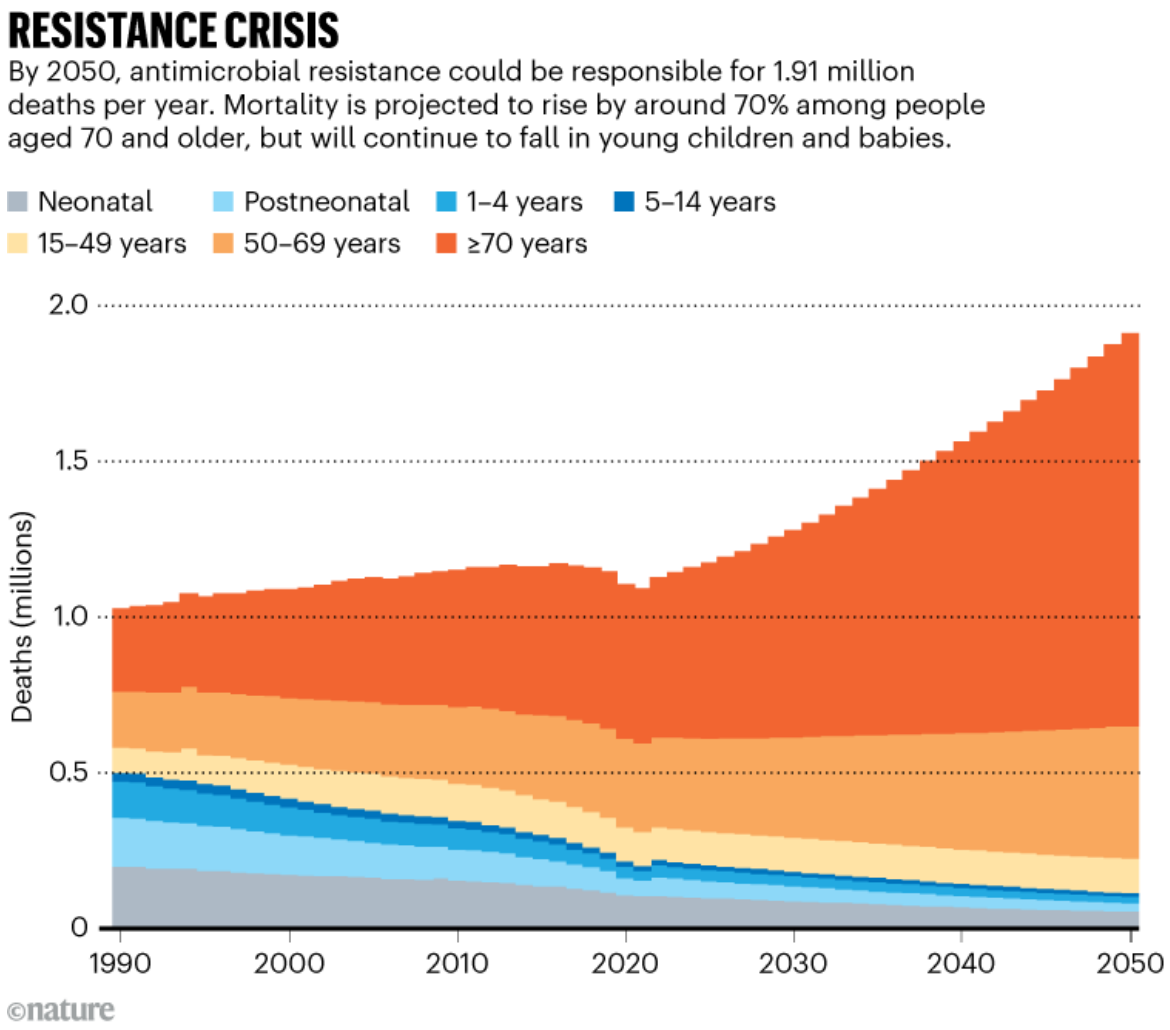
A Good Virus: Investigating Increased Antibiotic Sensitivity in *Pseudomonas chlororaphis* 14B11 Induced by PC2 Bacteriophage Resistance

Lauren St. Germaine

Overuse and Misuse of Antibiotics Results in Antibiotic Resistance

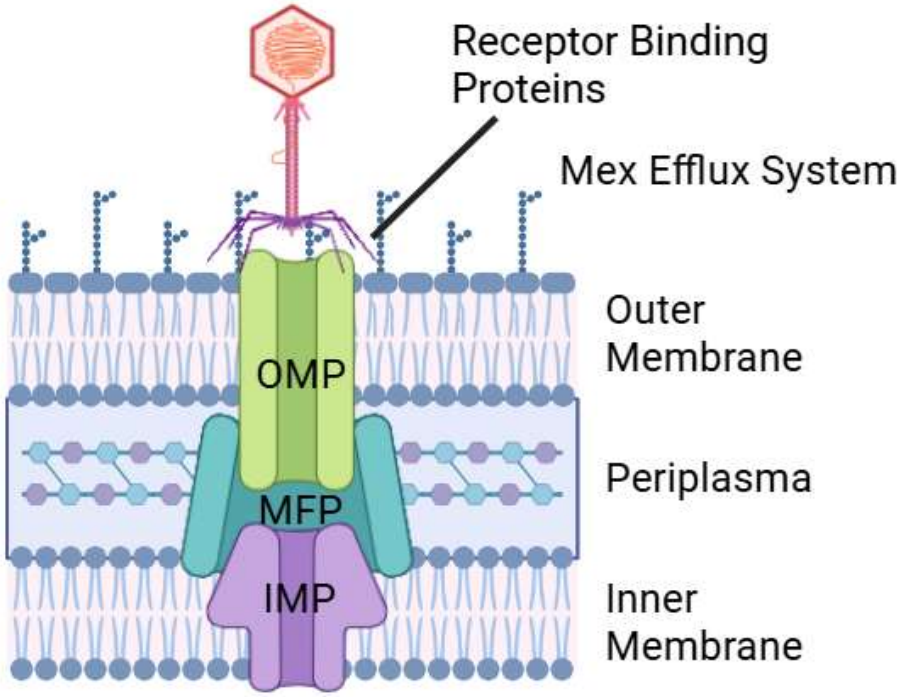


Naddaf, M. 40 Million Deaths by 2050: Toll of Drug-Resistant Infections to Rise by 70%. *Nature* **2024**

microbial Resistance 1990–2021: A Systematic Analysis with Forecasts to 2050. *The Lancet* **2024.**

