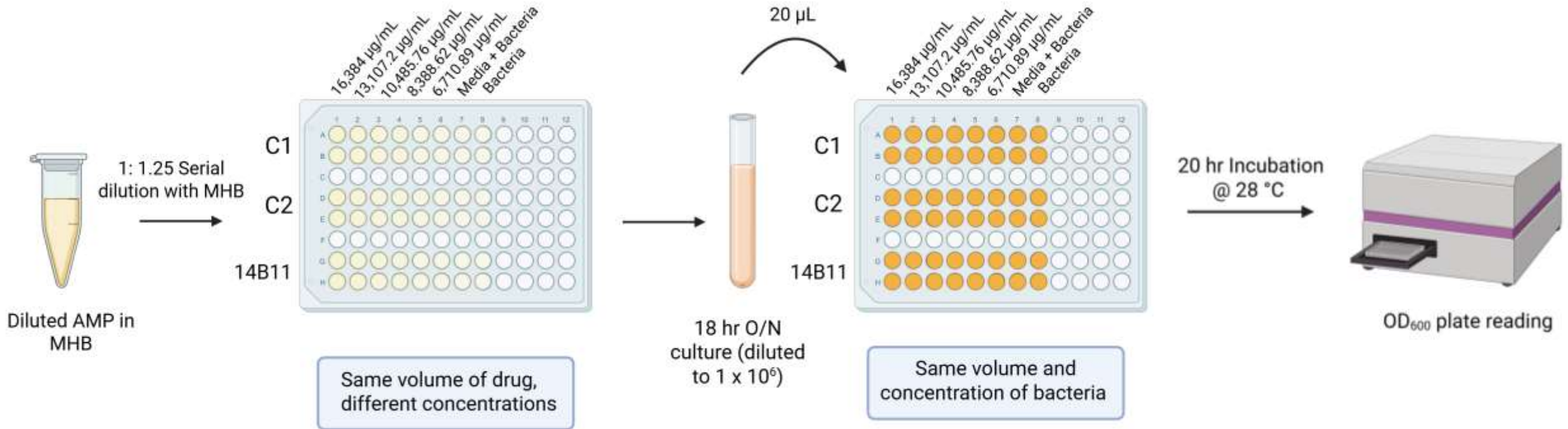
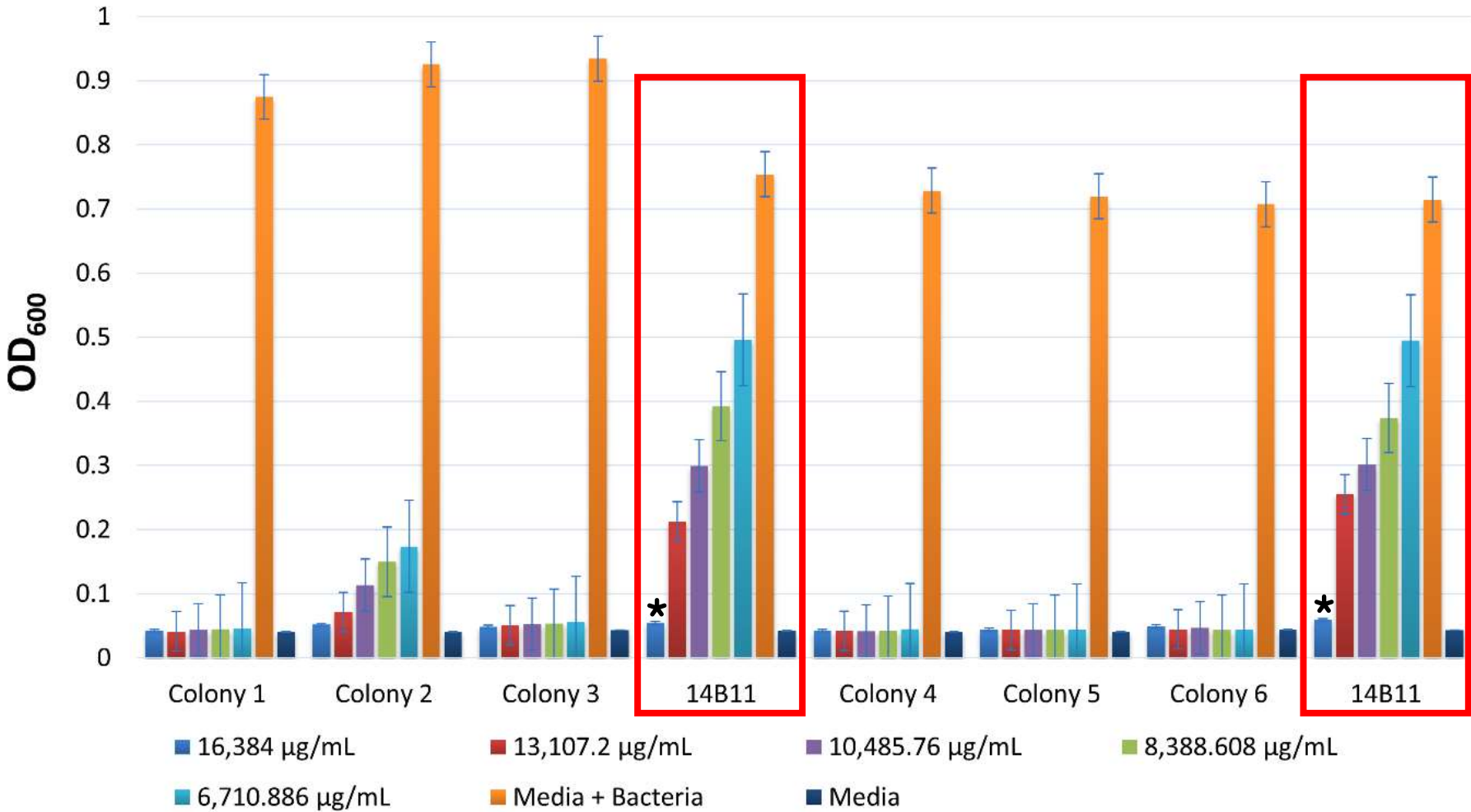


Image made with BioRender

V, L. B.; Heithoff, D. M.; Mahan, S. P.; House, J. K.; Mahan, M. J. Antimicrobial Susceptibility Testing to Evaluate Minimum Inhibitory Concentration Values of Clinically Relevant Antibiotics. *STAR Protocols* **2023**, 4 (3). <https://doi.org/10.1016/j.xpro.2023.102512>.

