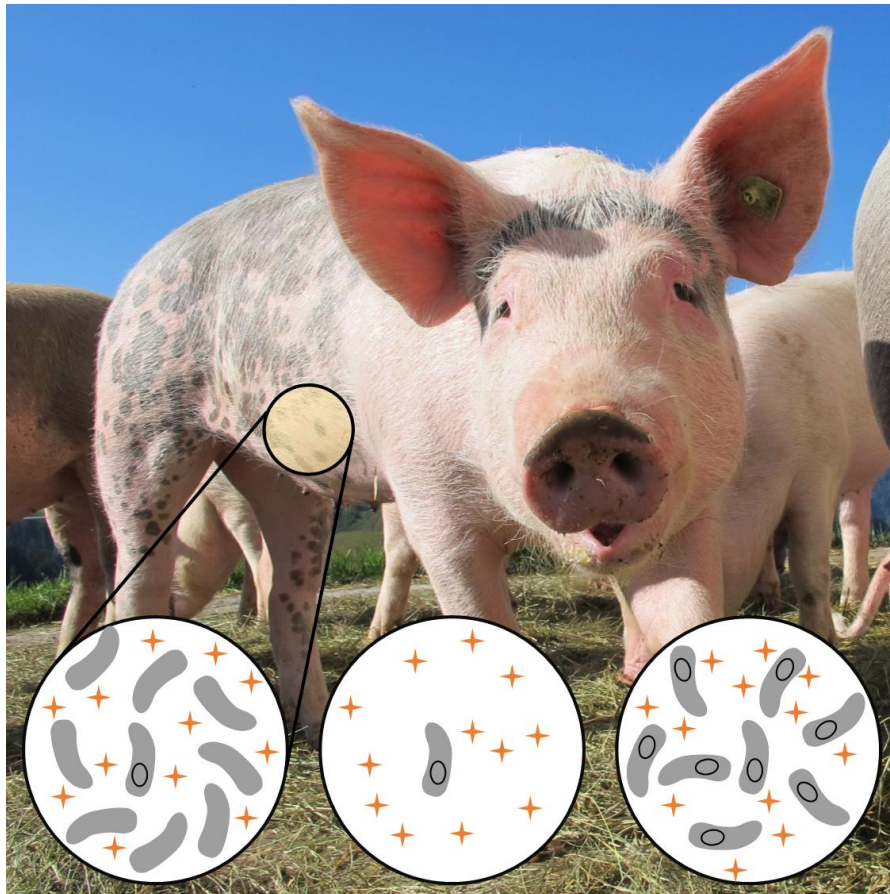




miniPCR™ Antibiotic Resistance Lab: Monitoring resistant organisms in the environment.

Produced in collaboration with

PARE – Prevalence of Antibiotic Resistance in the Environment



Student Guide Contents

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1. Background information

A) Antibiotic resistance in the environment

Bacteria are all around us. They live on you; they live inside you. They also live in the soil, water, and pretty much anywhere available carbon can be obtained. By some estimates, a single teaspoon of productive soil will have about as many living bacteria as there are people living in the United States¹. We normally think of bacteria as “germs” that make us sick, but the vast majority of bacteria living in the environment would be about as successful living inside of you as you would living with them under the ground.

But we all know that some rare bacteria do make us sick. And when we get sick with these, we can take an antibiotic drug for a few days that will typically get rid of the infection. This hasn't always been the case. Widespread use of antibiotics has only been around for less than 100 years. Alexander Fleming's discovery and characterization of penicillin in 1928 is widely seen as ushering in the modern world of antibiotic medicine. For the first time in human history, infections that would regularly kill were easily and routinely cleared up within a few days of beginning treatment. Within a few decades, several dozen varieties of antibiotics were introduced and available, and their use is thought to be responsible for saving the lives of hundreds of millions of people.

But as the use of antibiotics has spread, so has bacteria's resistance to them. It is fairly common today for routine infections to be resistant to antibiotics that were once used to treat them. And as bacteria gain resistance to more and more different drugs, a future where antibiotics can no longer treat some routine infections is a serious possibility that we may have to face sooner rather than later. In hospital settings, the fear of antibiotic resistant infections is a very real one. Hospitals regularly see sick patients and treat them with antibiotics. Because so many infections are brought to hospitals and resistant infections are so difficult to kill, hospitals become enriched environments for antibiotic resistance. In fact, hospitals are one of the places where people are most likely to become infected with resistant bacteria.

