Communicating Research to the General Public

The WISL Award for Communicating PhD Research to the Public launched in 2010, and since then over 100 Ph.D. degree recipients have successfully included a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance—as well as their excitement for and journey through their area of study—to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

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FUNCTIONAL CHARACTERIZATION OF ESSENTIAL GENES IN *ACINETOBACTER BAUMANNII*

by

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CHAPTER 5

Chapter for the Public: A Journey through the Genetics of a Drug-Resistant Bacterium

Introduction

As graduate students and scientists, we are traditionally trained to communicate primarily with other experts in our field, focusing on a niche audience. As a result, academic research can sometimes be insular, with researchers working in isolation within very specialized areas. However, to truly advance science, solve complex problems, and create meaningful real-world change, we need to break out of these silos and engage with diverse perspectives and disciplines. Clear communication not only inspires collaboration but also encourages more people to join and support science, contributing to a better and more inclusive scientific future.

My goal for this chapter is to share my six years of graduate work with the people in my life and beyond, who come from all walks of life, and hopefully spark an interest in microbiology. Many of these people may not consider themselves scientists but have the potential to be, or already are.

I'm thankful for the Wisconsin Initiative for Science Literacy (WISL) and Professor Bassam Shakhashiri, for giving me the opportunity to write this chapter, and Cayce Osborne and Elizabeth Reynolds for their writing guidance and editing assistance.

Discovering the World of Superbugs

While working towards my undergraduate degree in Biochemistry and Genetics at Texas A&M University, I had the opportunity to perform research in the lab of Dr. Ry Young. People often equate viruses and bacteria when thinking about infectious diseases, but my undergraduate research taught me that there are distinct differences. Bacteria, like *E. coli* or *Salmonella*, are single-celled microorganisms that can live, survive, and multiply independently. In contrast, viruses, like those causing the flu or common cold, require a host to infect. They hijack host cell machinery to multiply and eventually escape back into the environment to reinfect others. In the Young lab, I studied viruses that only infect bacteria, called bacteriophages or 'phages' for short. During my time there, members of the lab were asked to isolate phages as an experimental treatment for a patient with an immediately life-threatening bacterial infection that was unresponsive to any antibiotics or treatments. These phages targeted and killed the bacteria causing the infection and helped save the patient's life.[1]

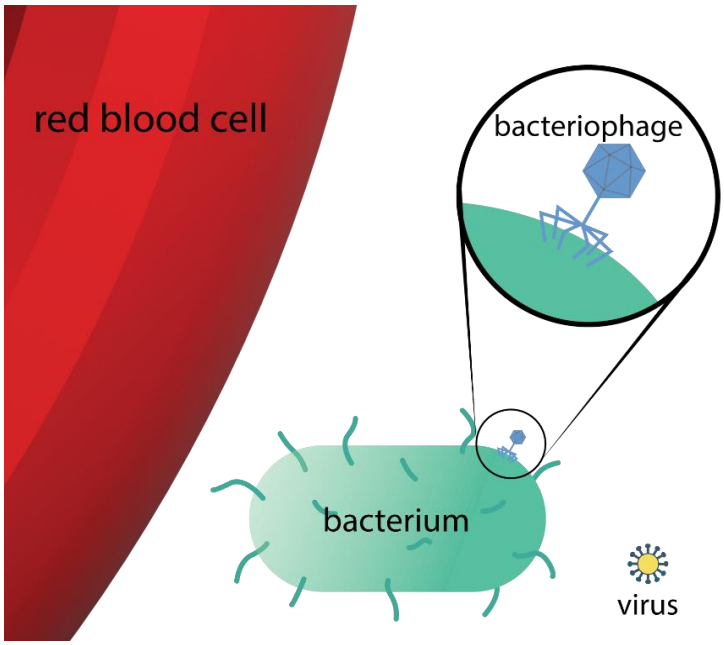

Figure 5.1 A bacterium and viruses to scale compared to a red blood cell. Bacteria are typically 1-5 micrometers, or microns, in length, where viruses are typically 0.02-0.5 microns. Red blood cells in comparison are ~25 microns.

This experience was my first real exposure to superbugs – disease-causing bacteria resistant to all known antibiotics that pose a significant threat to global public health. These pathogens have evolved to withstand multiple antibiotics, rendering standard treatments ineffective and leading to

[1] 1. Steffanie A. Strathdee, Teresa Barker, and Thomas L. Patterson, *The Perfect Predator: A Scientist's Race to Save Her Husband from a Deadly Superbug: A Memoir* (New York, NY: Hachette Books, 2020).

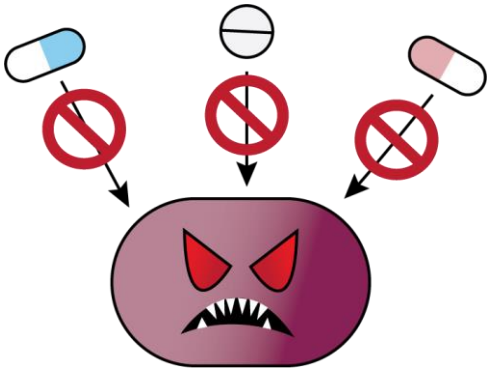

Figure 5.2 A cartoon depicting a superbug, resistant to antibiotic treatment.

persistent, hard-to-treat infections. The overuse and misuse of antibiotics have accelerated this resistance, resulting in bacteria that are nearly impossible to treat with existing drugs. According to the World Health Organization (WHO), antibiotic resistance is one of the biggest threats to global health, food security, and development today.[2] Superbug infections can cause severe illness, longer hospital stays, increased mortality rates, and higher healthcare costs. The rise of superbugs underscores the urgent need for new antibiotics and innovative treatment strategies to combat these resilient pathogens and safeguard public health.

I was inspired by my time in the Young Lab to pursue a PhD in Microbiology and further study these superbugs and their genetics. My thesis work in Dr. Jason Peters' lab, here at the University of Wisconsin-Madison, focuses on *Acinetobacter baumannii* or *A. baumannii* – the same bacteria that caused that deadly infection treated with Young Lab phages. By studying *A. baumannii* genetics and physiology and how it responds to antibiotics, our lab hopes to eventually develop better treatments.