# Phage-resistant Colonies Showed a ~ 2.5-fold Increase in Antibiotic Sensitivity to AMP



\* = MIC of 14B11

# Mutations in the OMP Results in Phage Resistance and Increased Antibiotic Sensitivity

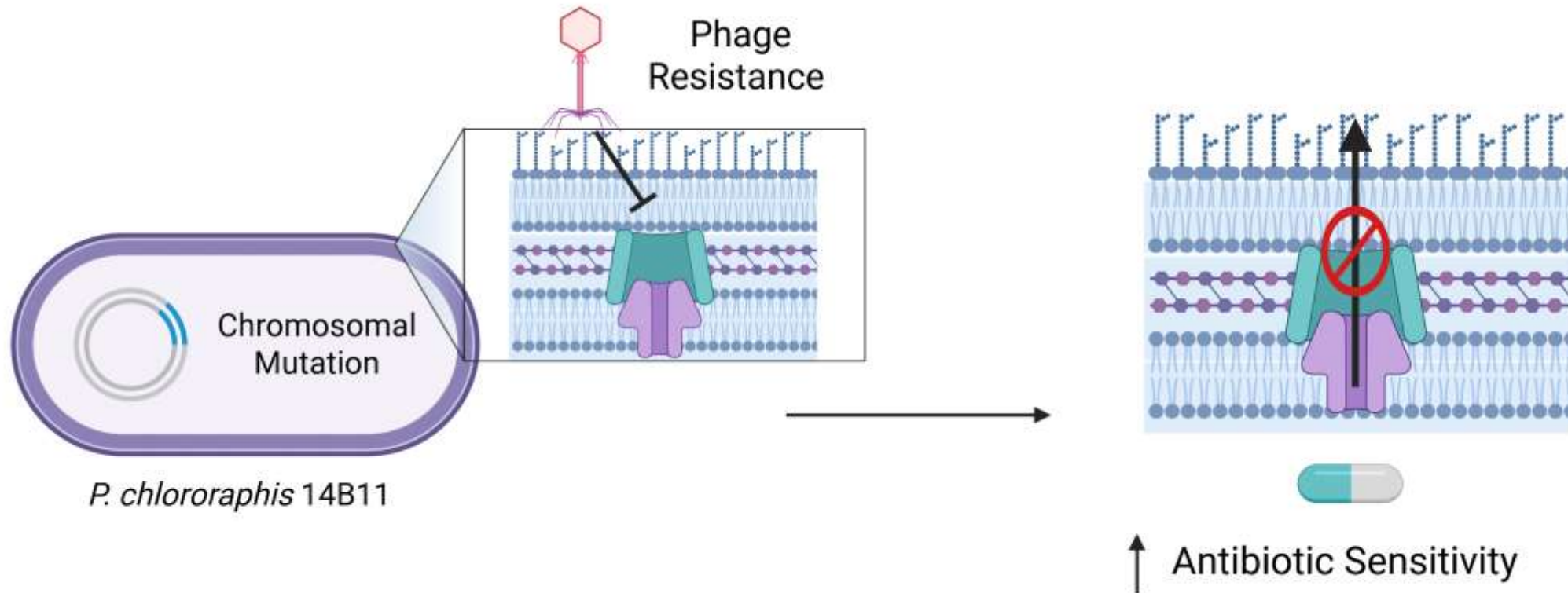
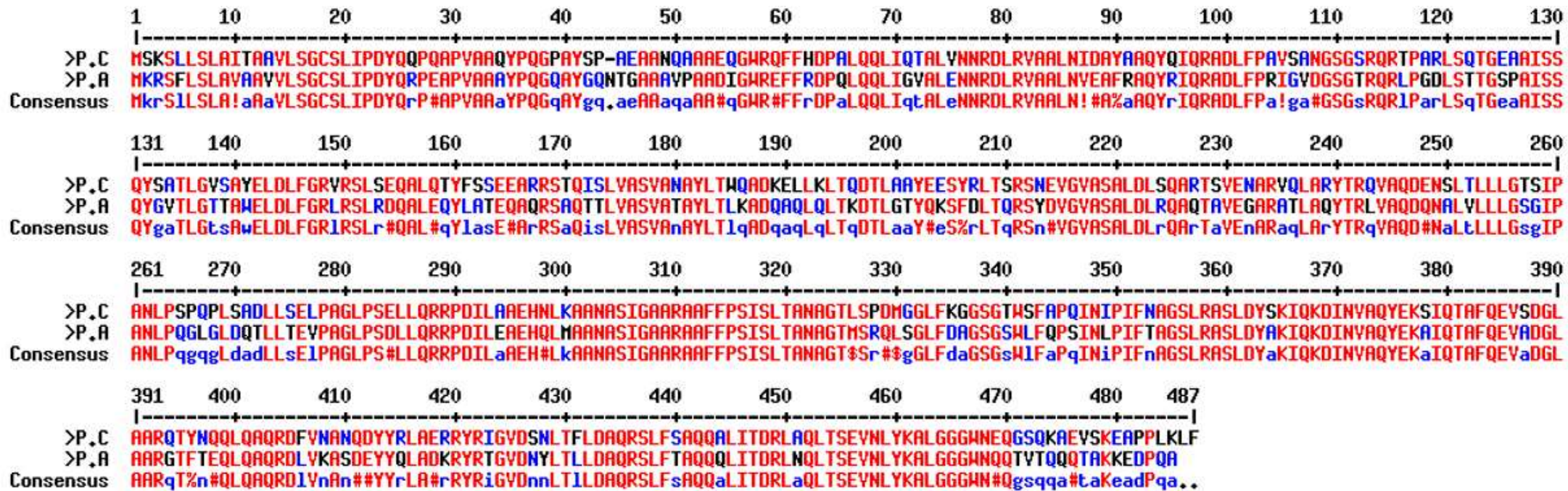


Image made with BioRender

Chan, B. K.; Siström, M.; Wertz, J. E.; Kortright, K. E.; Narayan, D.; Turner, P. E. Phage Selection Restores Antibiotic Sensitivity in MDR *Pseudomonas Aeruginosa*. *Sci Rep* 2016, 6, 26717. <https://doi.org/10.1038/srep26717>.