But not all resistant bacteria live in hospitals. More and more, antibiotic resistant bacteria are being found simply in the environment – living in the soil and water. Nobody knows for sure exactly how widespread resistant bacteria in the environment are, but as it is becoming more and more clear that environmental pathogens are an important source of infections in humans, the concern that we could be infected with resistant pathogens from our environment is a real

¹ https://www.nrcs.usda.gov/wps/portal/nrcs/detailfull/soils/health/biology/?cid=nrcs142p2_053862



one. In 2018, about 200 people across 36 states were sickened by a pathogenic strain of *E. coli* that had contaminated romaine lettuce; five people died. The source of the contamination was environmental; the bacteria had spread through an irrigation canal. Luckily, in this case, the bacteria were not resistant to antibiotics. But what if they had been?

B) Where does resistance come from?

Antibiotic resistance is one of the clearest examples of evolution by natural selection that humans have been able to view in real time. In under 100 years, we have gone from virtually no widespread antibiotic resistance to a world where antibiotic resistance is so widespread that it is viewed as a global health crisis. How did this happen so fast?

When we take an antibiotic, the goal is to kill all of the bacteria that are making us sick. But treatments often don't kill one hundred percent of the bacteria. The few surviving organisms after an antibiotic treatment were likely able to survive because they were more resistant to the antibiotics than all their counterparts that died. If their ability to survive treatment was due to a genetic variation, when these remaining bacteria reproduce, they will pass on this resistance to their offspring. When another dose is taken, the cycle will repeat itself. Over time, after successive antibiotic treatments, the only bacteria left in the population will be ones that were able to survive because they inherited the set of genes that made them resistant. This is why antibiotic resistance is now such a problem in hospital settings – all the non-resistant bacteria are routinely eliminated. The populations that remain are the ones that evolved to survive in a world where they regularly must overcome antibiotic treatments.

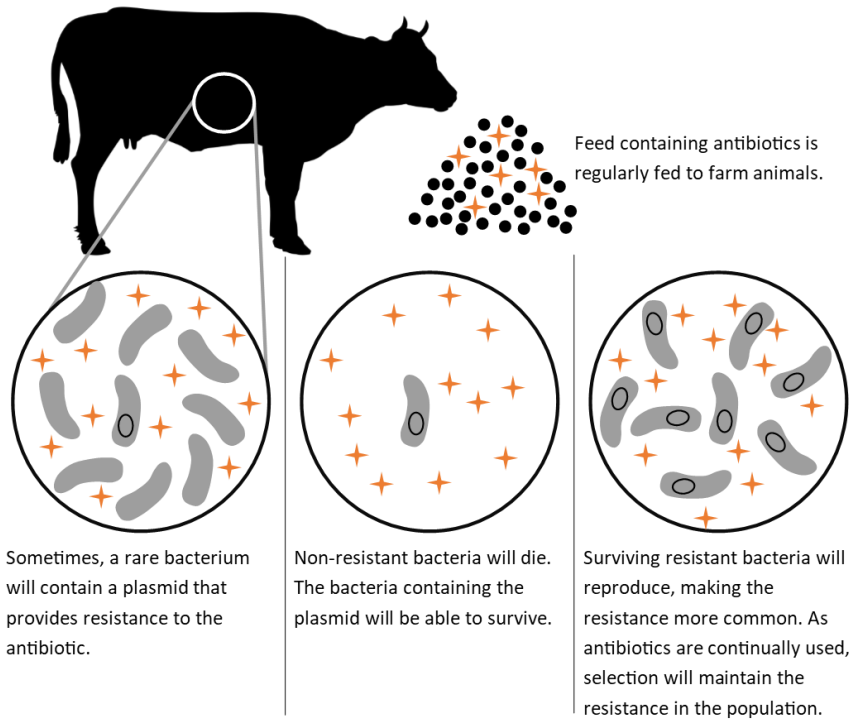
Humans, of course, are not the only animals that can get sick from bacteria. Farm animals are also susceptible to bacterial infections, and so farmers regularly treat their animals with antibiotics. In fact, over 70% of medically important antibiotics sold in the United States are used on animals.² This has led to some of the problems with antibiotic resistance in the environment that we see today. For a long time, a standard practice has been to include low levels of antibiotics in animal feed. This constant low-level use reduces the incidence of infections in farm animals and also, for mainly unknown reasons, often increases the growth rate of the animals. But the constant low-level use means that bacteria are under constant selective pressure. Every time antibiotics are administered, antibiotics will kill much, but not all, of the bacterial population. Those bacteria that survive to reproduce will do so because they possess a resistance to the antibiotic being used. As the same antibiotics are used in the feed, over and

² <http://www.cidrap.umn.edu/news-perspective/2017/01/farm-antibiotics-does-new-fda-policy-go-far-enough>



over, year after year, the bacteria that are able to survive can do so because they inherit genes that provide resistance.