The Good, The Bad, and The Neutral Bacteria: Where does *A. baumannii* fit in?

Not all bacteria are harmful. 'Good' bacteria in our intestines aid in digesting foods, producing vitamins, and even protecting us from infectious bacteria. Soil and compost bacteria help break down leaf litter and waste, while others help plants get nutrients by growing in their roots. These microorganisms are everywhere and play important roles in human health, agriculture, and more.

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[2] World Health Organization. *WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance*. World Health Organization, 2024.

In contrast, some bacteria are 'neutral' and free-living in the environment, not designed for human infection or benefit. Yet, these neutral bacteria can sometimes cause serious infections in individuals with weakened immune systems – these are known as opportunistic pathogens.[3] *A. baumannii* is one such pathogen. It can take advantage of the weakened defenses of immunocompromised individuals, such as those undergoing chemotherapy, patients with serious wounds or illnesses, or patients with chronic autoimmune diseases. Infections caused by *A. baumannii* can lead to severe conditions such as pneumonia, bloodstream infections (bacteremia), and septic shock. Commonly found in soil and water, *Acinetobacter* species have evolved to grow under a variety of conditions and protect themselves from harsh environments. This resilience makes *A. baumannii* a formidable pathogen, particularly in healthcare settings where it can infect patients

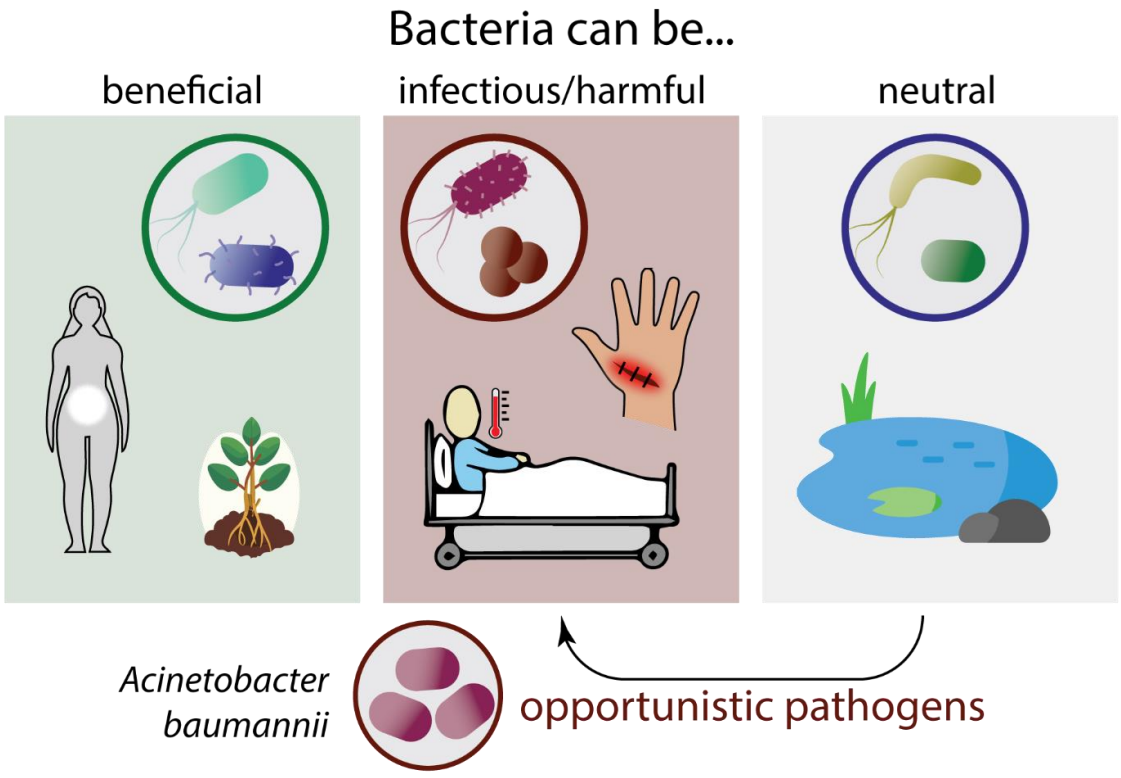

Figure 5.3 Depiction of beneficial or 'good' bacteria, infectious/harmful bacteria, and bacteria which are typically neutral and do not benefit or infect other organisms. An arrow depicts how opportunistic pathogens, which are typically neutral, can become infectious or harmful to immunocompromised individuals. *Acinetobacter baumannii* is one of these opportunistic pathogens.

[3] Wassenaar, Trudy M. 2012. *Bacteria : The Benign, the Bad, and the Beautiful.* Hoboken, N.J: Wiley-Blackwell.

with weakened immune systems and survive on hospital surfaces for long periods without nutrients or water.

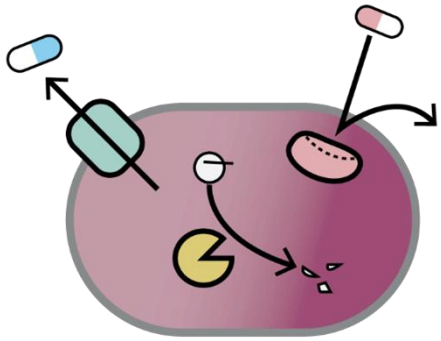

Figure 5.4 Antibiotic resistance mechanisms of *A. baumannii* include efflux pumps (green) that pump out antibiotics, proteins (yellow) that degrade or break down antibiotics, and changes to the target (pink) to prevent antibiotic binding.

The Challenge of Antibiotic Resistance

The difficulty in treating *A. baumannii* infections lies in its remarkable ability to develop resistance to antibiotics. Antibiotics typically work by targeting essential processes that bacteria need to survive, like DNA replication or production of the envelope that surrounds and encapsulates the cell. However, *A. baumannii* has evolved several mechanisms to evade these drugs. It can produce molecules that degrade antibiotics, pump out antibiotics before they can take effect, and alter its cellular targets to reduce drug binding. These survival tactics, coupled with its ability to acquire antibiotic resistance genes from other bacteria, have led to the emergence of multidrug-resistant superbug strains.[4]

Understanding the genetic and molecular basis of *A. baumannii*'s resistance mechanisms is crucial for developing new strategies to combat these infections. My research focuses specifically on genes involved in those essential processes necessary for the bacteria to survive, which are often the target for antibiotics. By pinpointing these essential genes, I hope to learn more about *A. baumannii* biology, find new ways to disrupt the bacteria's defenses, and develop more effective treatments.