# Is There a Homologous OMP Gene in *P. chlororaphis* 14B11?

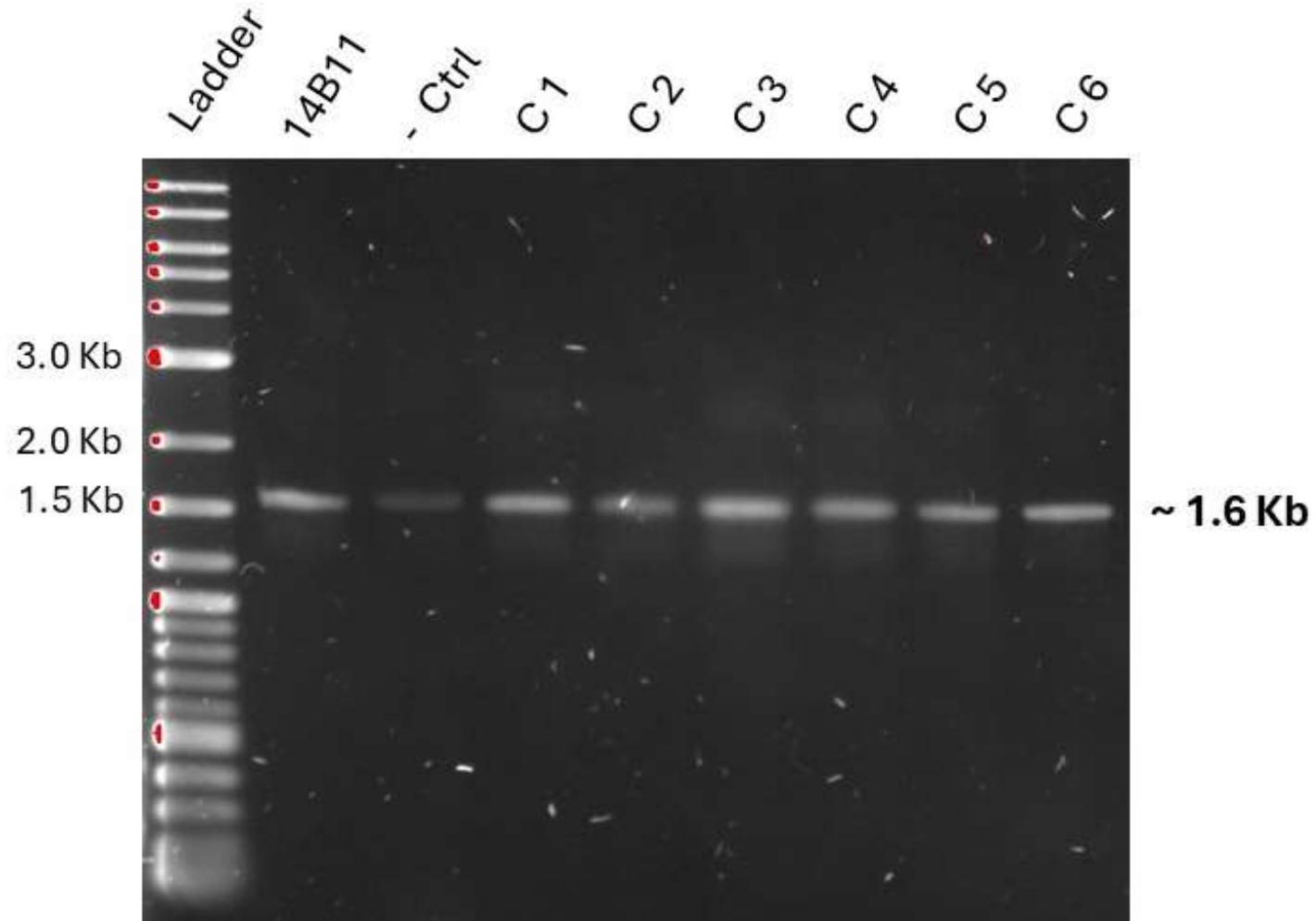


Sequence Alignment Reveals the AdeC gene is a Homologous OMP gene in *P. chlororaphis* 14B11

P. C = *P. chlororaphis* 14B11 AdeC protein sequence

P. A = *P. aeruginosa* OprM protein sequence

The *adeC* Gene was able to be Amplified in Parent 14B11 and Phage-resistant Colonies, via PCR and Gel Electrophoresis



# Is the AdeC Gene Altered When Bacteria Develop Resistance Under Selective Pressure From Phage PC2?

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Consensus . G T T G A C G A A G T C A C G C T G G G C C T G C A A C T C T G G T T G T A G G T C T G G C G T G C G C C A G G C C A T C G G A G A C T T C C T G G A A G G C G G T C T G A T G G A C T T C T G T A C T G C G C G A C G T T G A T G T C T T C T G G A T

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Consensus C G G G C A A C T C G C T G A C A G G T C G G C C G A C A A C G G C T G C G G G T C G G C A G G T T G G C C G G A T C A G G T A C C A G C A G C A G G G T C A G G T G T T T T C G C C T G G G C C A C C T G G C G G G T A T A C G C C C A G T T G