Of course, bacteria don't stay inside animals forever. Animals (and people) constantly spread the bacteria living inside them, for example, whenever they cough, sneeze, and, perhaps most importantly, through feces. For humans, this is often how bacterial infections spread, but the problem is largely mitigated by using modern sewage disposal and treatment systems. For farm animals, where there is no sewage treatment, this can be a major problem. The biota living inside farm animals are regularly



enriched for antibiotic resistance, and then, those surviving resistant organisms are released into the environment through the significant amounts of fecal matter produced on farms. These bacteria can then spread considerable distances by being carried in water run-off from rain or other sources.

This is all made even more troublesome because, in bacteria, these resistance genes can spread in a way that genes in you cannot. You get your DNA from your mom and your dad, and nowhere else. Bacteria, on the other hand, can be a little looser with where they get DNA from. Bacteria will often pick up DNA from the environment or exchange DNA from neighboring bacteria in the form of a plasmid. A plasmid is a small circular segment of DNA that contains an origin of replication and a few genes. Where most bacterial DNA is passed asexually directly from parent to offspring in a single circular genome (what we call *vertical* transmission), plasmids can be passed both vertically and *horizontally*, from one unrelated organism to another, often even across different species. This means that once resistance evolves in one species, if that resistance gene ends up as part of a plasmid, it can spread relatively quickly to many different species. Genes from plasmids can even be integrated into a bacterium's chromosome leading to more stable vertical transmission of the resistance. Today, many



plasmids are passed in the environment (sometimes referred to as eDNA, or environmental DNA) that contain not one, but several resistance genes.

Ultimately, we can expect populations of antibiotic-resistant bacteria to emerge and become more common any time the selective pressure of antibiotics is regularly applied. This, indeed, is a powerful example of evolution in action. This, however, does not mean that bacteria somehow change when antibiotics are applied; it just means that the bacteria that are susceptible to antibiotics die while bacteria that are resistant can expand. This also doesn't mean that resistance to antibiotics is a new thing. Even though antibiotics have only been used by humans for under a century, most antibiotics that we use were discovered already existing in natural sources. Penicillin, for example, is a molecule that is naturally produced by the fungus, *Penicillium chrysogenum*. Because certain types of bacteria have always lived in the specific environment where *Penicillium chrysogenum* occurs, selection for resistance to penicillin has been occurring in this environment for a very, very long time. Those bacteria that originally carried the penicillin resistance likely do not cause human infections, but as described above, horizontal gene transfer can pass resistance genes to bacteria that never had them before. Now, with humans using penicillin regularly, when a plasmid containing a resistance gene is passed into a human pathogen, natural selection leads to that now resistant strain of bacteria becoming more and more common.

So how does resistance work? There are a few ways that bacteria can become resistant to antibiotics. An enzyme can break down or change the antibiotic into a harmless molecule. A molecular pump can pump the molecule out of the cell, or other changes can make it more difficult for the antibiotic to enter the cell in the first place. Or, a change in the physiology of the bacteria or the binding site of the antibiotic can make the antibiotic lose efficacy. Molecular pumps and enzymes that inactivate the antibiotic are encoded on large chunks of DNA which can be passed around bacterial populations horizontally on plasmids and also through vertical transmission. Alternatively, changes that affect the binding site for the antibiotic often result from point mutations on the bacterial genome which can only be transmitted vertically.

Whenever an organism reproduces, it must copy its DNA. Occasionally, rare copying mistakes, or mutations, are made. Even more rarely, these mutations may lead to a random change in a gene that happens to provide an increase in resistance to an antibiotic. The important thing to remember is that these genes already existed, performing a function for the cell closely related to how it works with antibiotics. When an antibiotic is administered, the very rare bacterium that possesses this unique sequence of DNA is more likely to survive and spread that resistance trait on in the population. As the antibiotic is continually used, the bacteria that possess these



genetic sequences survive to reproduce, while other bacteria die. Over time, under the selective pressure of continued antibiotic use, resistance will become more and more common in the population until all the non-resistant bacteria have died off and only bacteria that contain the resistance DNA sequences remain.