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

Bacteriophage Binding to Cell Receptors



Phage Replication

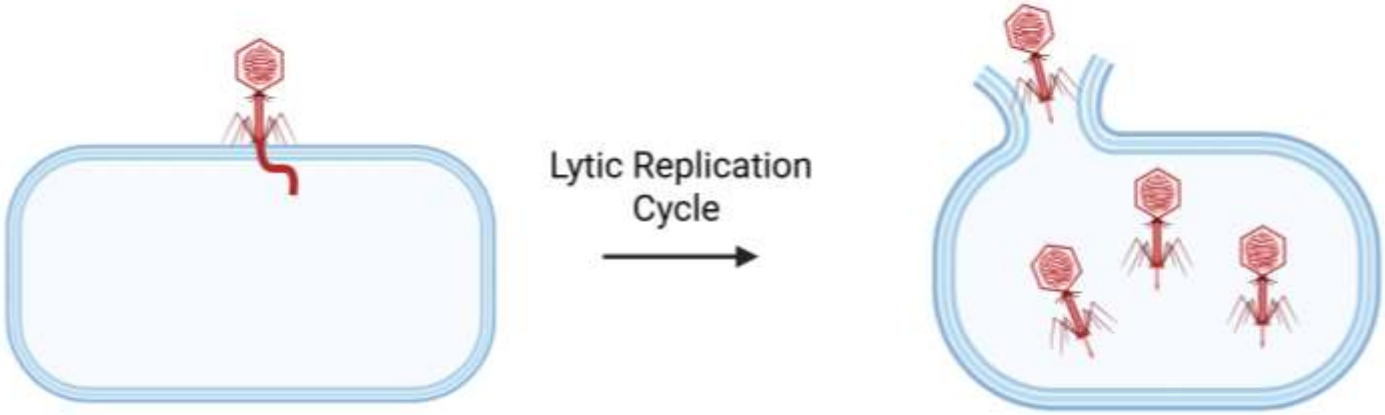


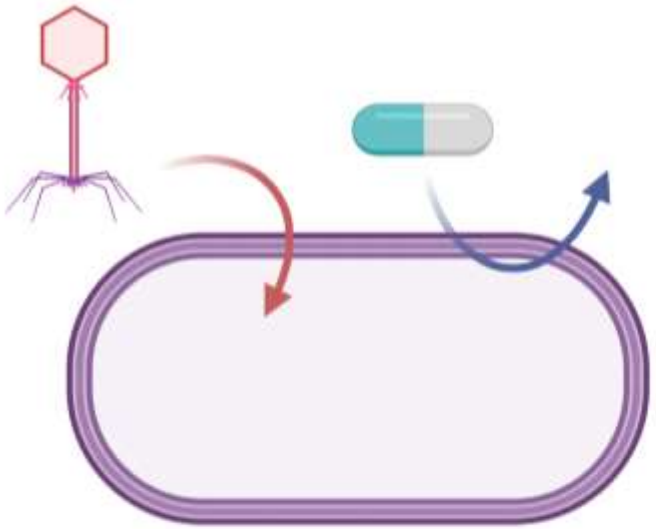
Image made with BioRender

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

Chan, B. K.; Sistrom, 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



Parent 14B11

Phage Sensitive,
Antibiotic Resistant

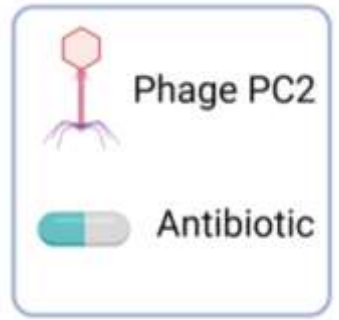
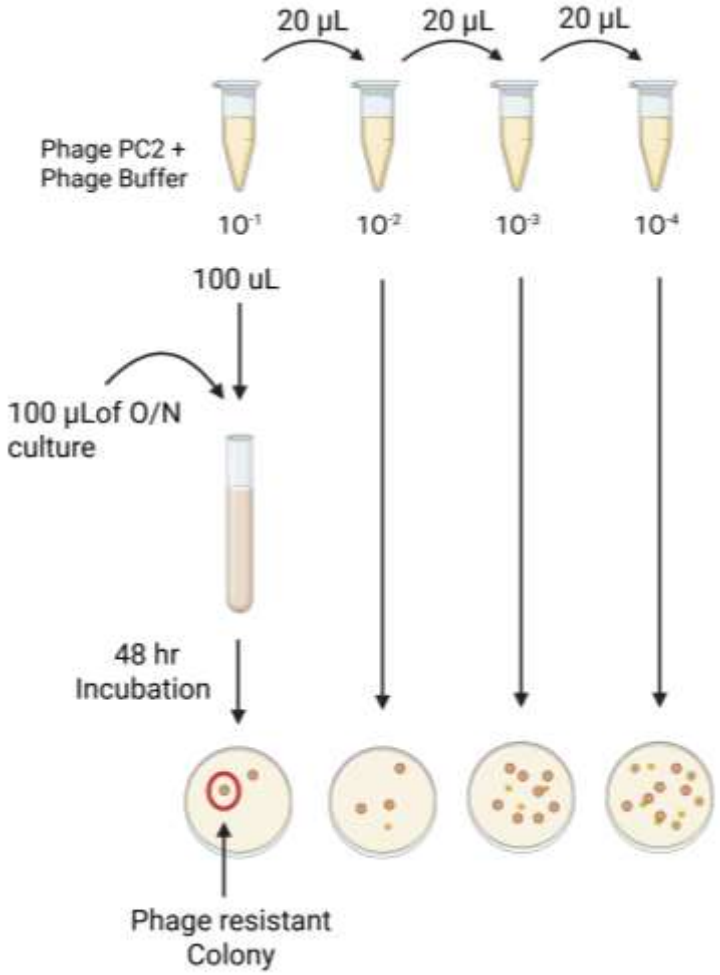


Image made with BioRender

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



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

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

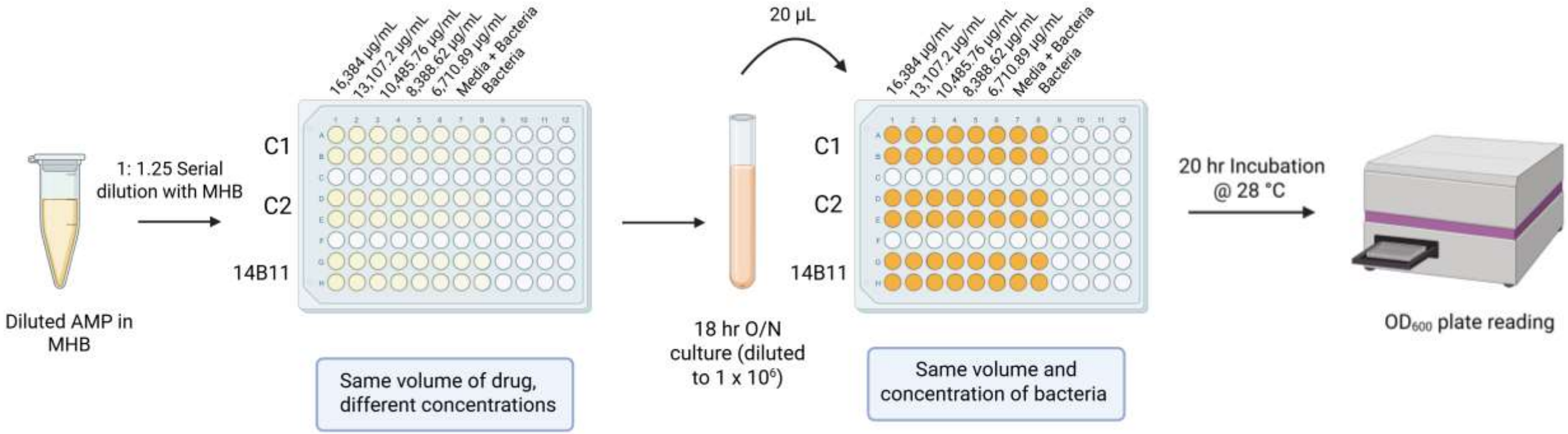
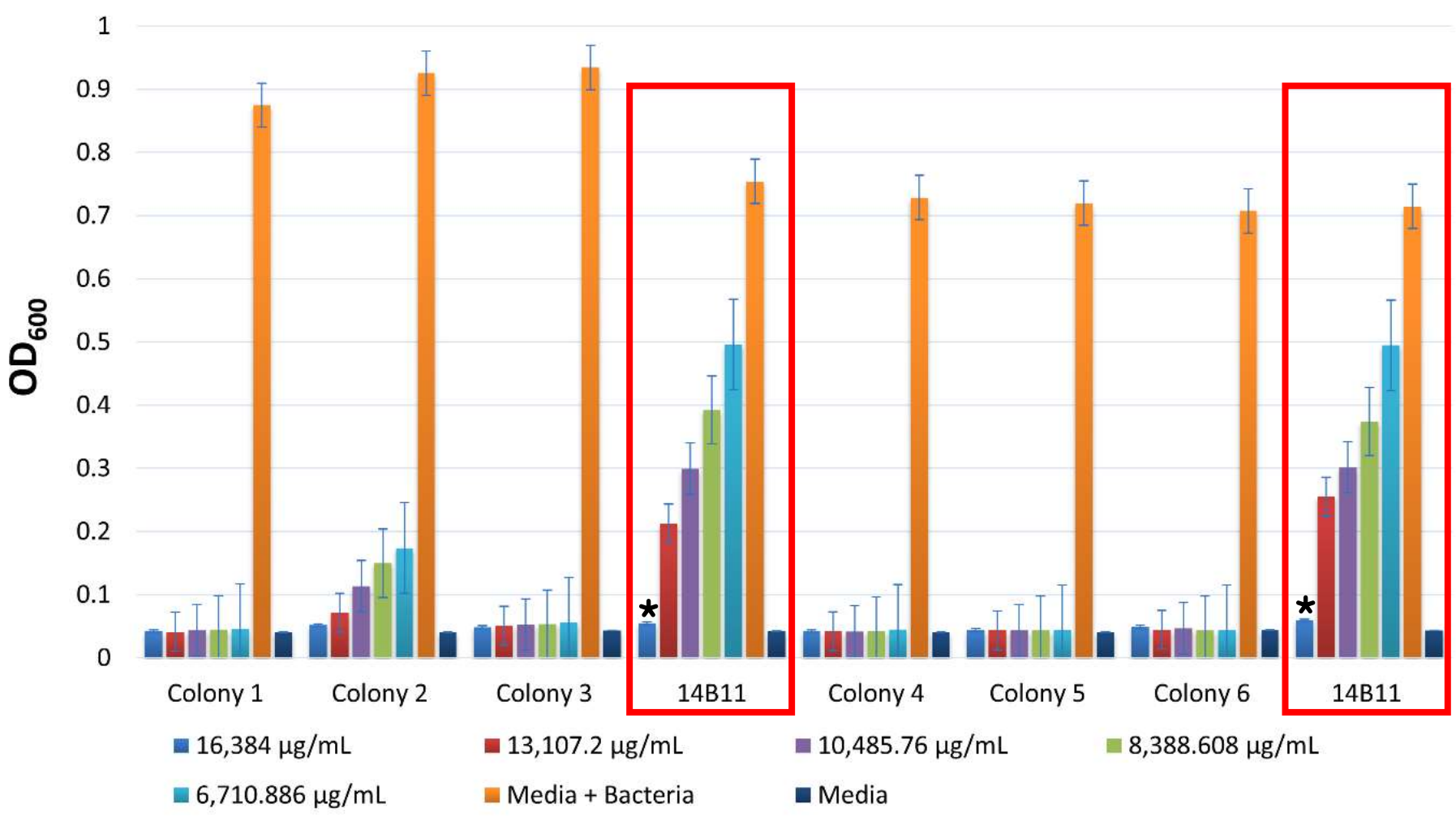