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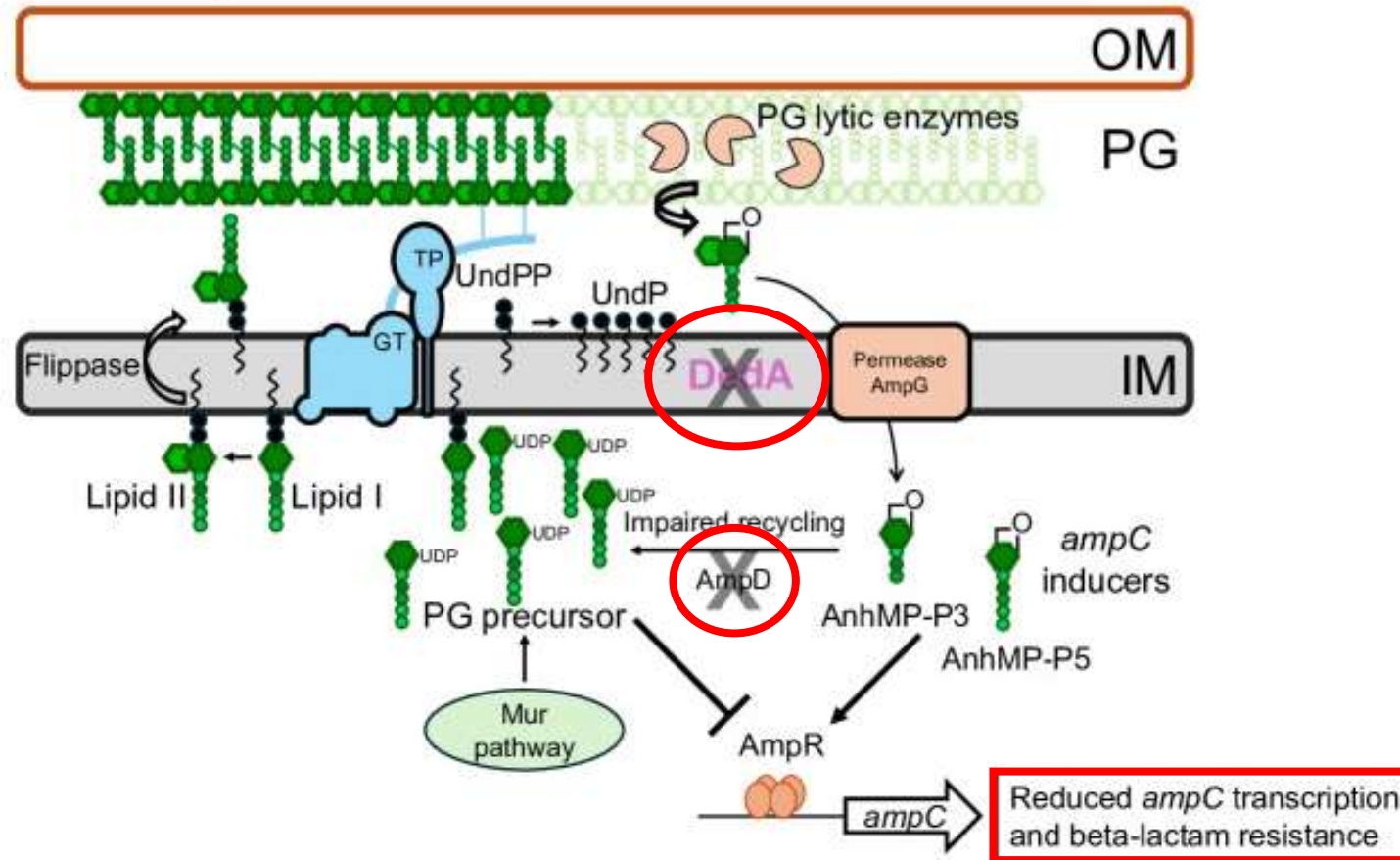
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Gene sequencing  
Revealed 5 out of  
the 6 Phage-  
Resistant  
Colonies  
Displayed no

Mutations to the  
AdeC Coding  
Region

# Lack of AdeC gene Mutations Suggests There are Other Mutations that Confer Phage Resistance and Increased AMP Sensitivity

In the  $\Delta ampD \Delta dedA4$  strain:



# Future Directions

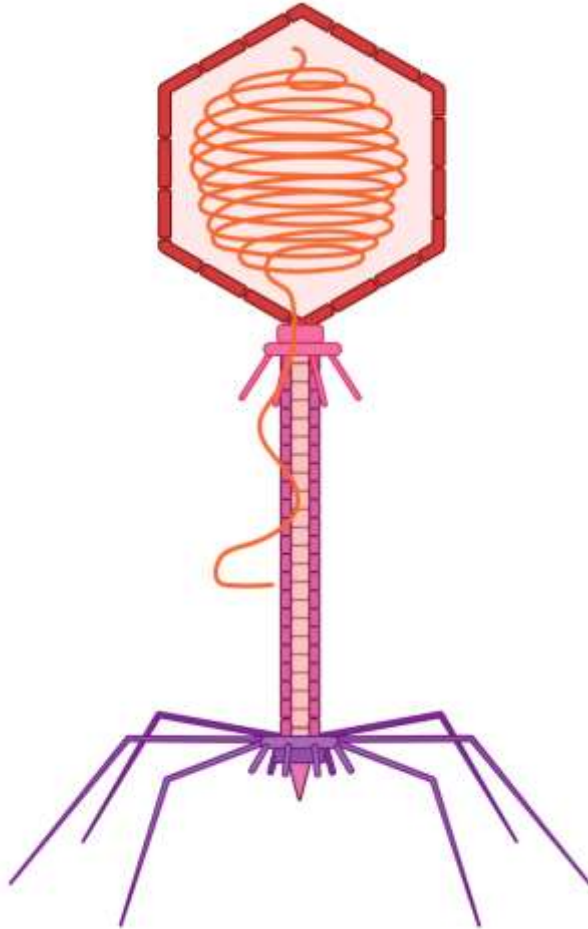
- Are the DedA and AmpD genes mutated in phage resistant colonies and are there other gene mutations that are contributing to phage resistance and increased AMP sensitivity?
  - Specific gene sequencing and Whole Genome sequencing
- Is there similar increased antibiotic sensitivity shown in other penicillin class antibiotics?

# Acknowledgements

Thank you to The College of Wooster Copeland Fund for providing the financial resources necessary to carry out this research