That the genetic code is universal and that DNA can spread between bacterial species on plasmids means that a single case of one of these DNA sequences in a single bacterium can lead to eventual worldwide antibiotic resistance in many different bacterial species. Today, many plasmids are circulating in the environment that contain several resistance genes linked together, allowing bacteria to become resistant to many different antibiotics by taking up a single piece of DNA from the environment. These resistant bacteria can be identified in different ways. Scientists can try to grow them, plating environmental samples on agar plates that contain antibiotics and looking for growth. They can also use molecular techniques such as PCR, amplifying DNA from environmental samples to try to identify the resistance genes being passed on plasmids.

C) What to do?

The problem with antibiotic resistance is a bit of a paradox. Using antibiotics leads to resistance spreading within a population of bacteria, which in turn makes our antibiotics no longer useful. If we want to stop new resistant strains from spreading, we need to stop using that antibiotic, but that isn't a great option for a person who is sick and in need of treatment.

Still, virtually all experts generally agree that the problem of spreading antibiotic resistance could be slowed significantly if antibiotics were used much less often and much more judiciously. When antibiotics are used too widely, bacteria are under constant selective pressure to develop and maintain resistance. But when antibiotics are not present, we know that non-resistant bacteria will regularly outcompete the resistant strains and populations will tend to become less resistant over time. The general consensus is therefore that antibiotics should be used much more sparingly than they generally are, and that specific "last line of defense" antibiotics should only be used when absolutely necessary. This is true for human use, but also especially true for animal health use. Already, use of antibiotics to promote growth of livestock has been banned in Europe and a directive from the FDA banned the practice in the United States in 2017. But many people think this does not go far enough. The drugs can still be used under the supervision of veterinarians to treat and even prevent diseases in animals, and in many countries around the world there are no restrictions at all. In preventing the spread of antibiotic resistance, we may know some important steps to take; actually, taking them is much more difficult.



2. Case study:

Note: The following case study represents a fictional outbreak of a real and spreading antibiotic resistance threat. It is presented as a possible scenario for students to investigate how antibiotic resistance in the environment can be seen as a real and growing problem.

Facts of the case

An outbreak of *E. coli* has infected 42 people across 12 different states. The individuals hospitalized showed severe symptoms of food poisoning including hemorrhagic diarrhea and some cases of kidney failure. Doctors treating the patients immediately administered the antibiotic imipenem, a powerful drug from the carbapenem class of antibiotics. Patients did not respond to the treatment. Doctors suspect that the *E. coli* were resistant to carbapenems and switched to another antibiotic, colistin. Luckily, most of the patients responded to the new treatment. Still, 8 of the 42 patients died.

Subsequent testing confirmed that the *E. coli* possessed a plasmid that contained the gene bla_{NDM-1} , a relatively new but spreading carbapenem resistance gene.

Public health officials have tracked the source of the infection to tainted pork that originated from a single farm. Hog farming produces considerable amounts of manure waste, and neighboring farms are now concerned that that waste may be spreading the genes responsible for carbapenem resistance into the soil and water. Two farms in particular, Apple Point Farms and Barrow Creek Farm have reached out to public health officials to try to assess their possible risk. Soil samples have been collected from these farms in order to test for the presence of the bla_{NDM-1} gene.

Information about carbapenems

Carbapenems are antibiotics that are generally used only in extreme, last resort settings and are considered one of our last lines of defense against antibiotic resistant bacteria. In 2007, however, a man was infected in India with a strain of bacteria that showed resistance to treatment with carbapenems. Subsequent testing identified that resistance was provided for by a gene coding for a protein that breaks down carbapenems. The protein was named NDM-1 (New Delhi metallo-beta-lactamase 1), a carbapenemase that hydrolyzes the carbapenems. The gene that codes for NDM-1, bla_{NDM-1} , was located on a plasmid that can be spread through horizontal gene transfer. The bla_{NDM-1} gene has since spread worldwide and has been found in settings ranging New Delhi drinking water to United States hospitals. The spread of this resistance gene



has been particularly alarming; in just ten years, it has gone from being completely unknown to being identified in samples worldwide.

Carbapenems are restricted to hospital use, so the identification of resistance in environmental settings is especially concerning. But while NDM-1 provides resistance to carbapenems, it also provides resistance to several other antibiotics³, some of which are used regularly in agriculture. It is possible that widespread use of these other more commonly used antibiotics is fueling the spread of *bla*_{NDM-1} in environmental settings.