Image made with BioRender

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

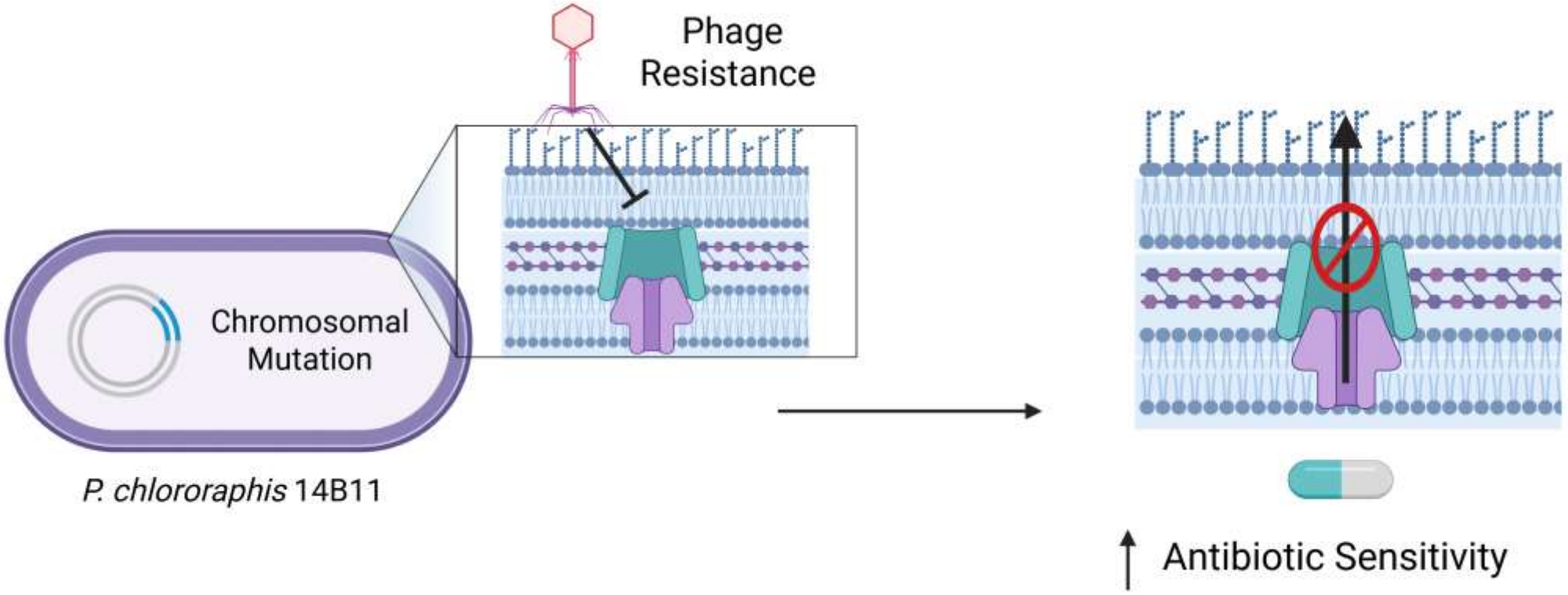
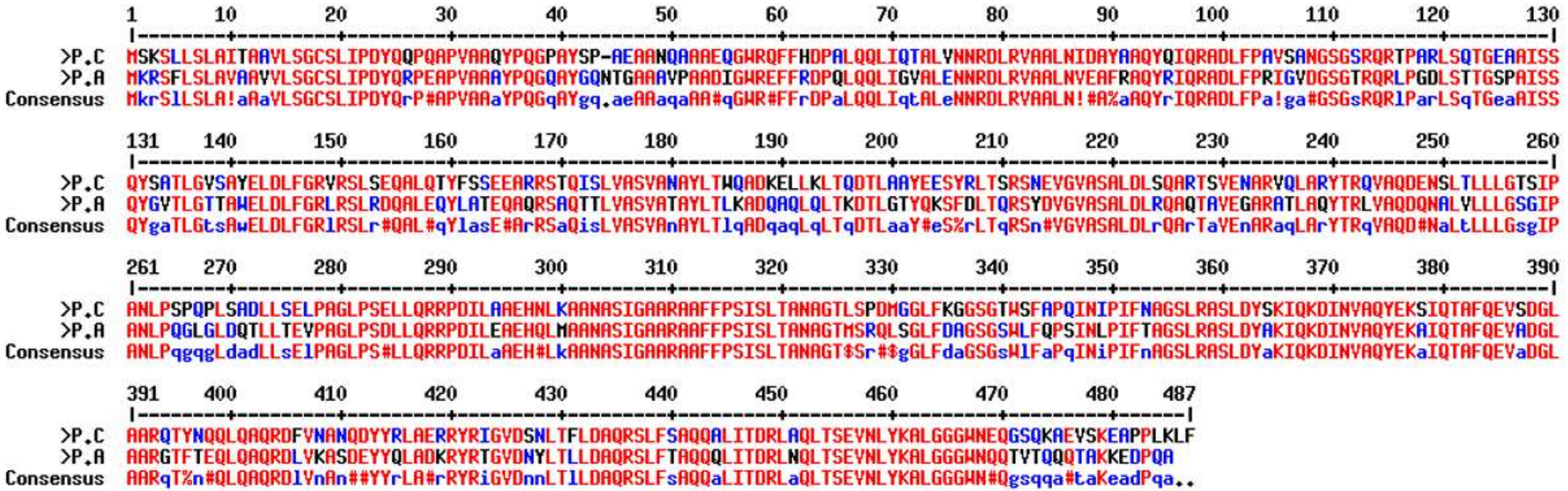


Image made with BioRender

Chan, B. K.; Sistrom, 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.

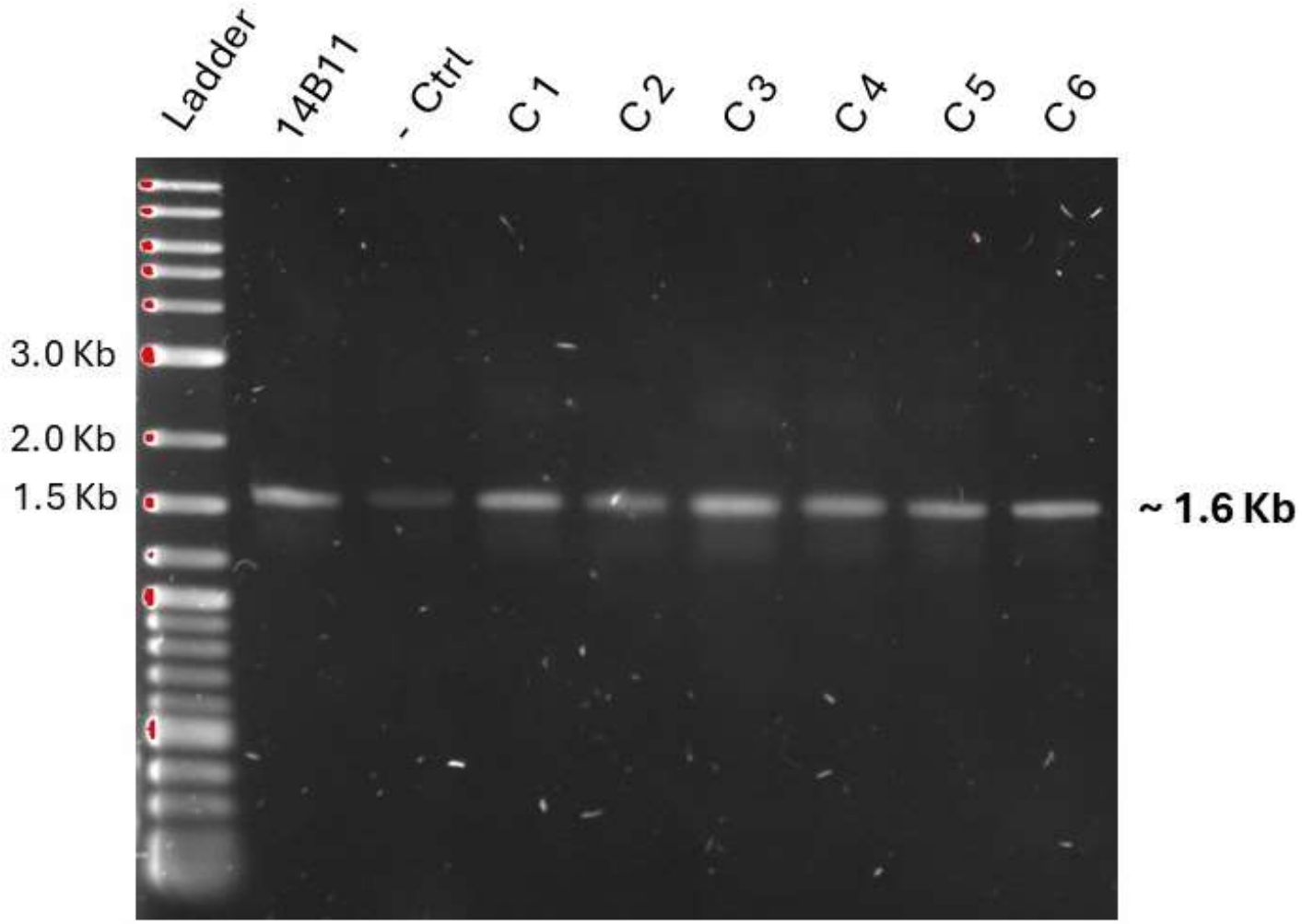
Is There a Homologous OMP Gene in *P. chlororaphis* 14B11?



