Online Case Study Report: Trying to Understand Antimicrobial Resistance

Ali Ahmed Diaz

Bacteria can infect us leading to illness, however for the past few decades antimicrobial drugs have allowed us to treat these illnesses easily. Bacteria are getting better at fighting these antimicrobial drugs, a phenomena know as Antimicrobial Resistance.

Antimicrobial Resistance is a major challenge to society. As it gets worse, it will be harder and potentially impossible to treat some bacterial infections. By researching how bacteria become resistant to antimicrobials by looking at single bacteria cells at a time, we hope to understand why antimicrobial resistance is getting worse, and what we can do to stop it

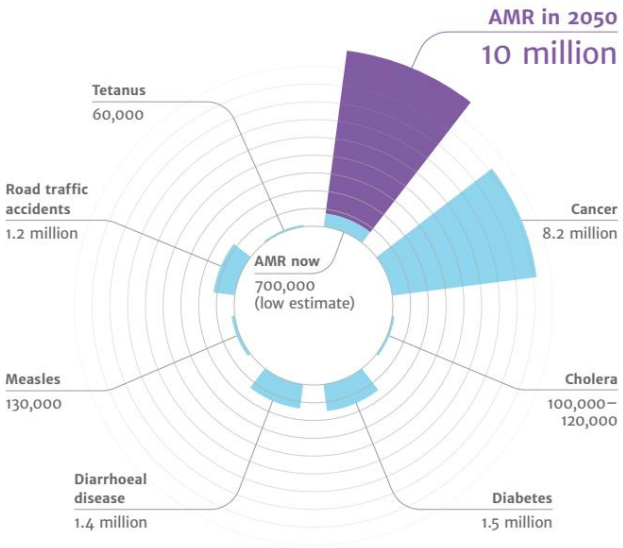
Background

Bacteria are tiny organisms that grow in number rapidly. Although there are many good bacteria which aid our wellbeing, many can become bad and make us sick. Usually, these bad bacteria can be killed using antibiotics - drugs which kill bacteria cells but not human cells. However, bacteria have learned to resist antibiotics, a phenomenon known as antimicrobial resistance.

Bacteria are tiny, look at how small they are compared to a human hair or grain of sand![1]

Antimicrobial resistance poses a major challenge to society in the coming decades. By the year 2050, deaths due to antimicrobial resistance are expected to increase more than 10-fold, exceeding those currently caused by cancer. Prior to the use of antibiotics, it was common for a bacterial infection from small wounds to be lethal for those in peak health. The antibiotic era sadly seems to be ending, and we need to find alternative solutions.

[1] *Zooming in: Visualizing the Relative Size of Particles*



More deaths by Antimicrobial Resistance (AMR) than Cancer currently[2]

Bacteria don't tend to live alone when infecting us, rather in very large communities, even with different species of bacteria. Understanding AMR requires understanding these communities and their behaviors. How bacterial cells interact with their environment, and one another determines how they respond to antimicrobial therapy.

To date, not many researchers have focused on looking at single bacteria cells under the microscope whilst giving then antimicrobial drugs, this is what I pursued in my project. By looking at the single cell isolated from all others, we can learn the building blocks behind the populations of bacteria. It is hard to understand a community without being able to understand an individual!

Why is antimicrobial resistance complicated?

Some mechanisms of antimicrobial resistance, such as resistance to ampicillin (an antimicrobial drug) result in complex cooperative relationships. Resistance to ampicillin is caused by the expression of the β-lactamase enzyme which breaks ampicillin, so it doesn't kill the bacterial cell. β-lactamase can the cell to help neighboring bacteria cells. Cells that have lost the ability to produce β-lactamase will thrive in an environment without ampicillin, since the production of the additional enzymes would put more burden on it.