Today's lab

You will be provided with DNA extracted from the soil from two farms, Apple Point and Barrow Creek, and will use PCR to identify if *bla*_{NDM-1} is present in one, both, or neither of the environmental samples. You will use primers that are specific to *bla*_{NDM-1} to test for the resistance gene. When *bla*_{NDM-1} is present in a sample, these primers will amplify a 700 base pair fragment of DNA. A second set of primers will be used to amplify a 400 base pair region of the 16S ribosomal RNA gene. This second set of primers will be used as a PCR control, to make sure that DNA was present in the sample and that DNA amplification in the PCR was successful. You will also be provided a sample of DNA extracted from an *E. coli* isolate known to contain *bla*_{NDM-1} and a second sample of DNA from an *E. coli* isolate known to be susceptible to carbapenem. These samples of DNA will serve as positive and negative controls for your experiment.

Your job: Determine if the *bla*_{NDM-1} gene is spreading resistance on either Apple Point or Barrow Creek farms.

³ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1932750/>



3. Laboratory guide

A. PCR set up

1. **Label 4 PCR tubes** (200 μ L tubes) per lab group. Label tubes on the **side wall**.
 - 1 tube labeled "A": DNA from Apple Point Farm
 - 1 tube labeled "B": DNA from Barrow Creek Farm
 - 1 tube labeled "N": 'Negative Control DNA' from non-resistant bacteria
 - 1 tube labeled "P": 'Positive Control DNA' from carbapenem resistant bacteria



Also label each tube with the group's name on the side wall

2. **Add PCR reagents** to each 200 μ L PCR tube

	Tube A	Tube B	Tube N	Tube P
Template DNA	DNA From Apple Point 10 μ L	DNA From Barrow Creek 10 μ L	Negative Control 'non- resistant' DNA 10 μ L	Positive Control 'resistant' DNA 10 μ L
PARE Primer Mix	10 μ L	10 μ L	10 μ L	10 μ L
5X EZ PCR Master Mix	5 μ L	5 μ L	5 μ L	5 μ L
TOTAL VOLUME	25 μL	25 μL	25 μL	25 μL




Use a micropipette to add each of the reagents.
Remember to change tips at each step!

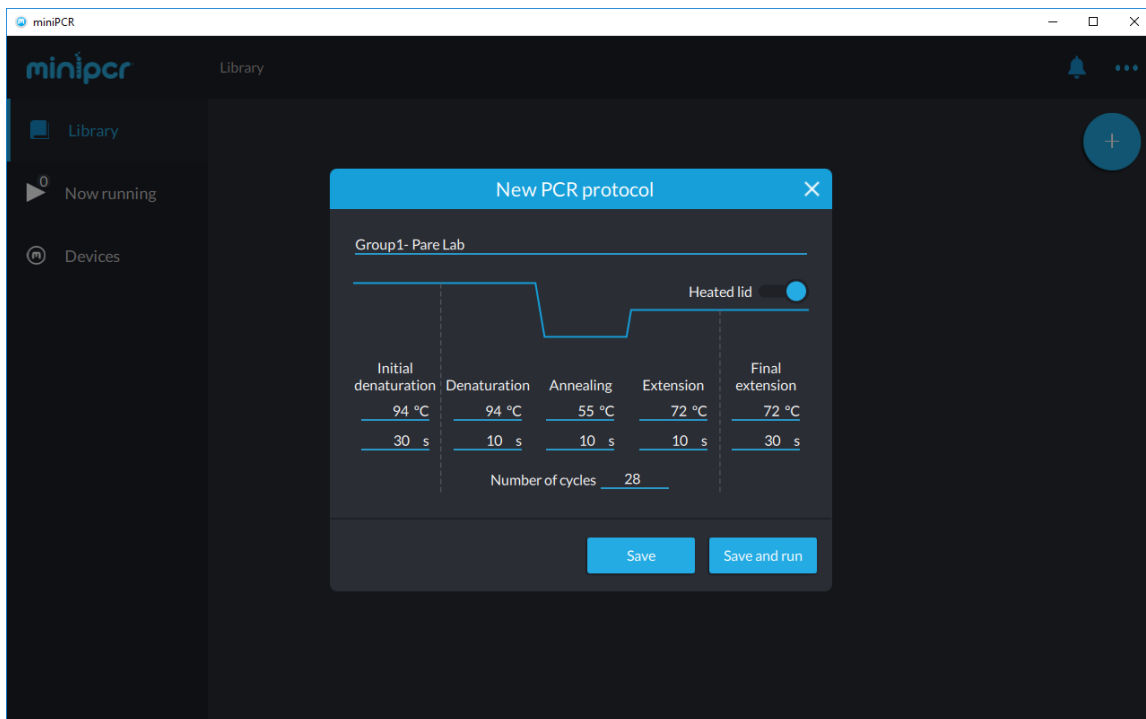
3. **Gently mix the reagents by pipetting up and down 3-4 times, cap the tubes**
 - Make sure all the liquid volume collects at the bottom of the tube (if necessary, spin the tubes briefly using a microcentrifuge)
4. **Place the tubes inside the PCR machine**
 - Press firmly on the tube caps to ensure a tight fit
 - Close the PCR machine lid and tighten it gently