[4] Kyriakidis, Ioannis, Eleni Vasileiou, Zoi Dorothea Pana, and Athanasios Tragiannidis. 2021. "Acinetobacter Baumannii Antibiotic Resistance Mechanisms." *Pathogens* 10 (3): 373. https://doi.org/10.3390/pathogens10030373.

The Scientist's Toolkit: how (and why) do we study *A. baumannii* genetics?

To understand and combat superbugs like *A. baumannii*, researchers use a variety of techniques from genetics and molecular biology - like DNA sequencing to determine the genetic code, PCR (Polymerase Chain Reaction) to amplify specific DNA segments, and gene cloning to introduce DNA copies or new DNA into a cell. We can use these techniques to create bacterial mutants that have undergone changes or mutations in their DNA, which allow us to study how specific genes contribute to *A. baumannii's* ability to cause disease, survive in hostile environments, or resist antibiotics.

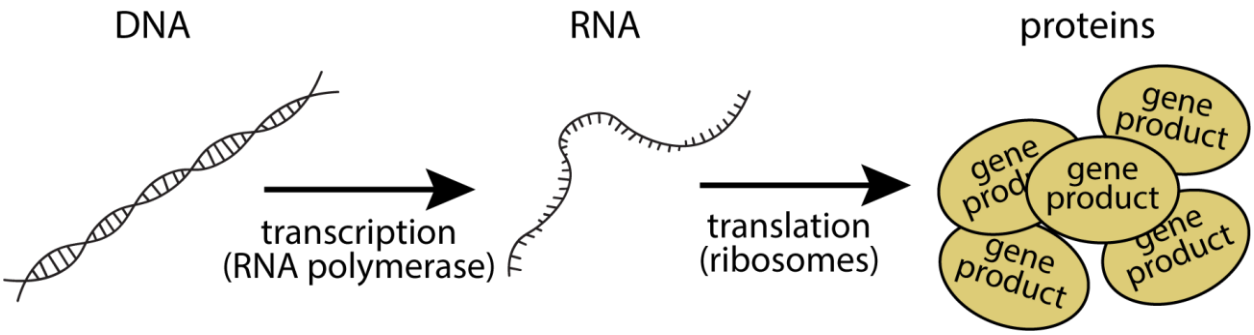

Figure 5.5 The Central Dogma of Biology. This illustrates the flow of genetic information within a biological system, where DNA or genetic material is transcribed by RNA polymerase into RNA, which is then translated by ribosomes into large molecules called proteins that perform functions in the cell. Changing or mutating the DNA affects everything downstream, causing cellular differences that can be seen or measured.

In the lab, my colleagues and I use additional CRISPR tools to create *A. baumannii* essential gene mutants. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats, after the DNA sequences that first pointed scientists to this naturally occurring system) is a revolutionary genetic engineering tool that allows scientists to make precise changes to the DNA of living organisms. It works like a pair of molecular scissors that can cut DNA at specific locations, allowing for the addition, removal, or alteration of genetic material. However, because these genes are essential, cutting them with CRISPR kills the bacteria, preventing further studies.

CRISPRi, or CRISPR interference, is a variation of the CRISPR technique.[5] CRISPRi does not cut DNA and is more like a broken pair of scissors. Imagine trying to cut paper with scissors that have blunt, non-cutting blades. You can still 'grab' the paper, but you can't cut it. Similarly, CRISPRi uses a modified version of the CRISPR system that binds to a gene without cutting the DNA. This method blocks gene expression, lowering the number of gene products made, almost like a genetic dimmer switch. This approach causes the bacterial cells to be sick but does not totally kill them when we target essential genes, allowing us to conduct more experiments and learn more about essential gene functions. We call these CRISPRi mutants "knockdowns."