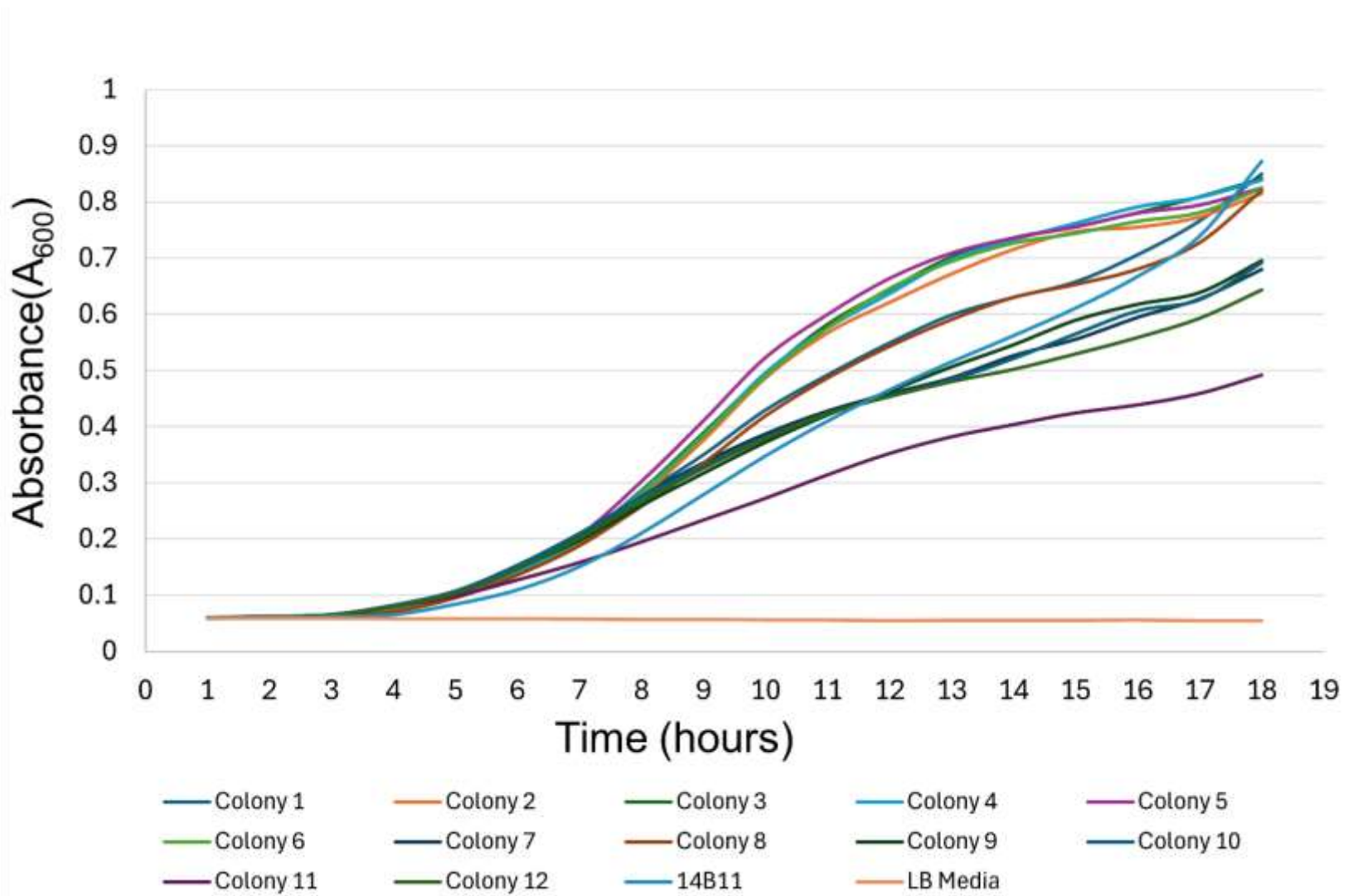
Thank you to Dr. Strand for her amazing mentorship and for her constant guidance, support, and valuable feedback throughout the course of this research

Thank You!! Any Questions?