B. **PCR programming and monitoring** (illustrated using miniPCR® software)

Note: depending on version of software used, programming procedures may vary slightly, but there are no changes to the temperatures, times, and number of cycles used.

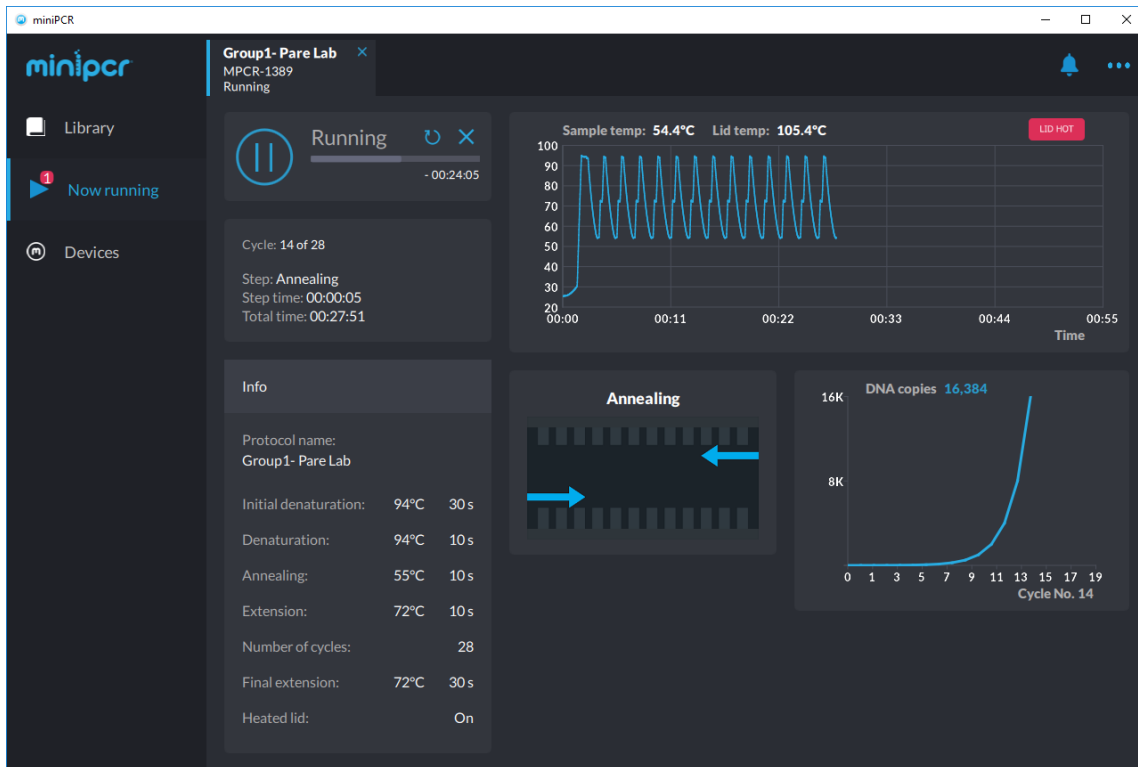
1. Open the miniPCR software app and remain on the "**Protocol Library**" tab
2. Click the "**New Protocol**" or  button
3. Select the **PCR "Protocol Type"** from the top drop-down menu
4. Enter a name for the Protocol; for example "**Group 1 – PARE Lab**"
5. Enter the PCR protocol parameters:
 - **Initial Denaturation** 94°C, 30 sec
 - **Denaturation** 94°C, 10 sec
 - **Annealing** 55°C, 10 sec
 - **Extension** 72°C, 10 sec
 - **Number of Cycles** 28
 - **Final Extension** 72°C, 30 sec
 - **Heated Lid** ON





6. Click **"Save"** to store the protocol or **"Save and Run"** to begin the protocol immediately.
 - Make sure that the power switch is in the ON position
 - For older software versions (v1.6) you will need to hit the **"Upload to miniPCR"** in the lower right-hand corner of the window.
 - If prompted, select the serial number of your miniPCR machine in the dialogue window. Serial numbers are located next to the USB port.