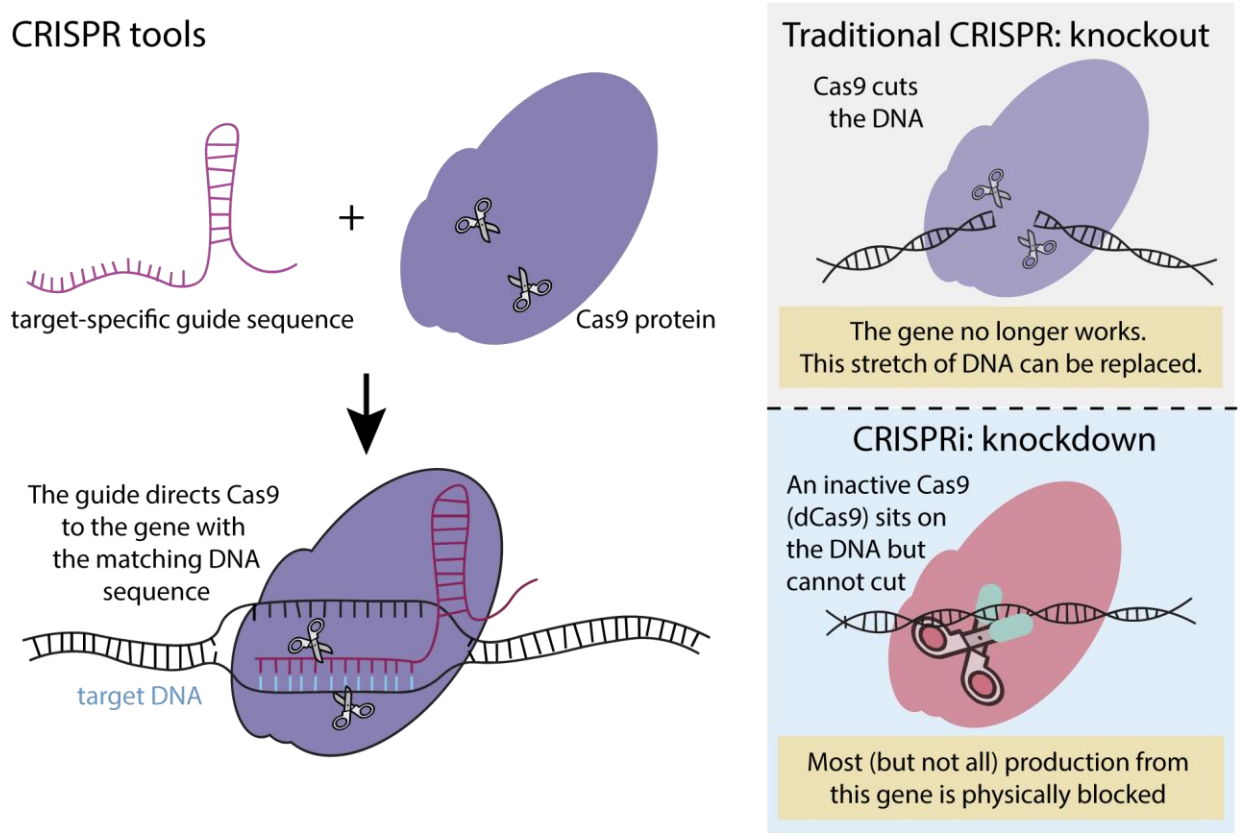

Figure 5.6 CRISPR involves a guide RNA and the protein Cas9. We can engineer the guide sequence to match any DNA target of interest. The guide will direct Cas9 to matching sequence, where it will cut the DNA and inactivate genes. In some systems, new DNA sequences can be inserted. In our experiments, using normal CRISPR would kill the bacteria we want to study. Instead, we use CRISPRi, which uses a deactivated Cas9 (dCas9). Instead of cutting, this dCas9 will sit on the DNA and block transcription of the gene.

[5] Qi, Lei S., Matthew H. Larson, Luke A. Gilbert, Jennifer A. Doudna, Jonathan S. Weissman, Adam P. Arkin, and Wendell A. Lim. 2013. "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression." *Cell* 152 (5): 1173–83. https://doi.org/10.1016/j.cell.2013.02.022.

I teamed up with other members of our lab to use CRISPRi to make a pooled collection of *A. baumannii* mutants, each with a different essential gene knocked down. This collection, known as a genetic 'library', allows us to study over 400 genes at once. By observing how well these mutants grow, we could identify which genes are crucial for the bacteria's survival. For example, if a mutant grows poorly or dies, it suggests that the gene we knocked down is important for the bacteria to thrive. Additionally, by treating the library with antibiotics, we can see which mutants are most affected. For instance, if a particular mutant becomes more susceptible to penicillin-like drugs, it indicates that the targeted gene plays a role in penicillin resistance. This helps us determine which genes are specifically involved in antibiotic resistance and could be targeted by new drugs. Studying over 400 genes at once, in multiple conditions, generates a lot of scientific data! While this data is a treasure trove of genetic information, I could not possibly study all of it in-depth within a single PhD. Instead, I focused on some key findings from these experiments that I will share here.

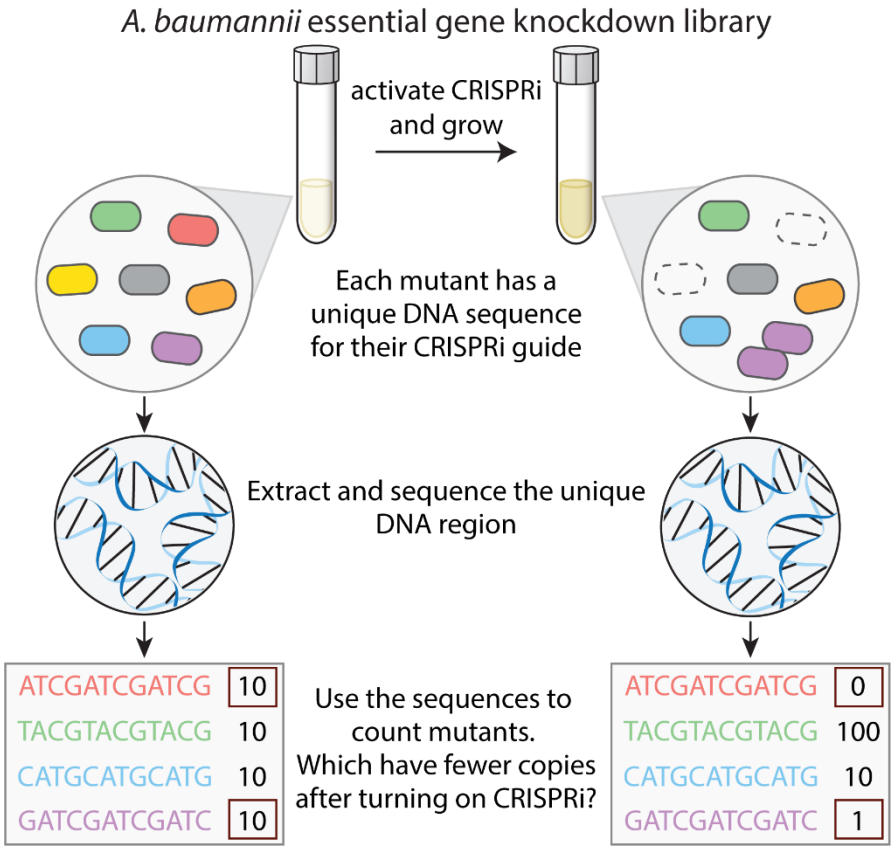

Figure 5.7 Experimental setup to find the essential genes most important for *A. baumannii* survival. Our group looked for knockdowns that were the most depleted after turning on the CRISPRi system.

Finding the best targets: the most vulnerable essential genes

For the first part of this project, our team grew the bacteria in our *A. baumannii* essential gene library, turning on the CRISPRi system and incubating the mutants in culture tubes filled with nutrient-rich liquid. We collected samples from before and after growth and used DNA sequencing to determine the composition of mutants in our samples, looking for gene knockdowns that were sicker compared to the other mutants in the mix. From this experiment, we discovered that *A. baumannii* cells became very sick when we knocked down genes for tRNA synthetases, which are needed for the cell to properly make proteins. Importantly, tRNA synthetases can be targeted with mupirocin, an antibiotic not currently prescribed for *A. baumannii* infections. Although mupirocin is used for different bacterial infections, our data suggested it could potentially treat *A. baumannii* as well. Our team also identified other genes that when targeted or knocked down also made *A. baumannii* sick.