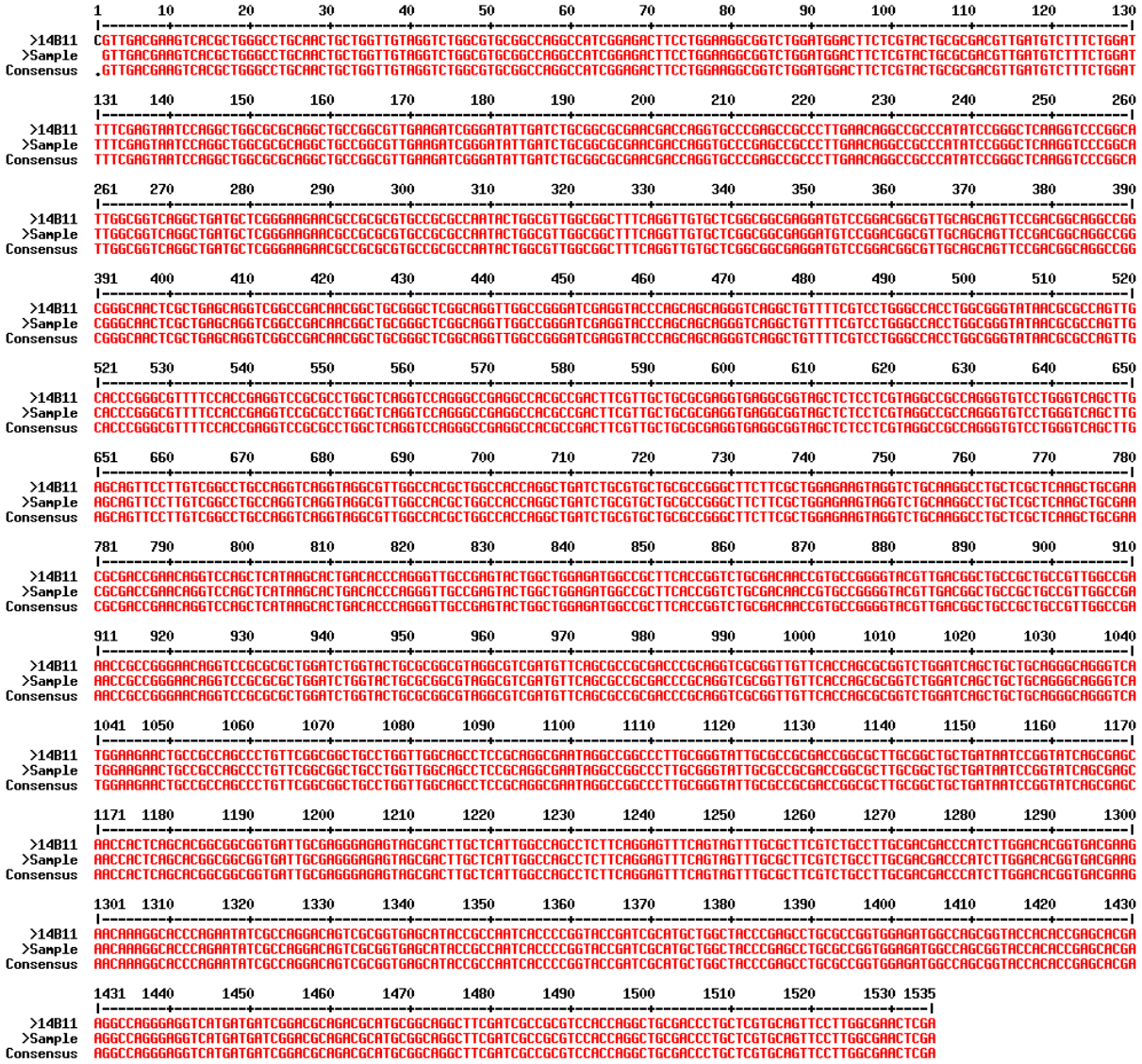
Sequence Alignment Reveals the AdeC gene is a Homologous OMP gene in *P. chlororaphis* 14B11

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?



Gene sequencing Revealed 5 out of the 6 Phage-Resistant Colonies Displayed no Mutations to the AdeC Coding Region

Sample = Mutant colony 4

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

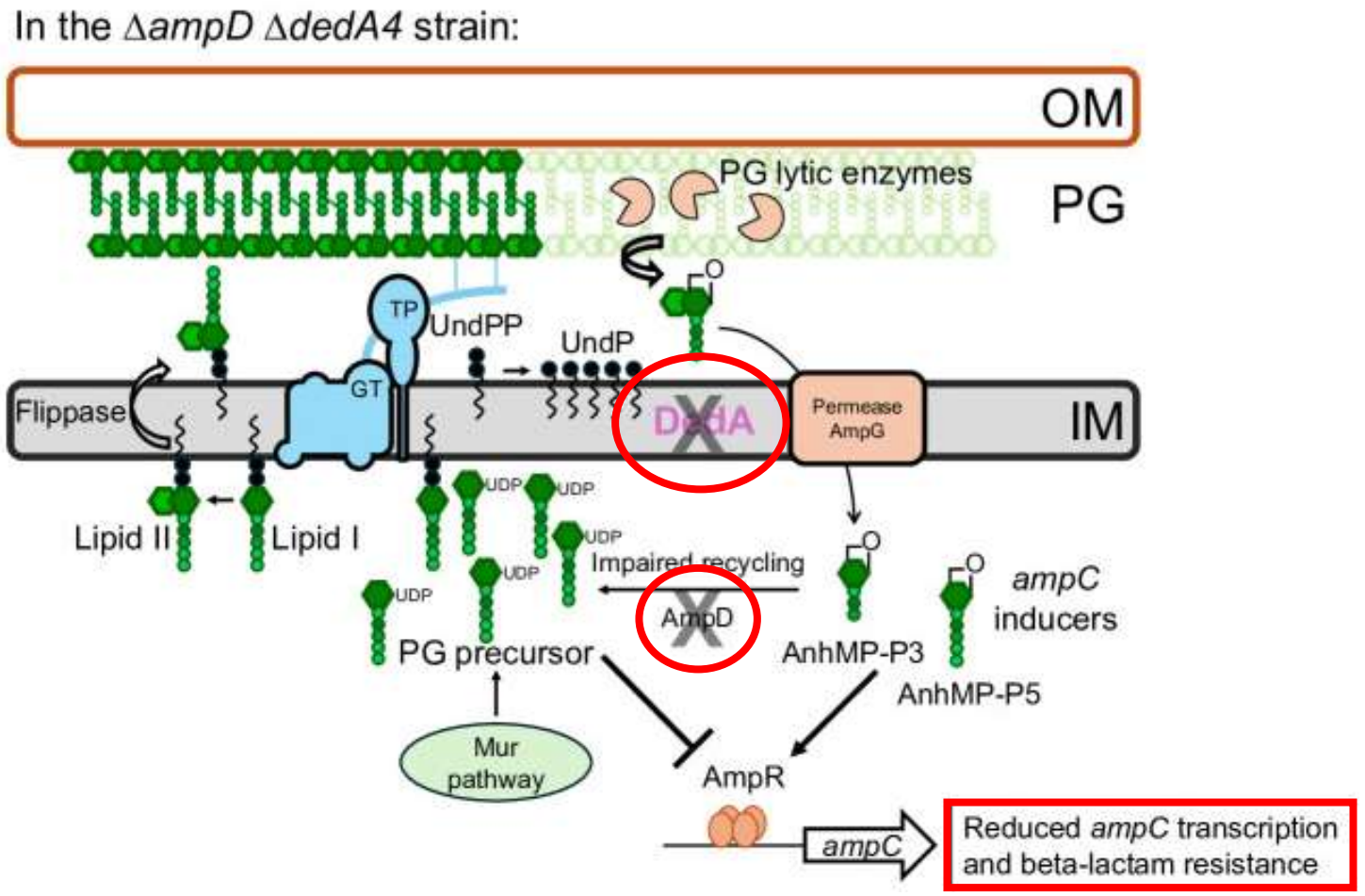


Image from: Klycheva, K.; Gyger, J.; Frund, M.; Torrens, G.; Cava, F.; Fumeaux, C. Disruption of Undecaprenyl Phosphate Recycling Suppresses ampC Beta-Lactamase Induction in Pseudomonas Aeruginosa. bioRxiv June 5, 2025, p 2025.06.03.657773. https://doi.org/10.1101/2025.06.03.657773.