7. Click **"miniPCR [machine name]"** tab to begin monitoring the PCR reaction



The miniPCR™ software allows each lab group to monitor the reaction parameters in real time, and to export the reaction data for analysis as a spreadsheet.

Once the PCR run is completed (approximately 30-40 min), the screen will show **"Status: Completed"** and all LEDs on your miniPCR machine will light up.

You can now open the miniPCR lid and remove your PCR tubes.



Be very careful not to touch the metal lid which may still be hot

The PCR product can now be stored for up to 1 week in the fridge or 1 year in a freezer.





TIME MANAGEMENT TIP


Gel electrophoresis – Pouring agarose gels (Preparatory activity)

If the lab is going to be completed in a single time block, agarose gels should be prepared during the PCR run to allow the gels to settle.


If the lab is going to be performed over two periods, gels can be prepared up to one day ahead of the second period and stored in a refrigerator, covered in plastic wrap and protected from light.

1. Prepare a clean and dry agarose gel casting tray
 - Seal off the ends of the tray as indicated for your apparatus
 - Place a well-forming comb at the top of the gel (4 lanes per group plus DNA ladder)

2. For each lab group, prepare a 1.6% agarose gel using 1X TBE buffer
 - Adjust volumes and weights according to the size of your gel tray
 - e.g., add 0.32 g of agarose to 20 ml of 1X TBE for blueGel™ system
 - Mix reagents in glass flask or beaker and swirl to mix
 - If using **blueGel Tabs™**
 - Use one tab per 25ml if making one gel.
 - Two tabs in 50ml can make up to three gels.

3. Heat the mixture using a microwave or hot plate
 - Until agarose powder is dissolved and the solution becomes clear
 -  Use caution, as the mix tends to bubble over the top and is very hot

4. Let the agarose solution cool for about 2-3 min at room temperature.
 - Swirl the flask intermittently

5. Add gel staining dye (e.g. GelGreen)
 - Follow dye manufacturer instructions
 - Typically, 1.0 µL of staining dye per 10 mL of agarose solution (2µl per 20ml gel)
 -  Note: Follow manufacturer's recommendations and state guidelines if handling and disposing of ethidium bromide

6. Pour the cooled agarose solution into the gel-casting tray with comb



7. Allow gel to completely solidify (until firm to the touch) and remove the comb
 - Typically, 15-20 minutes
8. Place the gel into the electrophoresis chamber and cover it with 1X TBE buffer

Gel electrophoresis – Running the gel

1. Make sure the gel is completely submerged in 1X TBE electrophoresis buffer
 - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged)
 - Fill all reservoirs of the electrophoresis chamber and add just enough buffer to cover the gel and wells
2. Load PCR samples onto the gel in the following sequence
 - **Lane 1:** 10 μ L DNA ladder
 - **Lane 2:** 15 μ L **Farm A** PCR
 - **Lane 3:** 15 μ L **Farm B** PCR
 - **Lane 4:** 15 μ L **Negative Control (N)** PCR
 - **Lane 5:** 15 μ L **Positive Control (P)** PCR



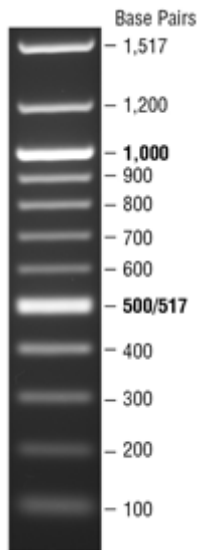
Note: there is no need to add gel loading dye to your samples. *miniPCR EZ PCR Master Mix* and *100 bp DNA Ladder* are Load-Ready™!

3. Place the cover on the gel electrophoresis box
 - Ensure the electrode terminals fit snugly into place
4. Insert the terminal leads into the power supply (not needed if using blueGel™)
5. If using blueGel™, simply press the “Run” button. Otherwise, set the voltage at 100-130V. Conduct electrophoresis for 15-20 minutes, or until the colored dye has progressed to about half the length of the gel
 - Check that small bubbles are forming near the terminals in the box
 - Longer electrophoresis times will result in better size resolution
6. Once electrophoresis is completed, turn the power off and remove the gel from the box (not needed if using blueGel™ which has a built-in illuminator)



Size determination and interpretation

1. Place the gel on the transilluminator (or turn on the blueGel™ illuminator)
 - Wear UV-protective goggles if using UV light (not needed with blueGel)
2. Verify the presence of PCR product
3. Ensure there is sufficient DNA band resolution in the 400-800 bp range of the 100bp DNA ladder



- Run the gel longer if needed to increase resolution
- DNA ladder should look approximately as shown

4. Document the size of the PCR amplified DNA fragments by comparing the PCR products to the molecular weight reference marker (100bp DNA ladder)
 - Capture an image with a smartphone camera
 - If available, use a Gel Documentation system



4. Study questions

Pre lab

1. When antibiotics were first introduced, there was virtually no human pathogen that had resistance. But resistance did exist in other non-pathogenic bacteria. Why may these other bacteria have possessed resistance?