Our team was surprised to find that the gene causing the largest and most significant growth defect was unidentified, its function unknown despite its crucial role in the bacteria's survival. Upon further investigation into the gene, I discovered that it was a phage repressor. Phages - those bacterial viruses I had studied in my undergraduate work - can have two lifestyles: a lytic lifestyle, where they replicate and then burst the host cell to escape and reinfect, and a lysogenic lifestyle, where they integrate their own DNA into the host's and lie dormant until later. This phage repressor was part of one of these lysogenic phages and was actively maintaining the phage's dormant lifestyle.[6] Targeting this phage repressor with CRISPRi caused the phage to switch to the lytic lifestyle, expressing toxic genes that killed the host *A. baumannii* cells. Phages that target and stow away in *A. baumannii* are surprisingly common, and this finding opens the door to discovering proteins produced by these

[6] Britannica, T. Editors of Encyclopaedia. "bacteriophage." *Encyclopedia Britannica*, May 31, 2024. https://www.britannica.com/science/bacteriophage.

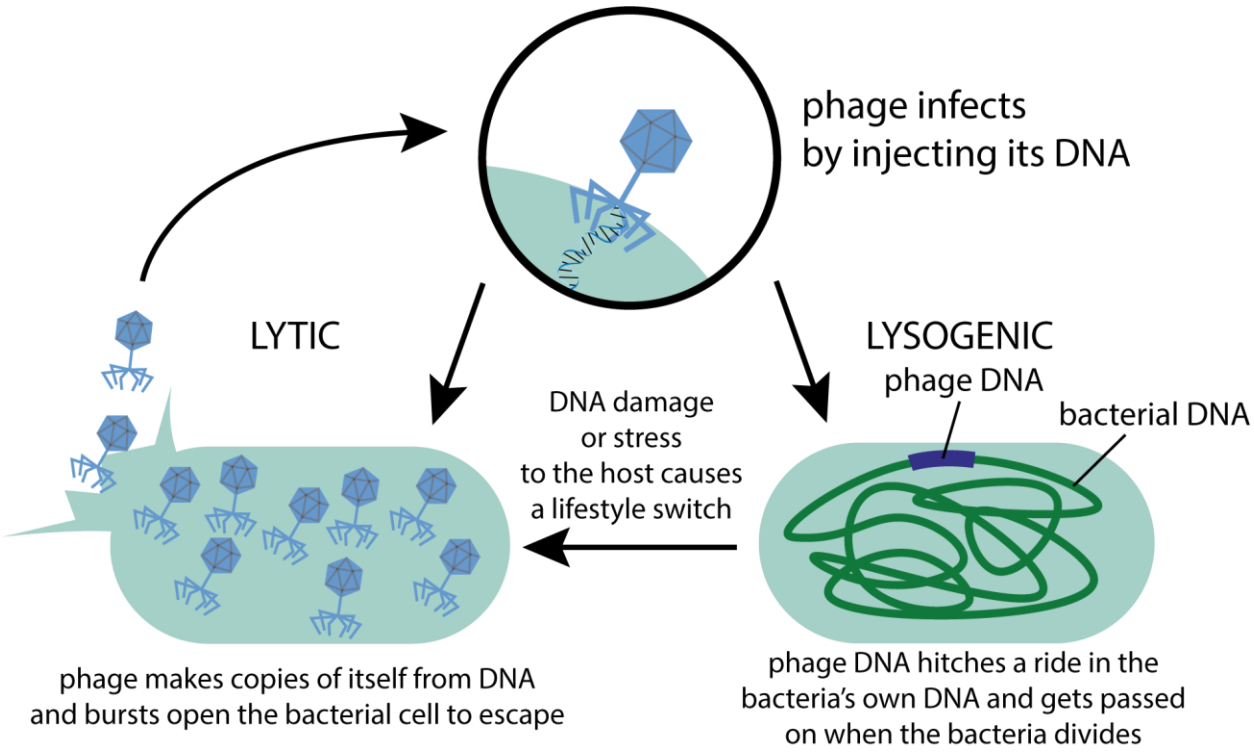

Figure 5.8 The two main lifestyles of bacteriophages. In the lytic cycle, phages infect bacteria, replicate, and then burst open or lyse the host to release new phage progeny. This requires phages to carry genes that can kill their bacterial host from the inside. Some phages have an alternate lifestyle known as lysogenic, in which they integrate their DNA into the bacterial genome. They remain dormant and pass on as the bacteria divides until they are triggered by DNA damage or other stress that causes them to switch lifestyles, like an "eject" button. The phage repressor in our screen was keeping the phage infecting our *A. baumannii* strain in the lysogenic lifestyle. By knocking it down, we essentially hit the "eject" button and killed the *A. baumannii* host.

phages that are exceptionally good at killing *A. baumannii*. Engineered versions of these proteins could potentially be used as future treatments for infection.

Genes in antibiotic resistance

I then wanted to see how *A. baumannii* essential genes interact with antibiotics. To do this, I grew this library of *A. baumannii* mutants again, but this time with low levels of antibiotics added. I tested 45 different antibiotics in separate

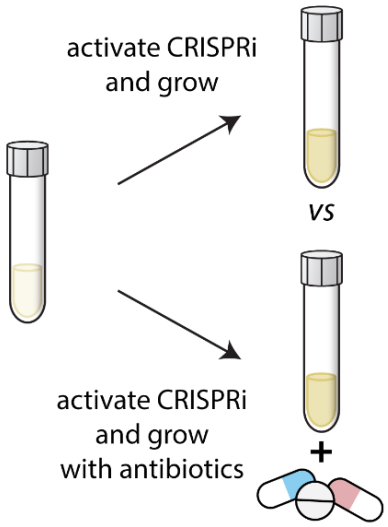

Figure 5.9 I compare the library grown with antibiotics to the library grown without them to find genes especially sensitive to antibiotics.

tubes, looking specifically for gene knockdowns that became sicker, or died, in antibiotics. This would indicate that these genes are needed for drug resistance.