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

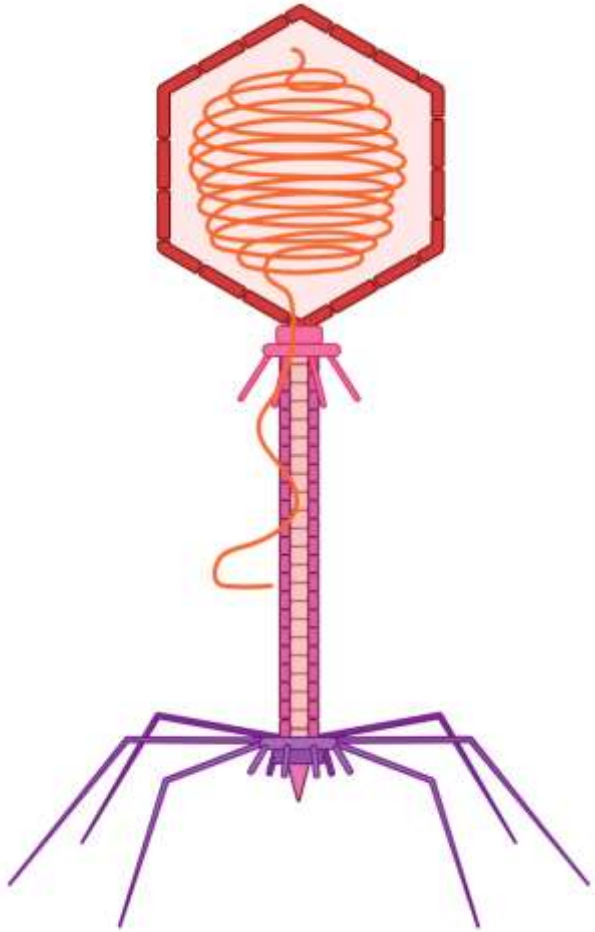
- Is there similar increased antibiotic sensitivity shown in other penicillin class antibiotics?

Acknowledgements

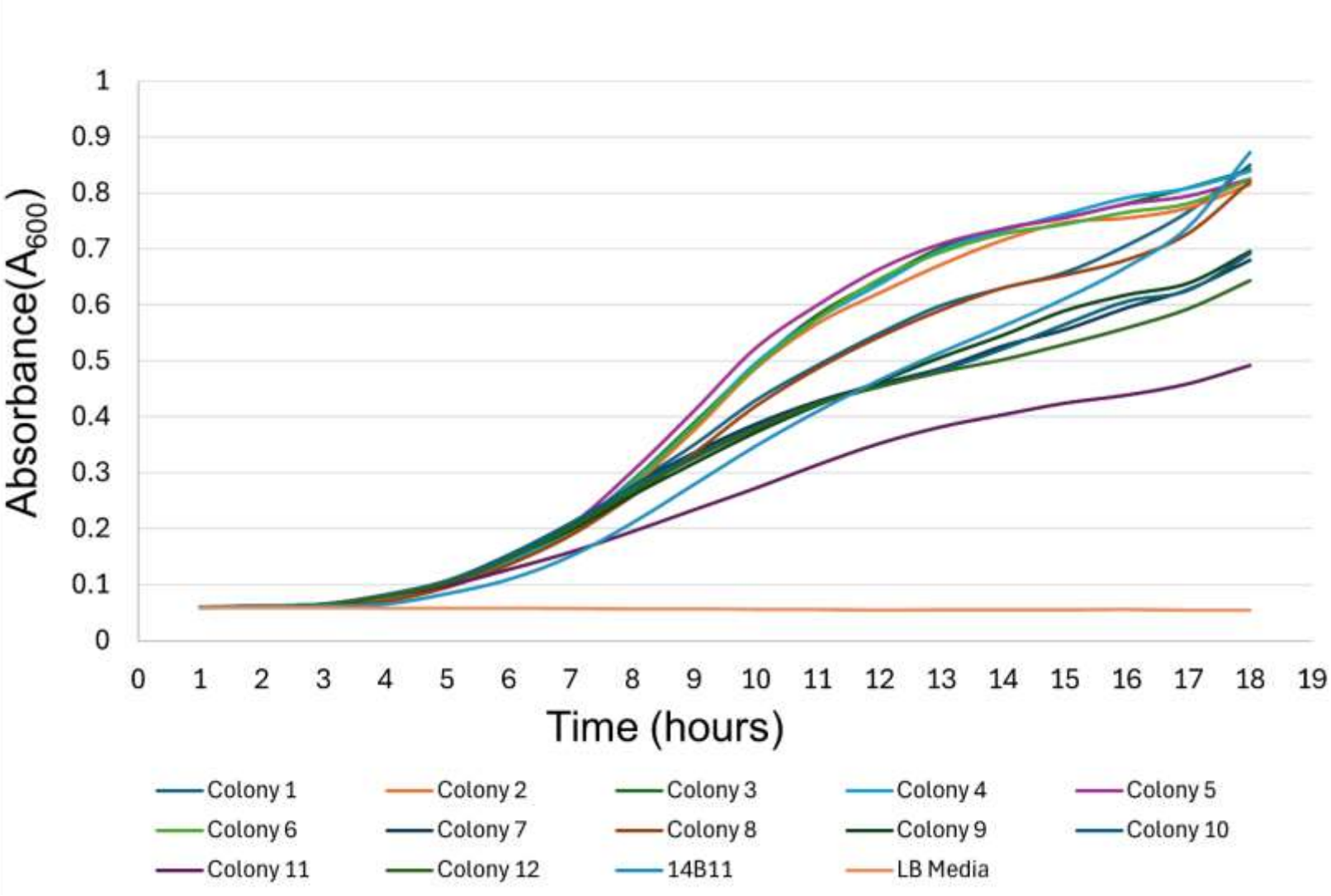
Thank you to The College of Wooster Copeland Fund for providing the financial resources necessary to carry out this research

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

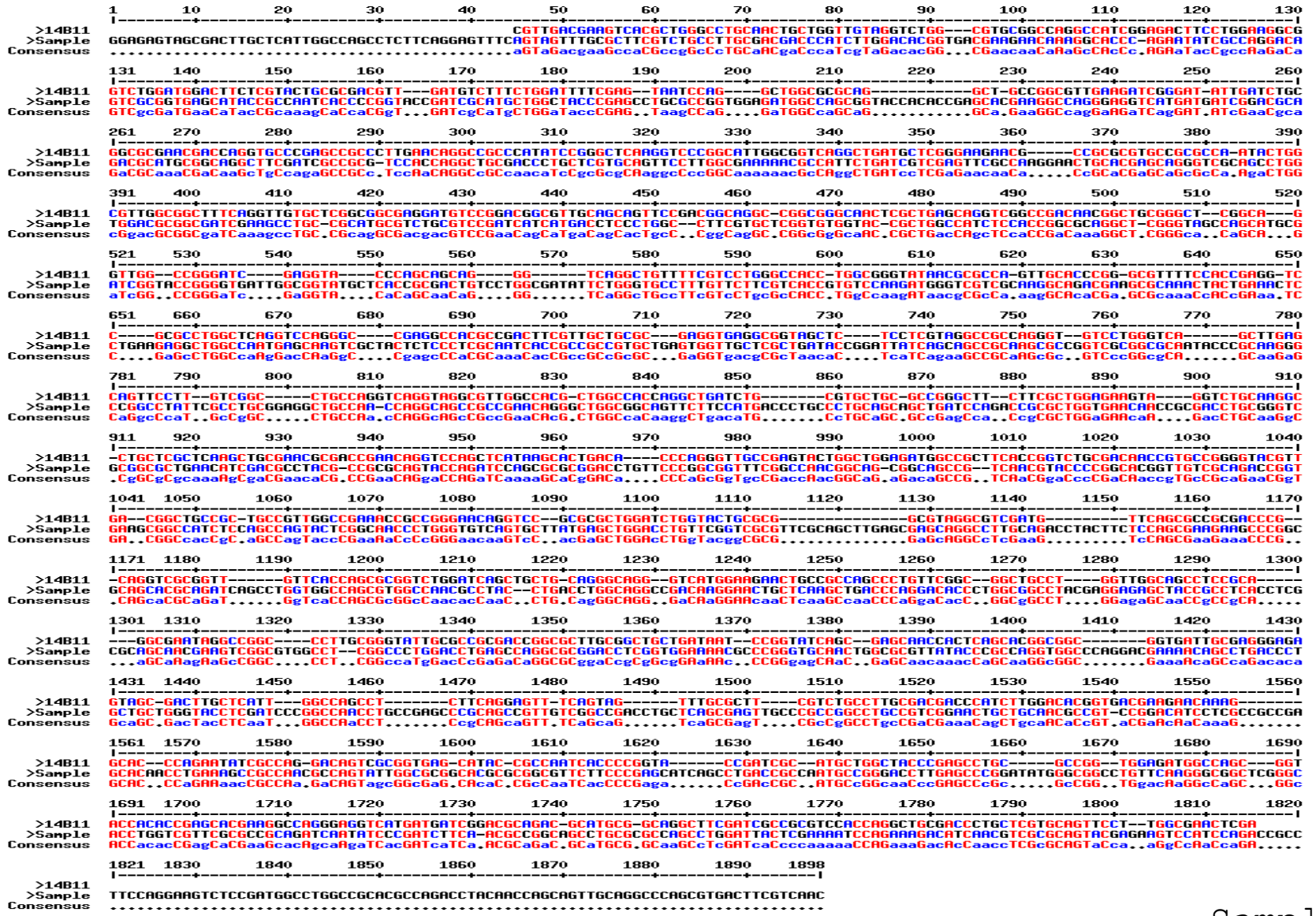
Thank You!! Any Questions?