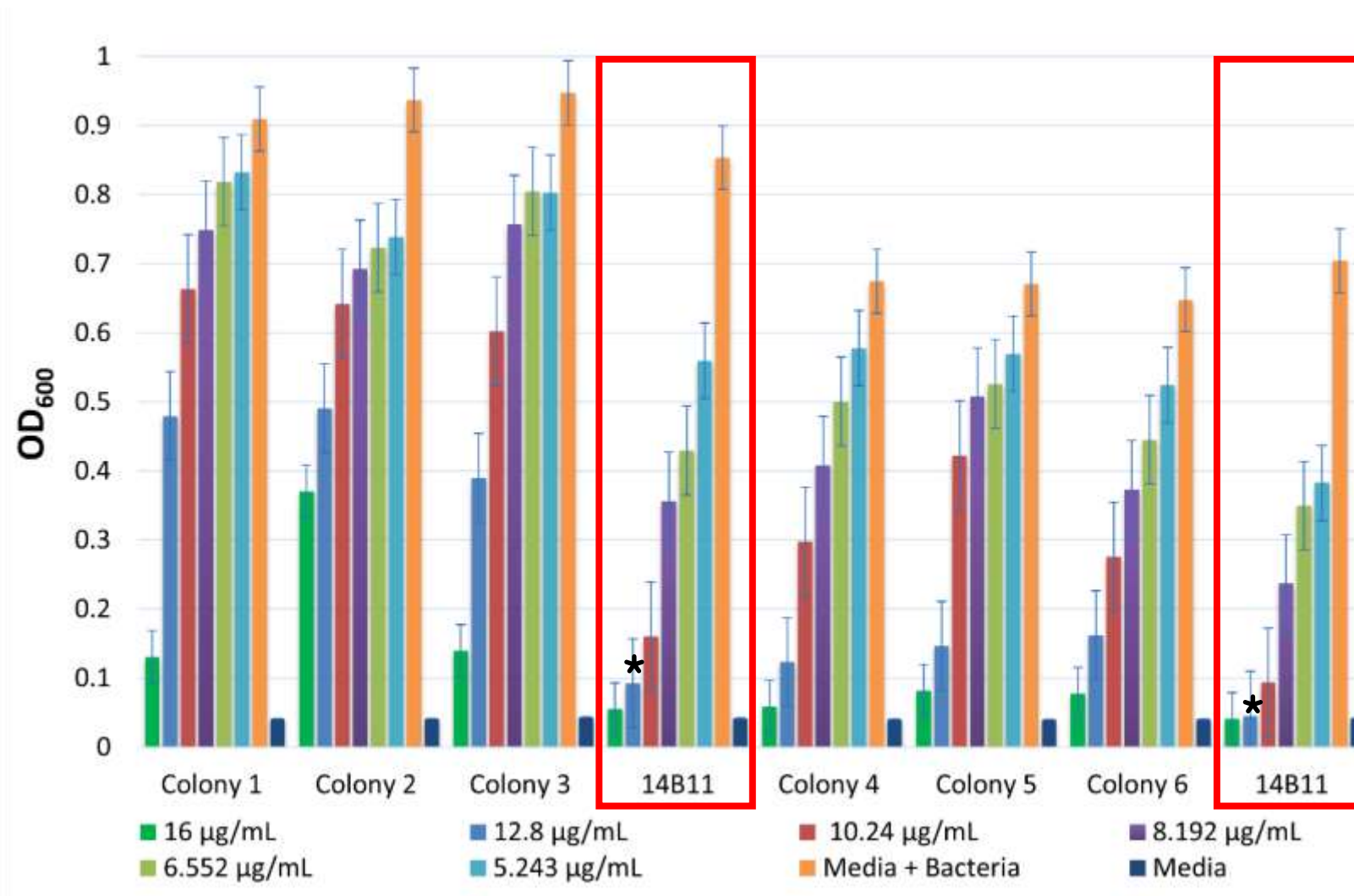


# Growth Curve Determined Phage-Resistant Mutants Acquired No Growth Mutations



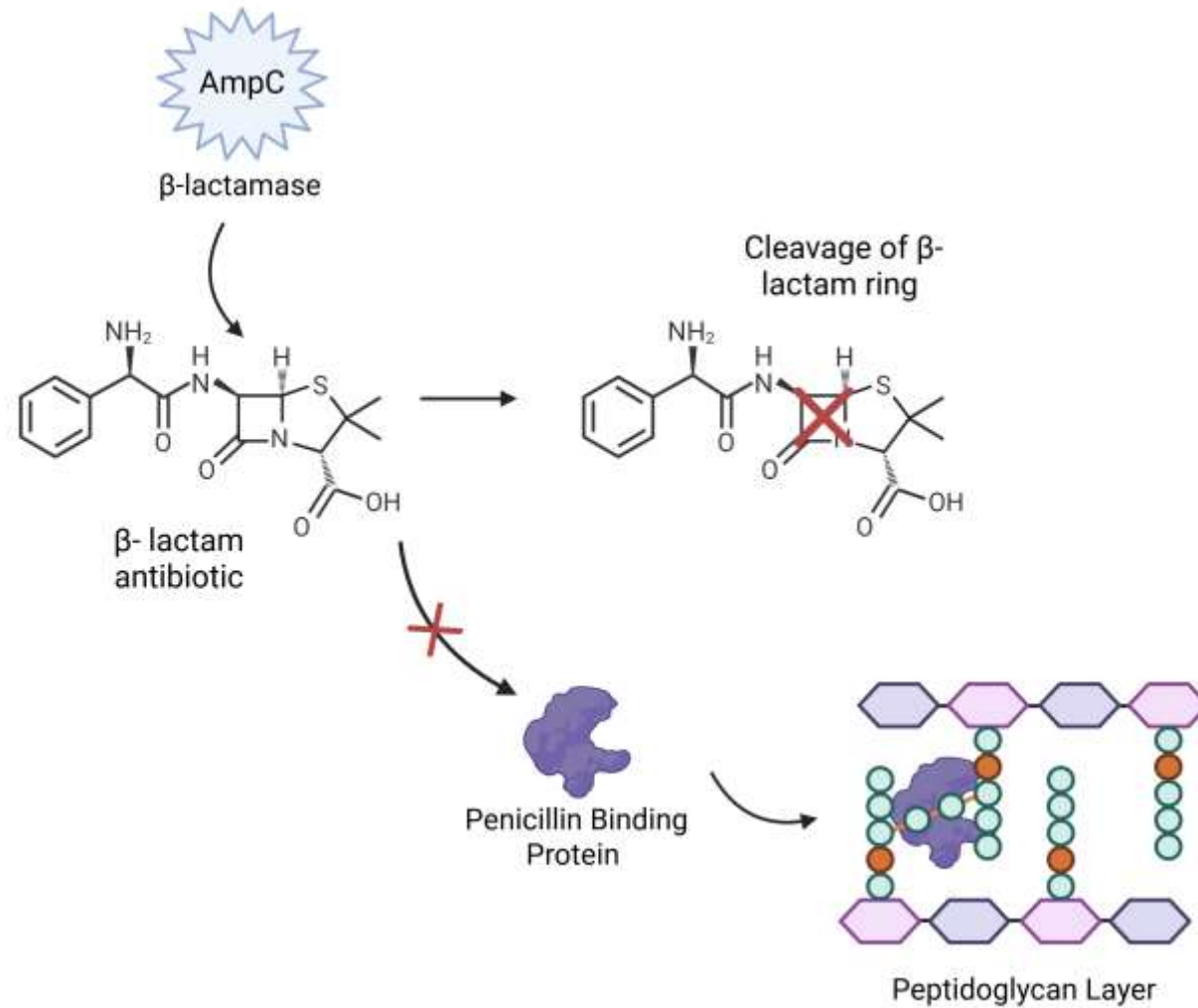


# Phage-resistant Colonies Showed a Decrease in Antibiotic