I discovered that knocking down genes involved in lipooligosaccharide (LOS) transport caused *A. baumannii* to be sensitized to a wide array of antibiotics. LOS makes up the outermost layer of the envelope that surrounds and protects *A. baumannii* cells. This LOS layer, the outer membrane, acts as a crucial barrier, preventing harmful substances – like antibiotics – from entering the bacterial cell. The LOS transport system functions like a shuttle, moving LOS from its production site inside the cell

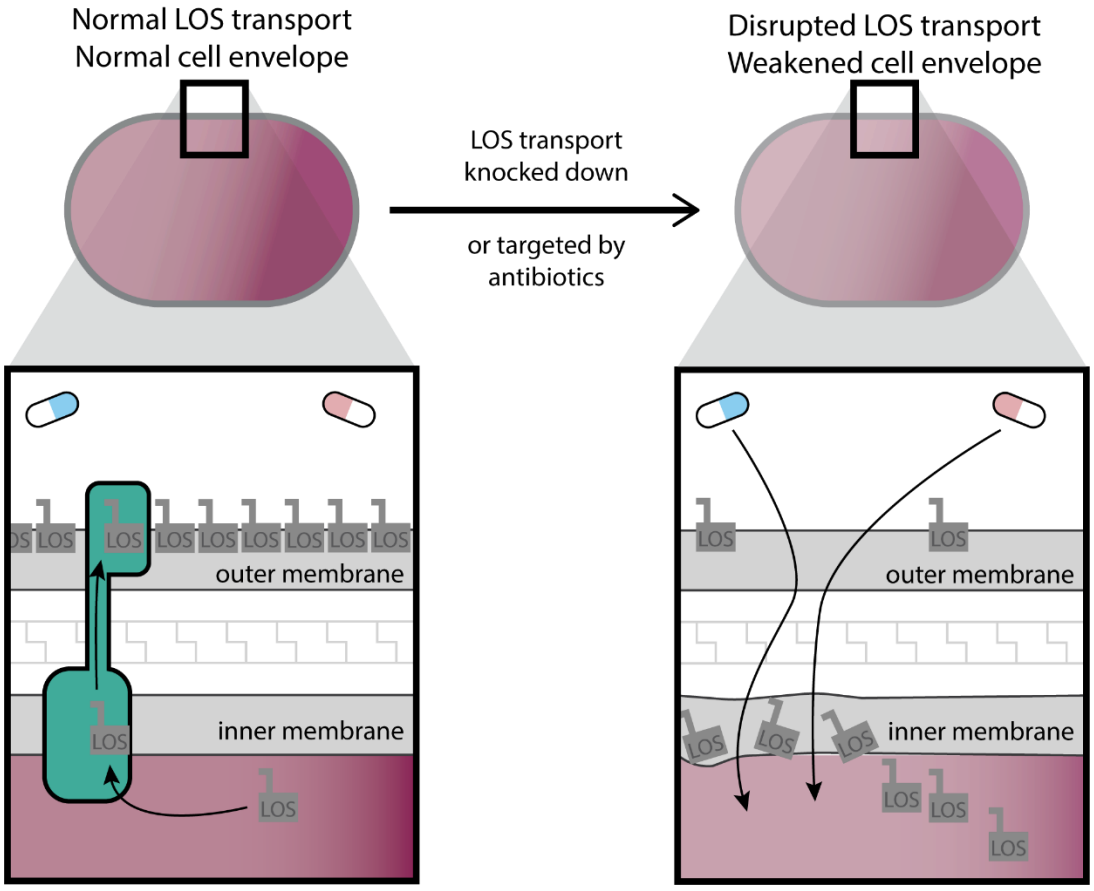

Figure 5.10 *A. baumannii*'s cell envelope, which separates the inside of the cell from the outside, has an inner membrane and an outer membrane. The outer membrane contains LOS that forms a tight barrier to antibiotics. These cartoons depict the cell envelope with normal LOS transport (left) or with disrupted LOS transport (right). LOS is typically transported to the outer membrane by the LOS transport machinery (teal). When LOS transport is knocked down or targeted with antibiotics, LOS builds up in the inner membrane and inside the cell. The inner membrane is weakened by the unexpected backup of LOS, and the outer membrane is missing its important outer component, allowing other antibiotics to pass through both barriers.

to its proper place in the outermost layer. I found that knocking down the LOS transport system causes a traffic jam of LOS in the cell. Surprisingly, this backup doesn't just disrupt the destination of LOS (the outer membrane) but also the stop along the way (the inner membrane), allowing antibiotics to get through both barriers and reach the cell's interior more easily. This finding is especially important in the context of other scientists' work. Recently, new drugs have been developed that specifically target this LOS transport system.[7] My discovery suggests that these drugs, which also cause an LOS backup, could actually enhance the effectiveness of other antibiotics, helping them reach their targets. This tells us that these new drugs targeting LOS transport could be extremely useful in combination therapies – where clinicians prescribe multiple antibiotics together for patient infections – and work simultaneously with existing antibiotics to create a more powerful strategy to combat *A. baumannii* infections.

Connecting the dots with gene functions

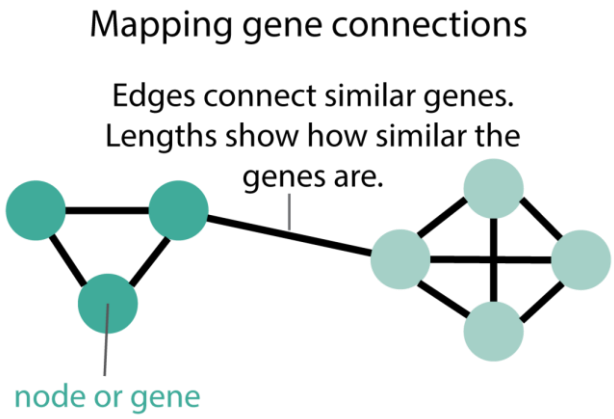

Figure 5.11 I used this large dataset to draw an essential gene network for *A. baumannii*, where each node is a gene. Genes are connected by lines or edges when they behave similarly. Lengths of those edges are related to how similar the genes are. Connected genes are likely to be involved in the same cellular processes.