Growth Curve Determined Phage-Resistant Mutants Acquired No Growth Mutations

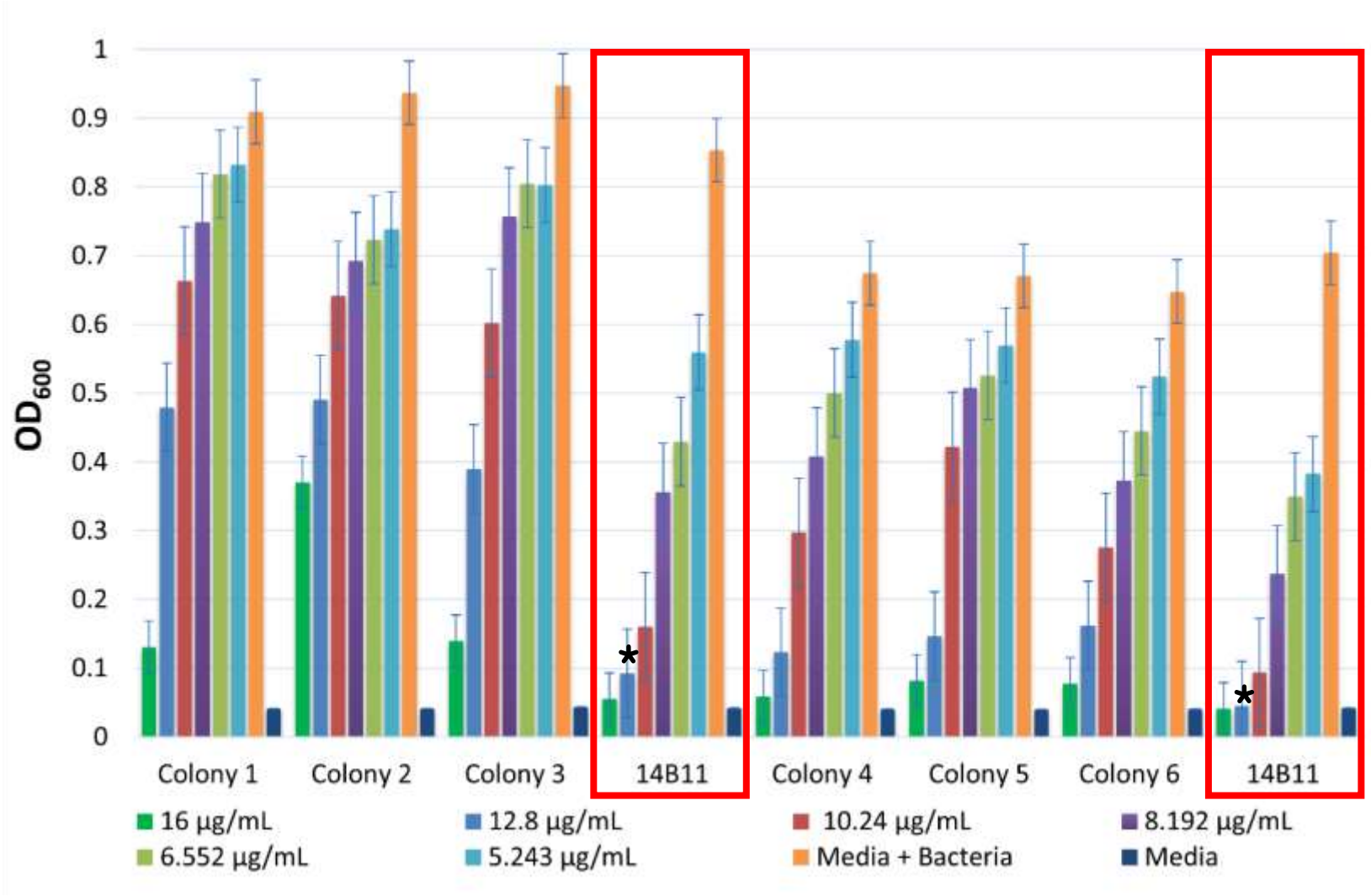


One Mutant Colony Displayed Mutations to the AdeC Coding Sequence



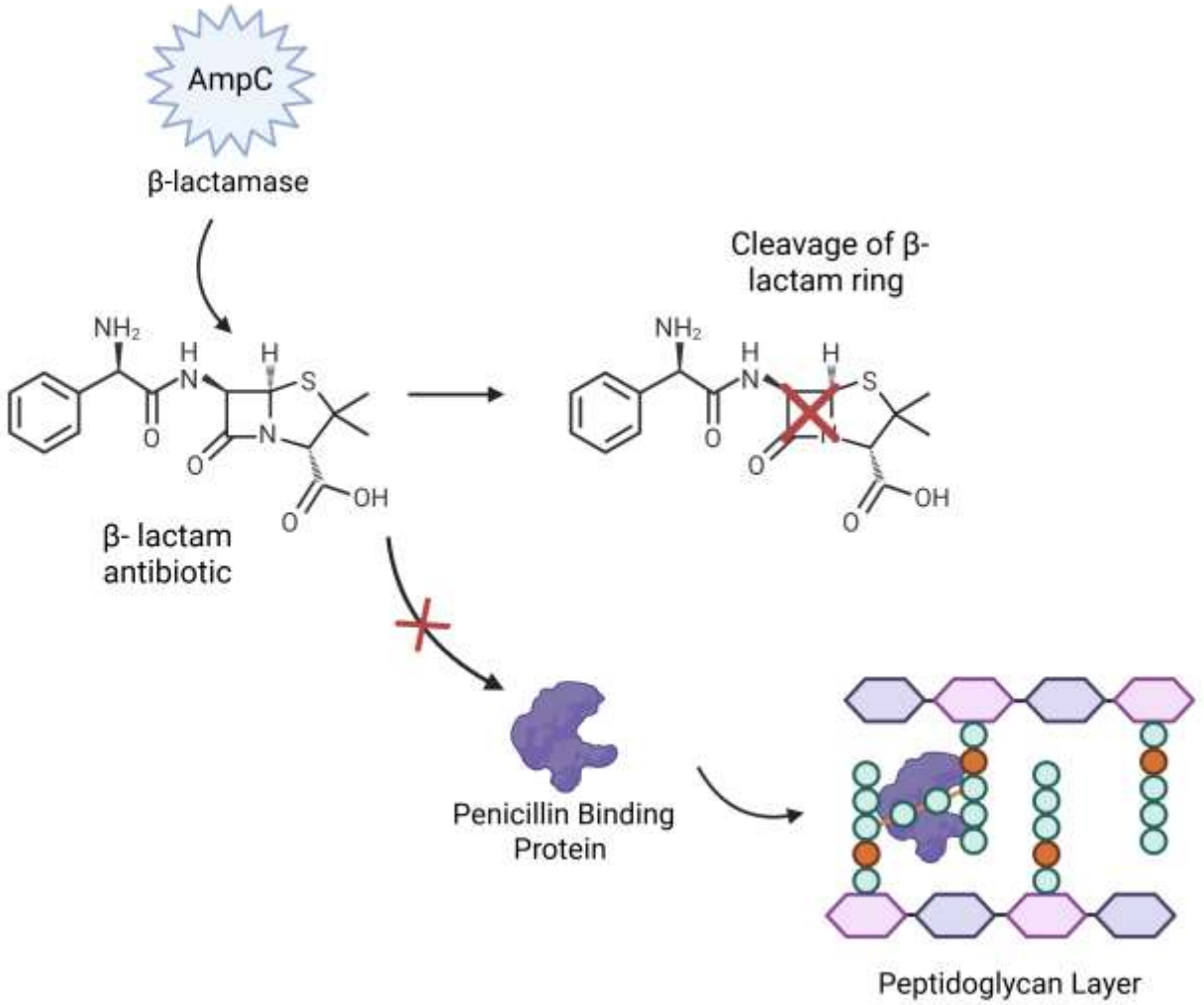
Sample = Mutant Colo

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

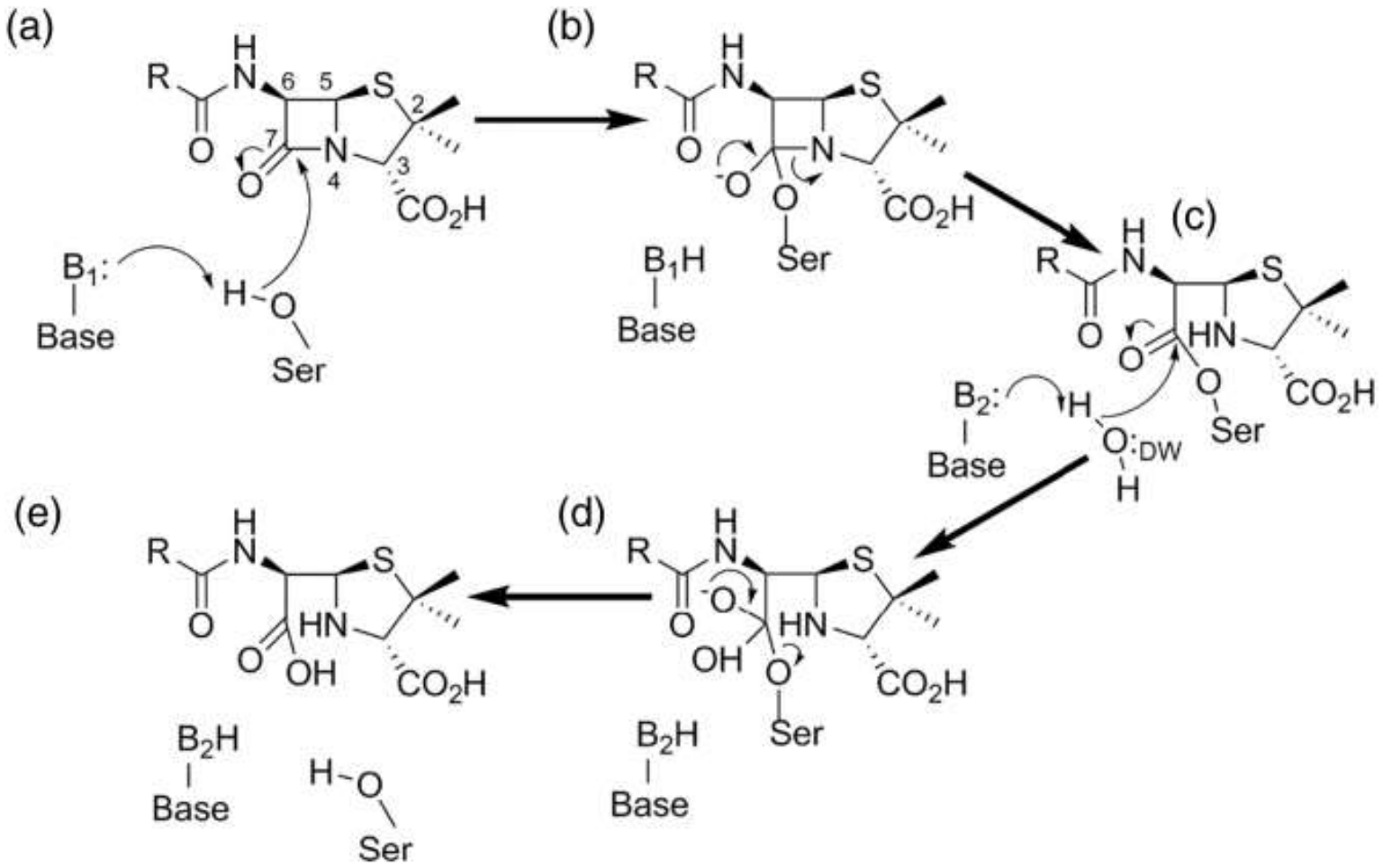


* = MIC of TET

High AMP Resistance for 14B11 is Likely Due to Genetically Encoded β-lactamase Enzymes

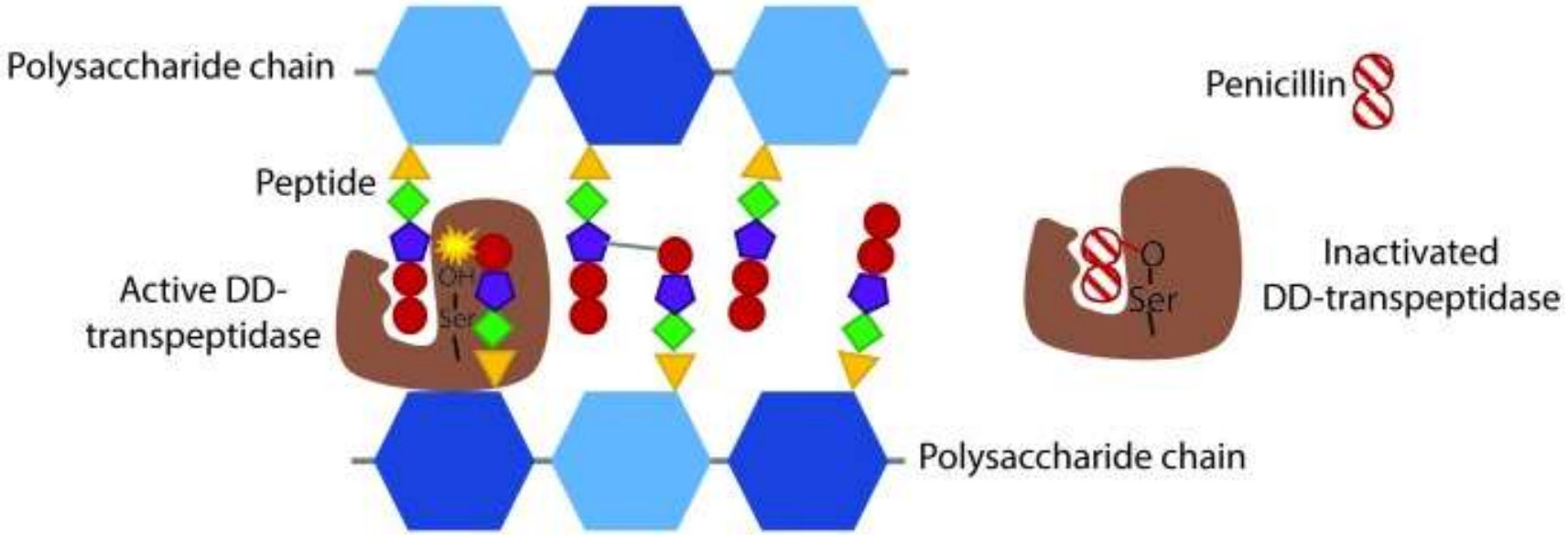


Mechanism of β-lactamases



Tooke, C. L.; Hinchliffe, P.; Bragginton, E. C.; Colenso, C. K.; Hirvonen, V. H. A.; Takebayashi, Y.; Spencer, J. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *J Mol Biol* **2019**, *431* (18), 3472–3500. https://doi.org/10.1016/j.jmb.2019.04.002.

Penicillin Mechanism of Action



Lobanovska, M.; Pilla, G. Penicillin's Discovery and Antibiotic Resistance: Lessons for the Future? *Yale J Biol Med* **2017**, *90* (1), 135–145.