Sensitivity to TET

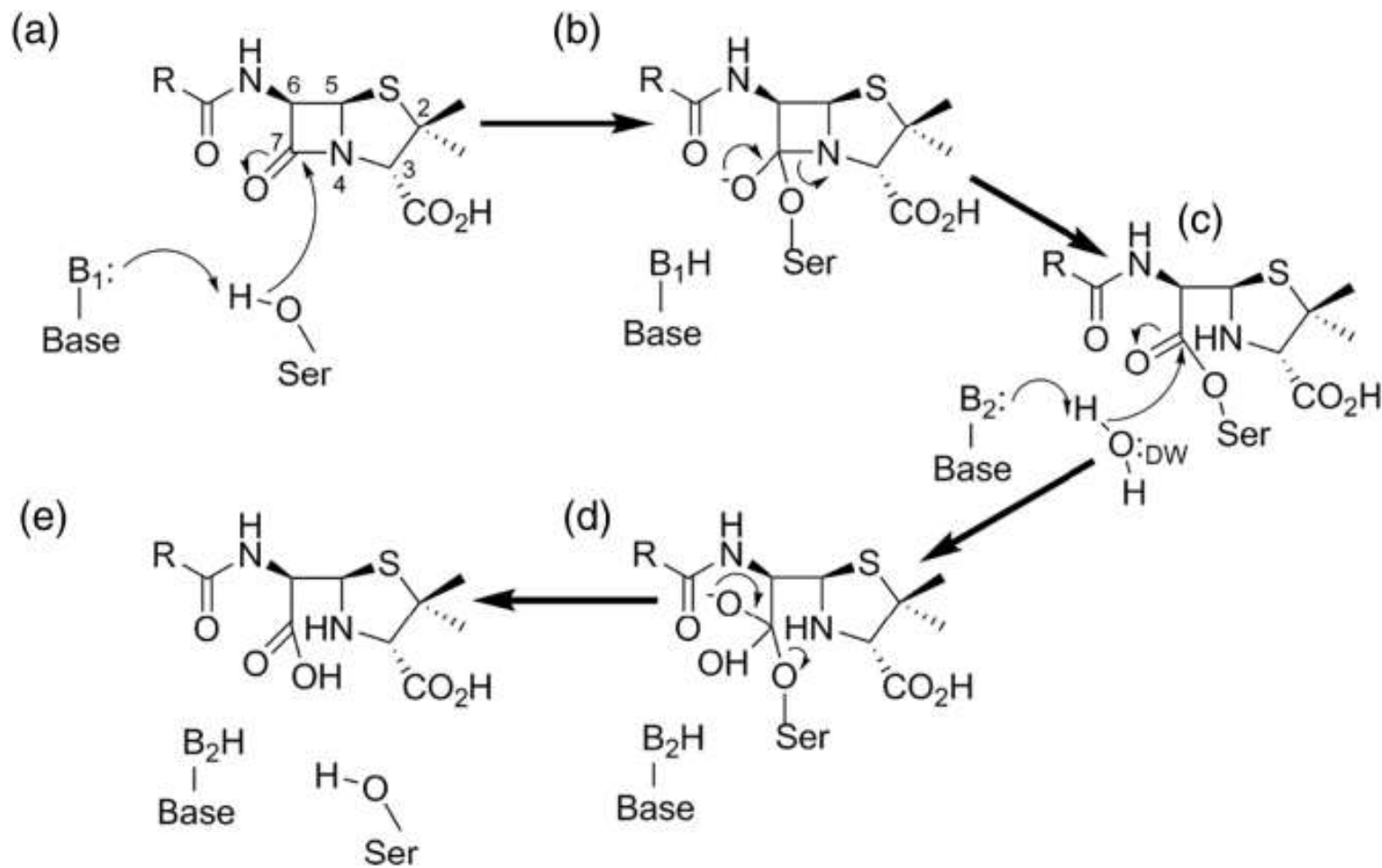


\* = MIC of TET

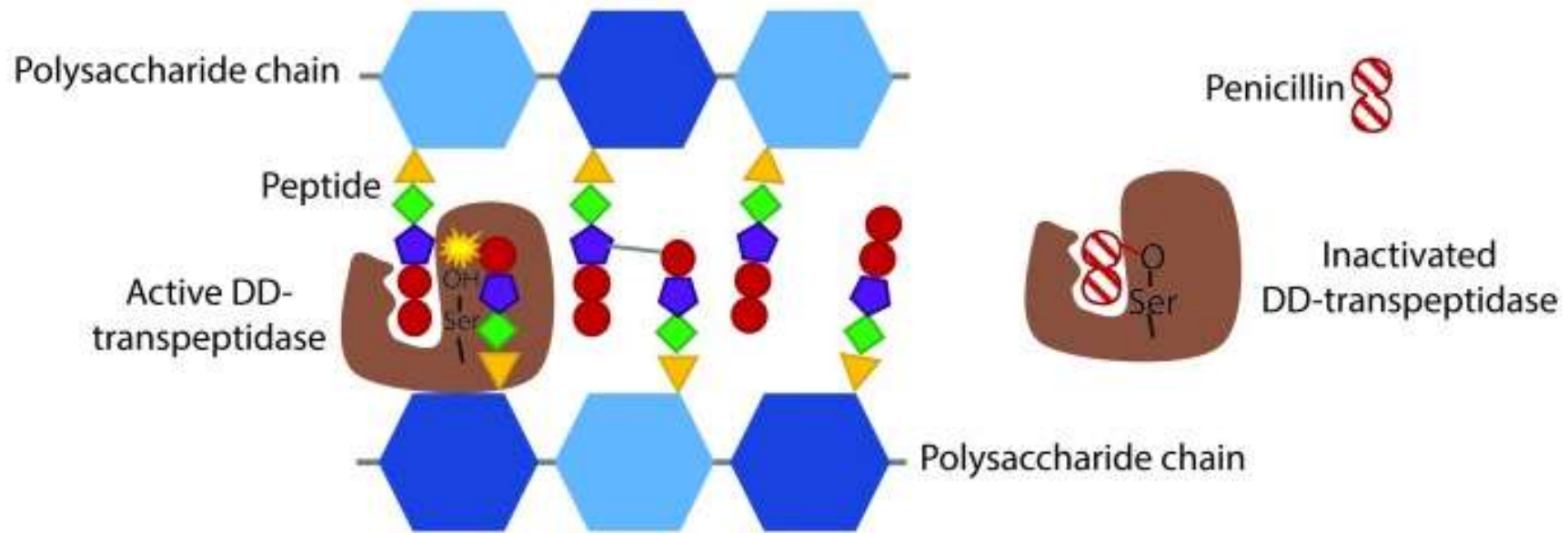
# High AMP Resistance for 14B11 is Likely Due to Genetically Encoded $\beta$ -lactamase Enzymes