These types of experiments generate large amounts of data; for each of the 400+ gene knockdowns, I know if they are sensitized, more resistant, or have no change when treated with 45 different antibiotics. Because of the sizable number of observations, patterns start to emerge across the antibiotics. Some of the genes in

[7] Pahil, Karanbir S., Morgan S. A. Gilman, Vadim Baidin, Thomas Clairfeuille, Patrizio Mattei, Christoph Bieniossek, Fabian Dey, et al. 2024. "A New Antibiotic Traps Lipopolysaccharide in Its Intermembrane Transporter." *Nature* 625 (7995): 572–77. https://doi.org/10.1038/s41586-023-06799-7; Zampaloni, Claudia, Patrizio Mattei, Konrad Bleicher, Lotte Winther, Claudia Thäte, Christian Bucher, Jean-Michel Adam, et al. 2024. "A Novel Antibiotic Class Targeting the Lipopolysaccharide Transporter." *Nature* 625 (625): 1–6. https://doi.org/10.1038/s41586-023-06873-0.

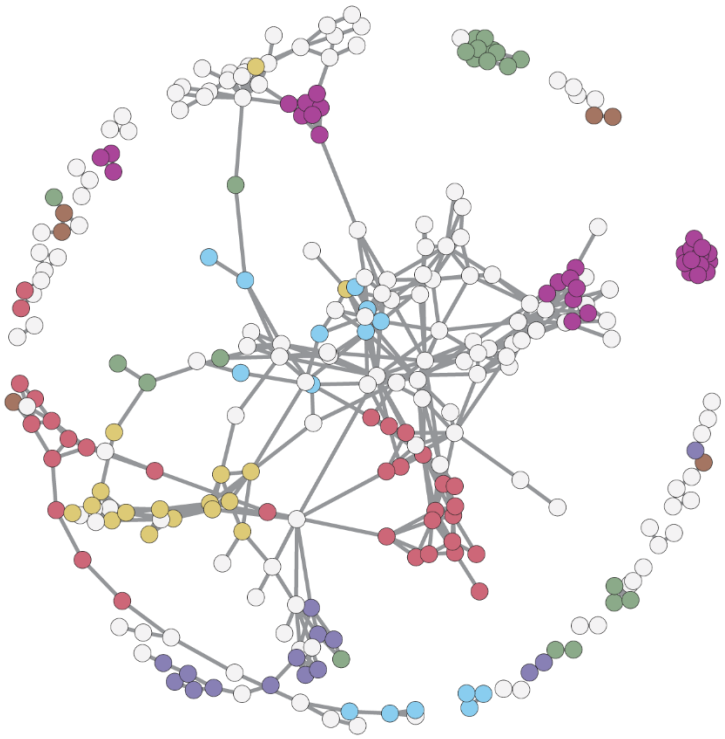

Figure 5.12 An *A. baumannii* essential gene network created using this dataset. Genes that are the same color are involved in the same well-known processes and are mostly interconnected. Uncolored genes fall into an "other" category.

our essential gene library behave similarly to each other when they are knocked down– that is, the knockdown mutants react in the same way across all the antibiotics. Genes with similar patterns have similar functions, or are working towards the same end goal in the cell.[8] I visually represented these similarities in a network, which is like a map of genes, that shows which genes are closely related and interact with each other. By analyzing these connections, I was able to identify the functions of several previously unknown genes.

I additionally focused on a group of genes involved in cell shape and cell division. Cell shape and cell division are closely related processes in bacteria. Cells grow lengthwise before cell division, during which the cell splits into two daughter cells. This process is

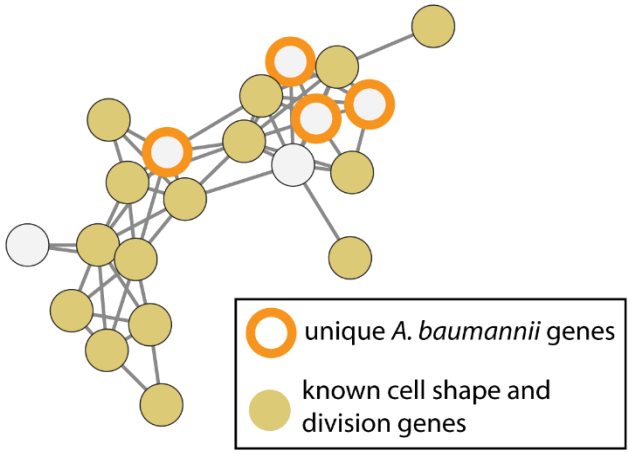

Figure 5.13 The cell division genes from the larger essential gene network. Unique *A. baumannii* genes are circled in orange and are clearly connected to known cell division genes (yellow).

[8] Peters, Jason M., Alexandre Colavin, Handuo Shi, Tomasz L. Czarny, Matthew H. Larson, Spencer Wong, John S. Hawkins, et al. 2016. "A Comprehensive, CRISPR-Based Functional Analysis of Essential Genes in Bacteria." *Cell* 165 (6): 1493–1506. https://doi.org/10.1016/j.cell.2016.05.003.

tightly regulated to ensure that each new cell receives the correct genetic material and maintains the proper shape and size.[9] While the process of cell division has many commonalities across bacterial species, I found that *A. baumannii* has unique genes for cell shape and division machinery that are distinct from other species. *A. baumannii* has a unique potato-like shape, and my research shows that this different machinery for cell division could be the explanation. I used microscopy to confirm that these *A. baumannii*-specific genes were indeed involved in division, observing that knocking down these genes significantly prevented the cells from dividing. Cell division is an important target for antibiotics, such as beta-lactams (like penicillin), but clinical strains of *A. baumannii* are often resistant to the drugs currently available. This discovery opens avenues for developing new, effective antibiotics that target these unique cell division genes.