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

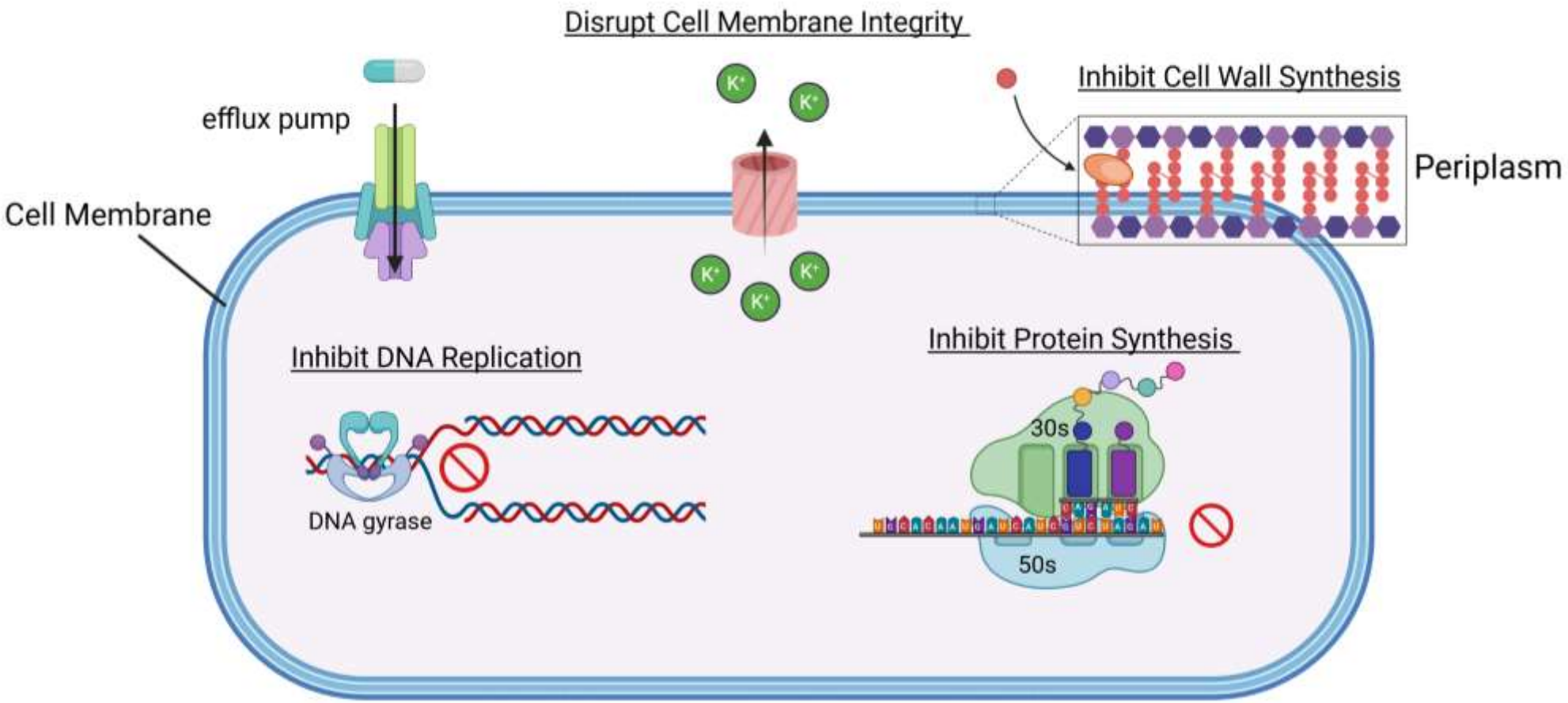
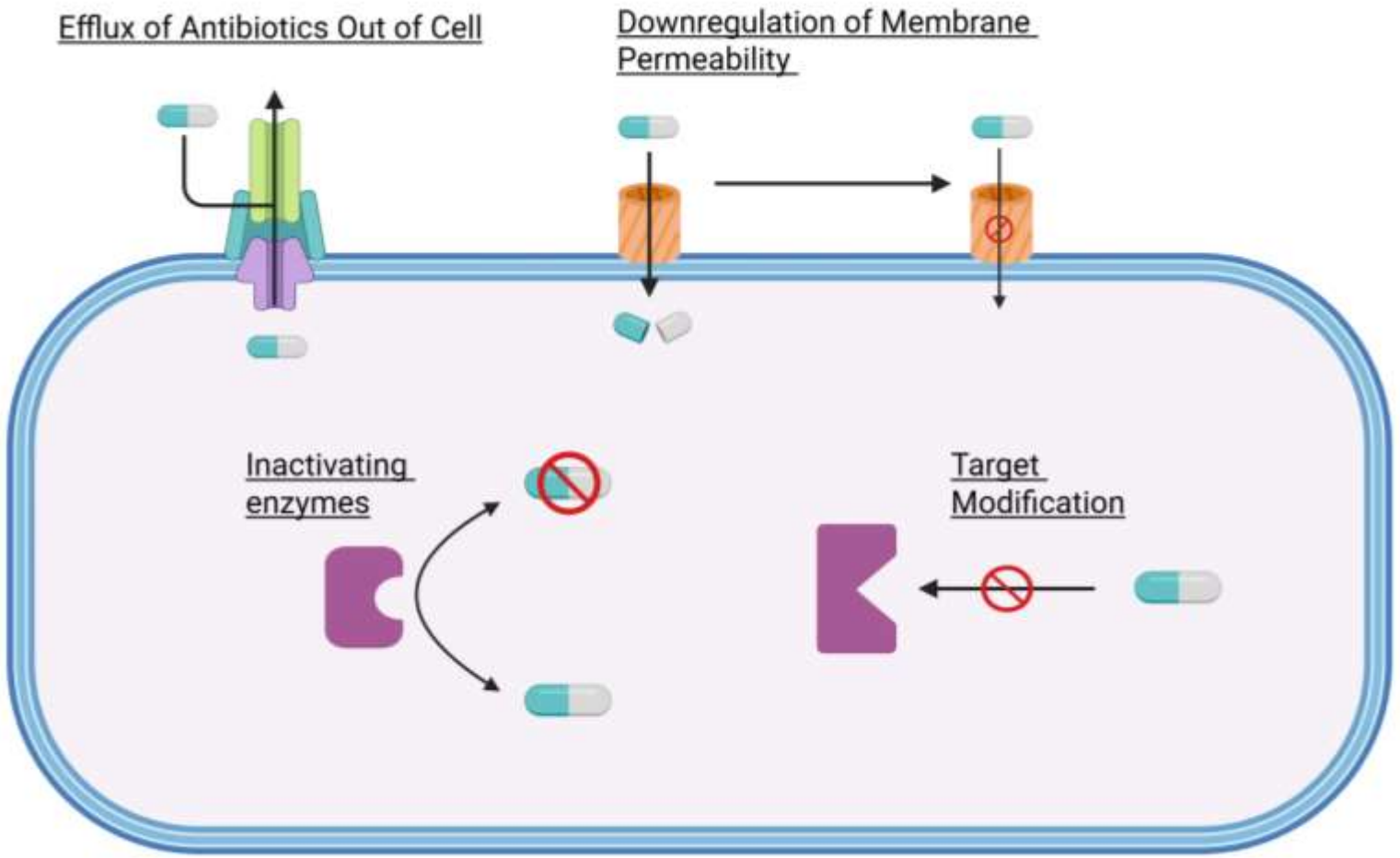


Figure made with BioRender

Zhang, F.; Cheng, W. The Mechanism of Bacterial Resistance and Potential Bacteriostatic Strategies. *Antibiotic* **2022**, *11* (9), 1215. https://doi.org/10.3390/antibiotics11091215.

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



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

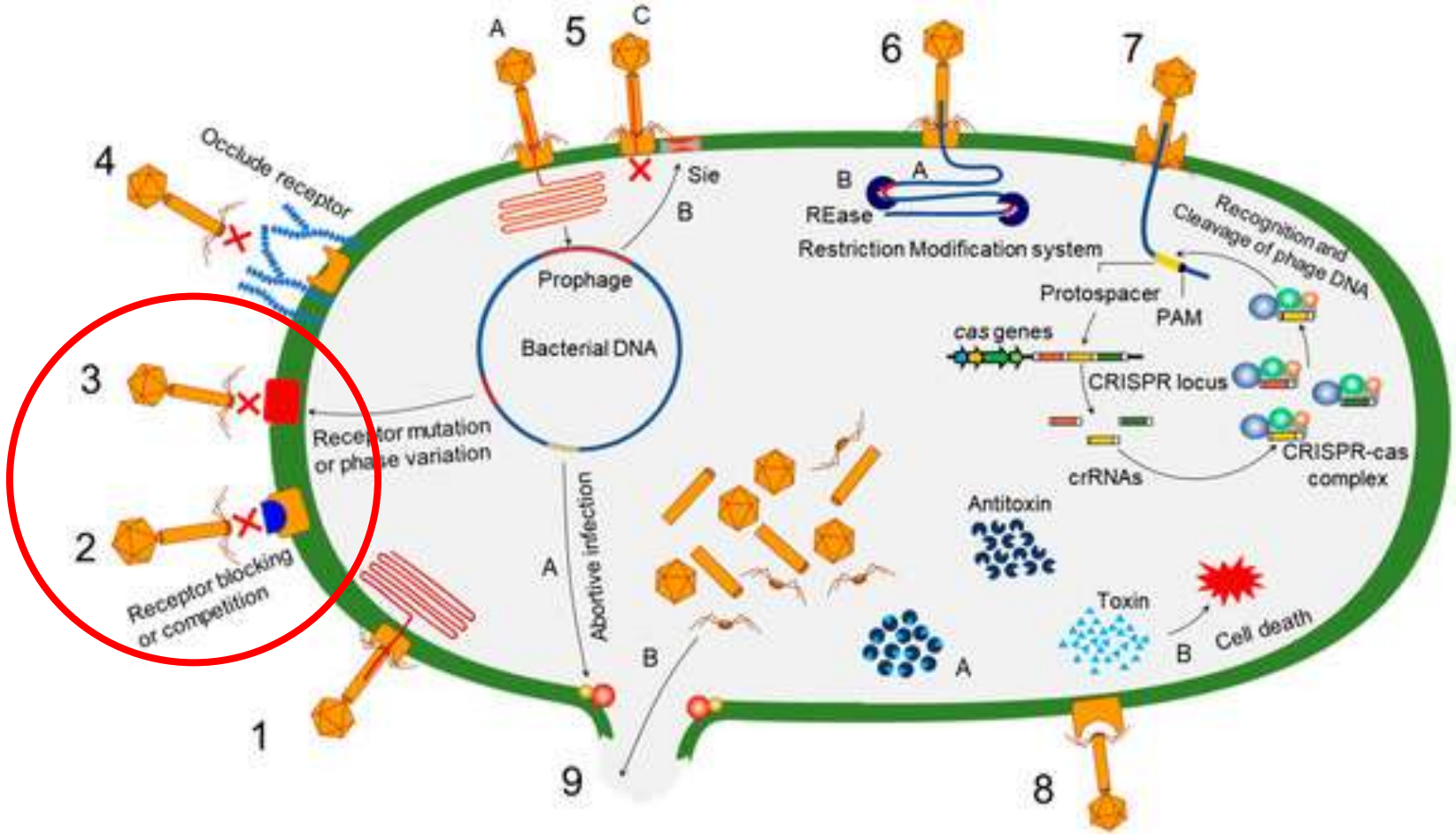

Image from: Hasan, M.; Ahn, J. Evolutionary Dynamics between Phages and Bacteria as a Possible Approach for Designing Effective Phage Therapies against Antibiotic-Resistant Bacteria. *Antibiotics* **2022**, *11* (7), 915. https://doi.org/10.3390/antibiotics11070915.