This is like if your get homework assignments, you will have to spend more time on the homework, which you could otherwise use for extracurricular activities. Similarly, if bacteria must work to produce this enzyme, they won't have much spare energy to do other things, such as growing and dividing. However, neighboring cells release β-lactamase and create an environment free of ampicillin allowing "cheaters" who don't produce β-lactamase to emerge and thrive. This is like if a friend does your homework for you, so you can go do something else instead!

[2] *Final Review of Antimicrobial Resistance: UK department of health*

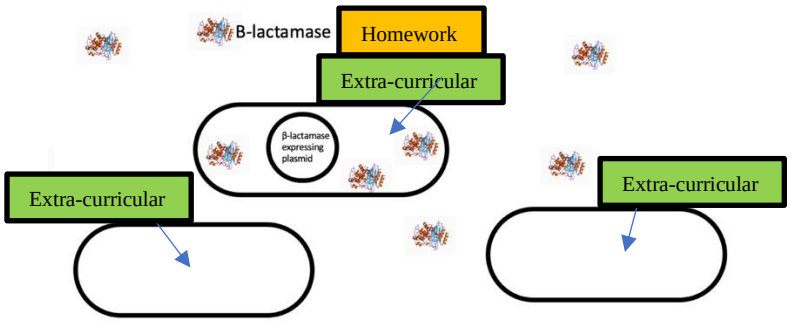
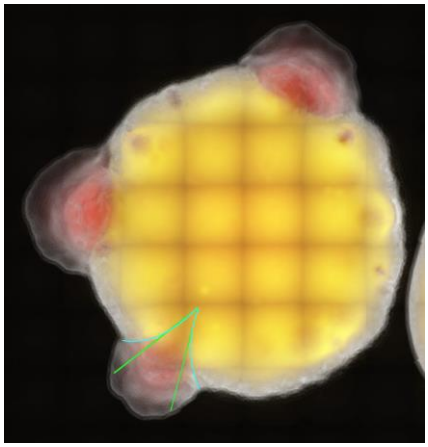


Figure 2: Diagram of a culture of two strains, one with a plasmid expressing β-lactamase resistance to ampicillin, the other lacking the plasmid due to a partitioning error during cell division or may be a different species.

The cell that has to produce β-lactamase because it has the plasmid will be burdened, like when you are assigned homework!

Bacteria typically express resistance to antibiotics in the form of a plasmid, a circular piece of DNA. These plasmids replicate independently from the cell's genome, and at times randomly during division cells may not inherit any plasmids, resulting in a new plasmid free strain. This bacterial cell will be incapable of producing β-lactamase, like if you broke your arm and couldn't do your homework. The only issue is that the bacterial cell needs β-lactamase if there is ampicillin nearby, so it either would need other bacteria to produce it for them or hope there is no ampicillin in order to survive.

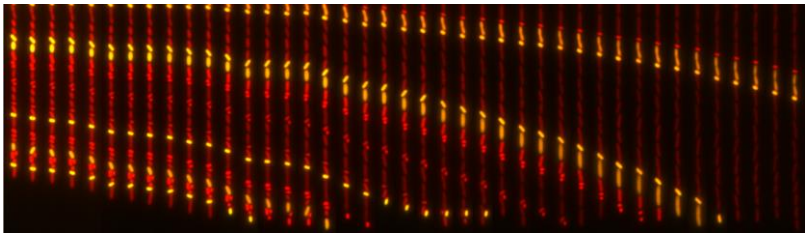
Bellow if a picture of some bacteria I grew. I put a few cells that have the plasmid (the yellow ones) in the center and let them start growing. When plasmid loss occurs, cells which can't express β-lactamase (the red ones), forming red sectors. These grow faster, as can be seen by the bulging sectors. Plasmid loss is like saying you won't do your homework – after deciding that you will have more time for you extra-curriculars!



Biofilm Yellow part of image is of many overlapping cells that are producing β-lactamase. Randomly, some cells lose the plasmid, and start to grow quicker because they no longer are producing β-lactamase – these are the cells that are red. We can see they are growing quicker because they are bulging out more. This is a single image in a movie, but you can imagine that as time goes on, these red sectors will keep growing faster than the yellow ones, bulge out more and eventually take over all the yellow cells!