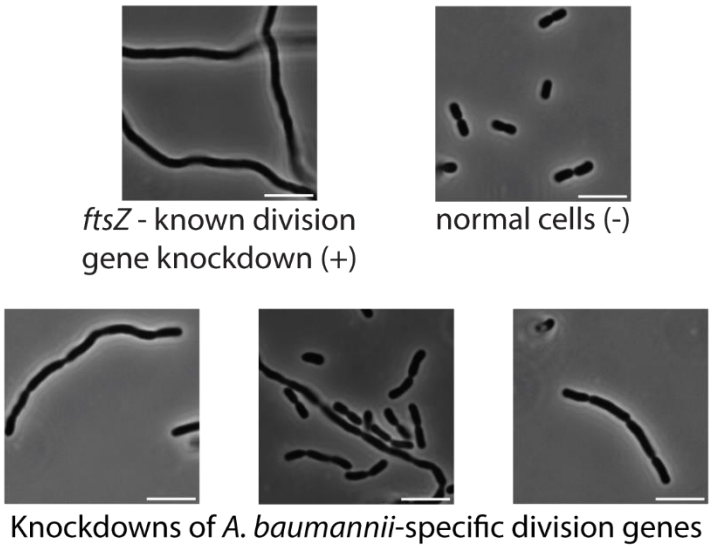

Figure 5.14 Microscopy of the knockdowns of unique division genes in *A. baumannii*. The top left shows a known division gene knockdown. These cells cannot divide and form long strings, compared to normal cells (top right), which are short and almost rounded. Knockdowns of these *A. baumannii*-specific division genes are also elongated and chained, confirming that these genes are needed for proper division. The small line in the bottom right indicates 5 microns.

New weapons in the arsenal: from genetics to therapeutics

My thesis research, using CRISPRi to investigate essential genes in *A. baumannii*, revealed important insights into the bacterium's unique biology and weaknesses. This work is part of a broader trend in genetic research that's opening new doors for drug development against superbugs. By

[9] Margolin, William. 2009. "Sculpting the Bacterial Cell." *Current Biology* 19 (17): R812–22. https://doi.org/10.1016/j.cub.2009.06.033.

understanding how bacteria resist antibiotics and survive in harsh conditions, scientists can find new strategies to target and treat bacterial infections. For instance, by identifying genes that are particularly sensitive when knocked down, our lab pinpointed specific weaknesses that can be used to develop new drugs. These discoveries pave the way for creating new antibiotics to target these critical pathways. I also showed that targeting one pathway can make other antibiotics more effective, potentially guiding future combination therapies for these infections.

This research, which combines large-scale, 'one-step' genetic screening with antibiotic testing, not only has the potential to lead to more effective treatments against multidrug-resistant bacteria, but also exemplifies collaborative scientific efforts. Researchers across academic institutions, pharmaceutical companies, and biotech startups are working together and using these techniques to develop powerful new tools in the fight against antibiotic-resistant infections. With a continued focus on exploring and targeting the ways bacteria survive, infect, and resist antibiotics, we can hopefully overcome multidrug resistance and one of the biggest public health challenges of our time.

Reflections on the PhD journey

My thesis research, presented here, was the backbone of my PhD. I've only shared a small part of our findings here as there was far more work (and struggle) beneath the surface: learning how to ask the right questions, designing experiments to answer them, and handling the large amounts of data these experiments generated. Over the past six years, I've learned to manage, analyze, and interpret vast and confusing data, turning it into coherent stories and visual presentations. Research doesn't happen in a vacuum either. One of my favorite aspects of my thesis project has been collaborating with others who have complementary skills and discussing my research and experiences with peers.

While my thesis research was a major focus during my PhD, it was far from the entirety of my graduate experience. Alongside my research, I embraced various roles that broadened my perspective and

skills. I became actively involved in my graduate program's committees, collaborating with students, staff, and faculty to plan annual student retreats and recruitment and address important program issues like diversity, equity, and inclusion. I delved into the world of entrepreneurship and the intersection of science and business through the Morgridge Entrepreneurial Bootcamp and through the Biotechnology Training Program (BTP). Through BTP, I interned with the Air Force Research Labs, offering me a glimpse into US government research. In more recent years, I've become an active member of WiSolve Consulting Group, a nonprofit run by UW-Madison graduate students and postdocs, helping local startups address challenges through market research, grant writing, and more. I've even had the chance to fundraise and organize major events for fellow graduate students, like UW-Madison Life Sciences Career Day and the Midwest Healthcare Case Competition. These experiences provided unique opportunities that only a graduate student could have, and greatly enriched my personal and professional development. I strongly encourage anyone pursuing a PhD to seek out experiences like this as well. For me, they have been invaluable.

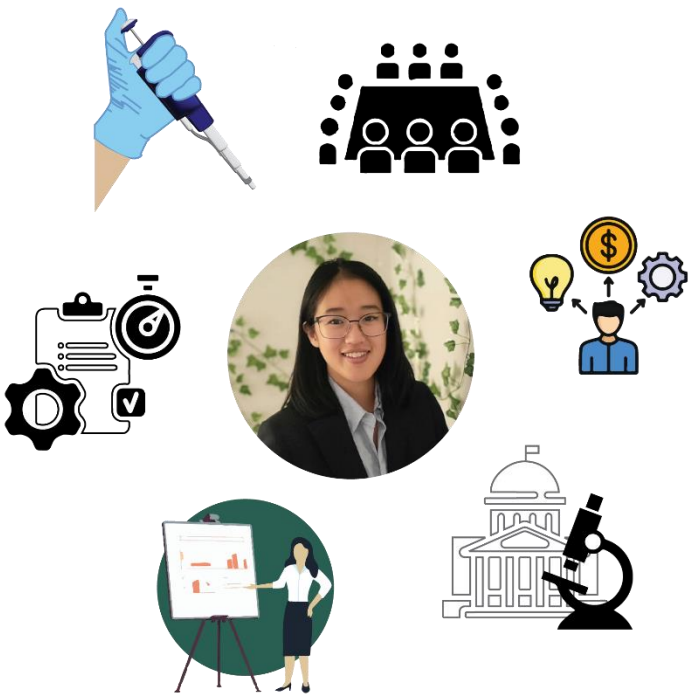

Figure 5.15 My thesis project is only one aspect of my time in graduate school. My experiences outside of the lab helped me build additional soft skills, meet students in other programs, and work with professionals outside of UW-Madison.

All these experiences have led me down a slightly different path than I initially planned when I started graduate school. I found that I genuinely enjoyed computational analyses, giving presentations, and meeting and chatting with diverse teams far more than spending time at the lab bench in my lab coat.

After my PhD, I will be starting a position as a management consultant, applying the skills I've gained in graduate school to areas outside of science experiments. While my thesis project focused on a specific scientific question and allowed me to become an expert in that niche, I now look forward to broadening my experiences across various business sectors. Nevertheless, microbiology, especially bacteria and bacteriophages, will always hold a special place in my heart.