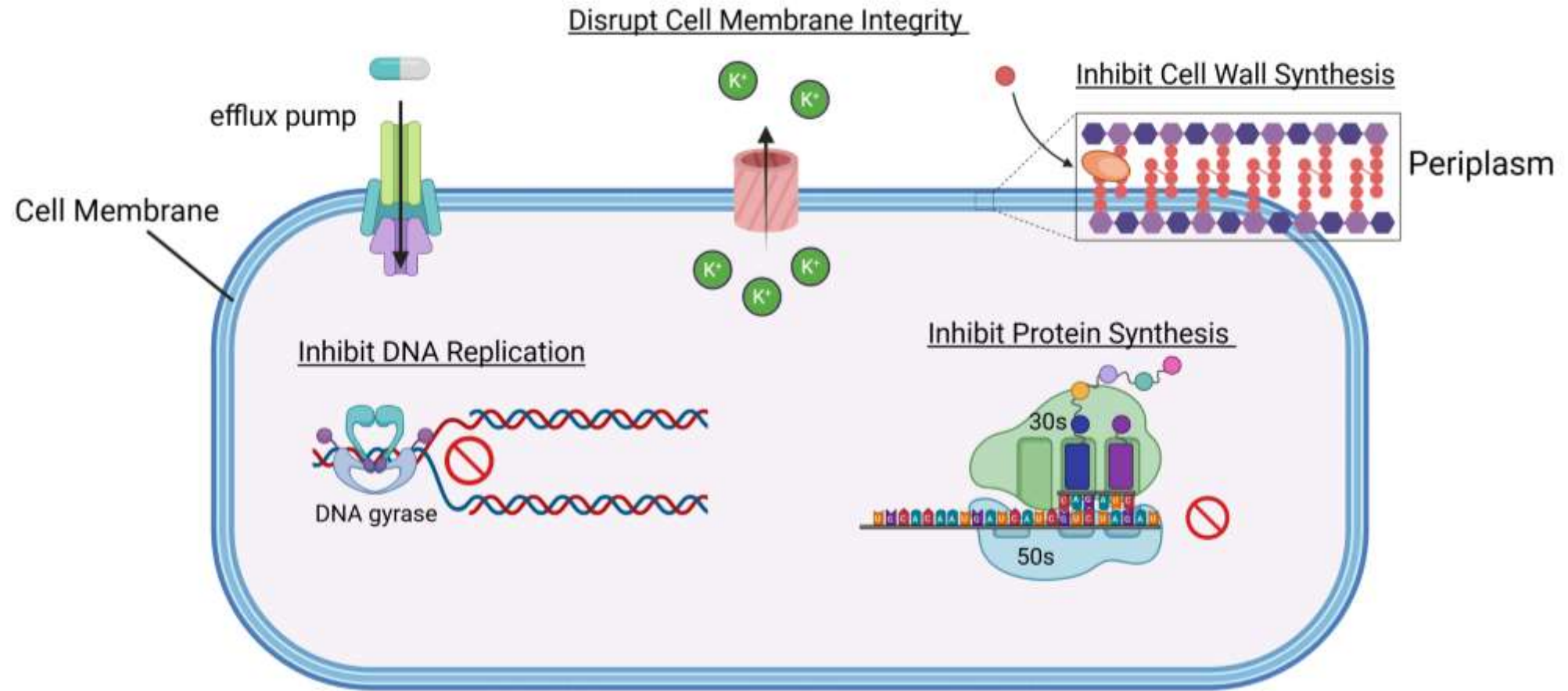
# Mechanism of $\beta$ -lactamases



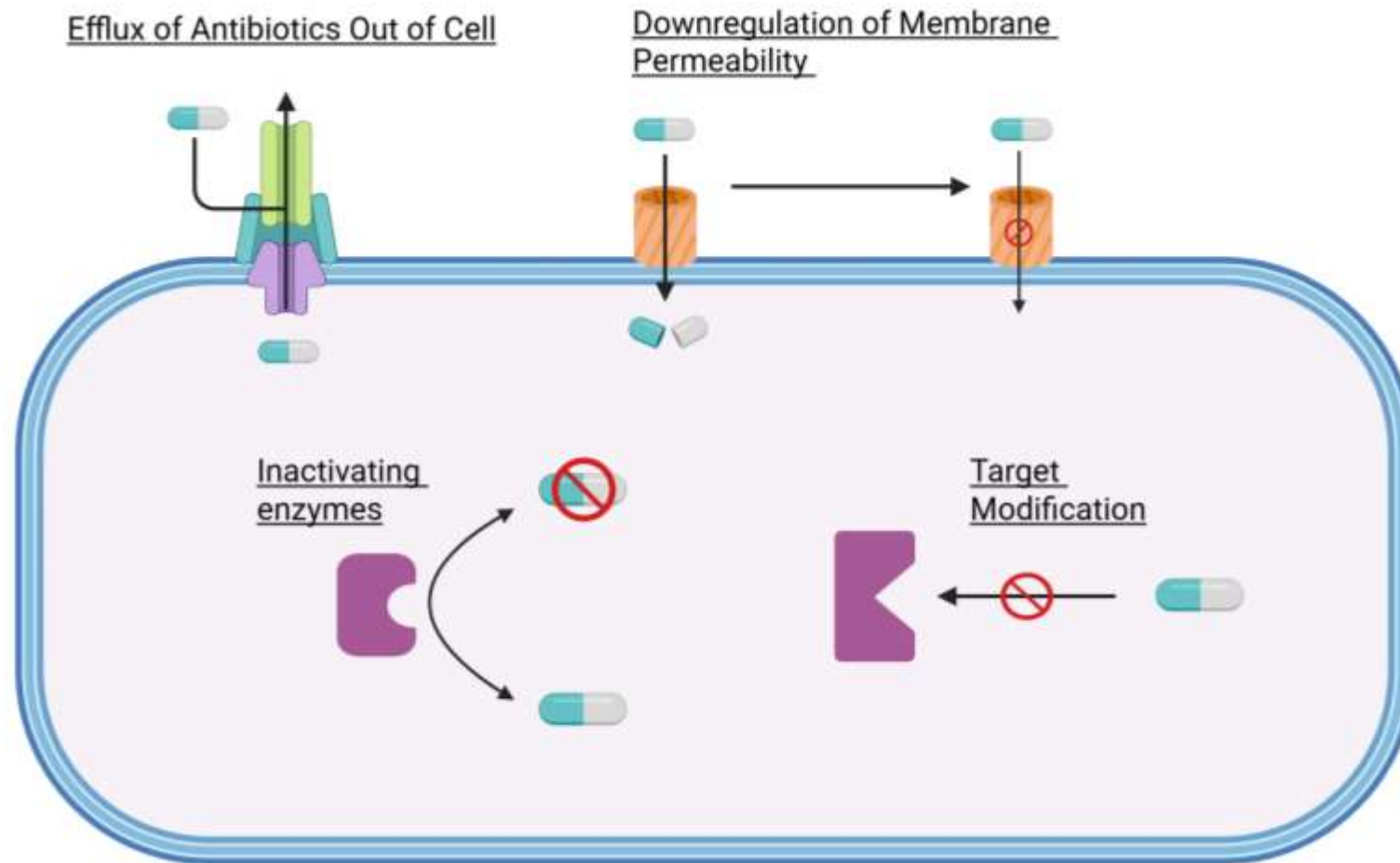
# Penicillin Mechanism of Action



Antibiotics kill bacterial cells through Inhibiting cell wall synthesis, protein synthesis, DNA replication, and disrupting the cell membrane integrity



# Bacteria Form Resistance to Antibiotics via Efflux, Downregulation of Membrane Porins, Inactivating Enzymes, and Modification to the Target Site



# Bacteriophage Resistance Occurs Through a Variety of Mechanisms, the Main Mechanism is Alteration of Bacterial Cell Surface Receptors