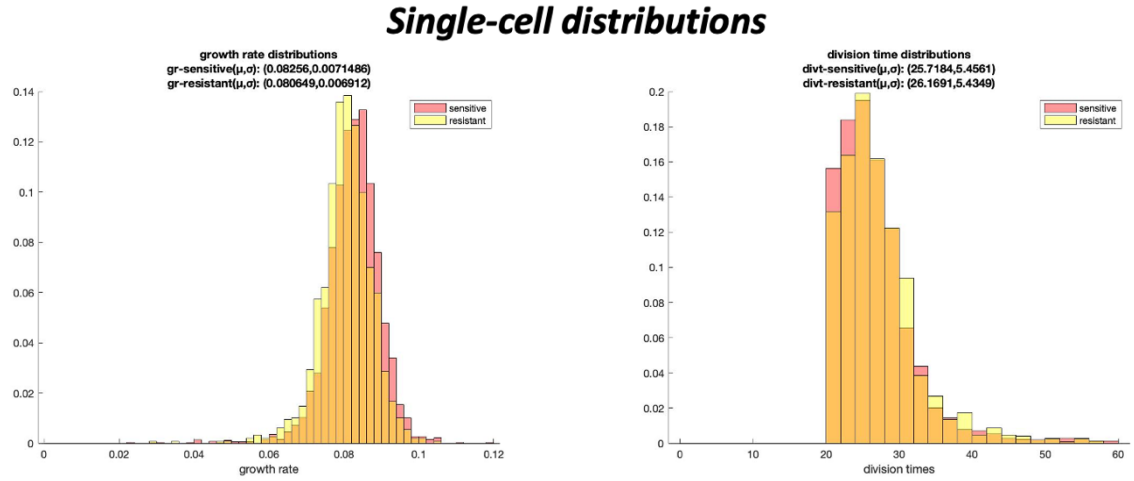
For my project, I wanted to quantify the exact effect of forcing a cell to have this plasmid, comparing those cells with plasmid to those without. Going back to the homework analogy, it's like saying I'm trying to determine how much more stuff can you do if your teacher doesn't assign you homework. By comparing some students that have homework and those that don't, we can see on average how long does it take you to do your homework?

To do this, we use something called the mother machine which allows us to monitor single bacteria under the microscope with high precision. This is achieved by trapping single bacteria so that as they divide, all of those new cells are washed away while we hold on to the original bacteria. Taking a video of this happening under the microscope for a long time, I did it for 24 hours, we can see these single bacteria divide many times. By inspecting these movies using a machine learning technique called computer vision, I was able to get a computer to recognize every time a cell splits in two without sitting and watching the 24 hour movie myself – there were many 24 hour movies to watch and they weren't worth going to the cinema for!



A single mother machine chamber, where the cell at the very top is trapped. Each repeated image is a new timepoint in the movie. Most of the cells start moving down and disappearing, these are the cells that are washed away, as the one on the top starts to grow and push them out. The one at the top will be there for the whole experiment, and will be the one we inspect – again, yellow cells are producing ampicillin, red ones are not

After getting the computer to watch the bacteria grow for me, I was able to take the notes the computer took, in particular how big the cells were every time they divided, and at what times did they divide. This was then used to find the difference in growth rate, and the metabolic burden by expressing ampicillin!



$$metabolic\ burden = \frac{\mu(division\ time_{resistant}) - \mu(divisiontime_{sensitive})}{\mu(divisiontime_{sensitive})}$$

$$metabolic\ burden = 2.26\%$$

Conclusion

This project focused on using engineering principles, such as data analysis, producing tiny devices like the mother machine to trap single cells, and machine learning tools like computer vision to try and understand the behavior of these really small bacteria! Engineering has become and will continue to become a very important aspect of biological research as we have to deal with large amounts of data and finding ways to analyze and experiment with small scales.