2. If a particular type of bacteria is resistant to antibiotics, does that mean that it is bad for you?

3. Evaluate this statement: "The use of antibiotics causes bacteria to become resistant."

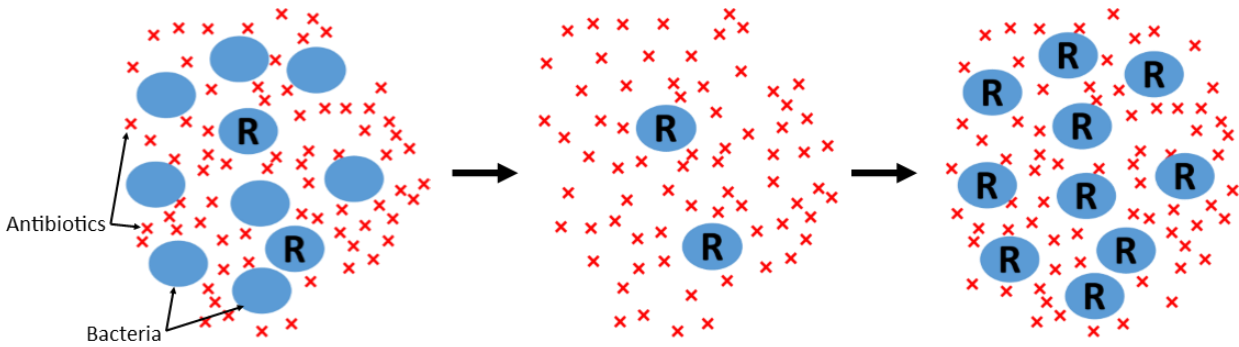
4. Explain the difference between vertical and horizontal transmission of DNA. Why does horizontal transmission potentially make the problem of antibiotic resistance worse?

5. If you were to sample bacteria from a healthy human gut, do you think that you would find antibiotic resistance genes? Explain your answer.

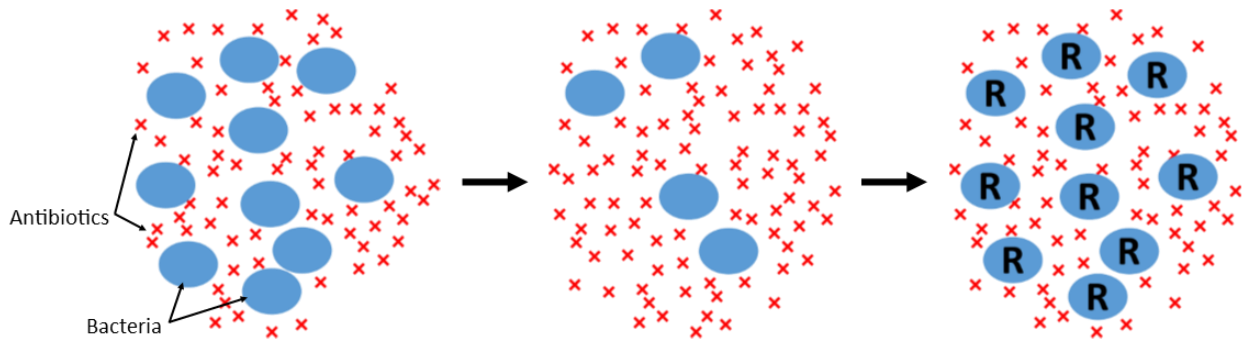


- Why would constant low-level usage of antibiotics on farms be more problematic for the development of antibiotic resistance than occasionally administering very high doses only when animals are sick?

- Presented below are two models for the evolution of antibiotic resistance. Which model do you think is more accurate? Justify your answer with evidence from the text or other sources.



Model 1: Antibiotic resistance already exists in the population, but in low numbers. Use of antibiotics eliminates the non-resistant bacteria, allowing the resistant bacteria to proliferate.

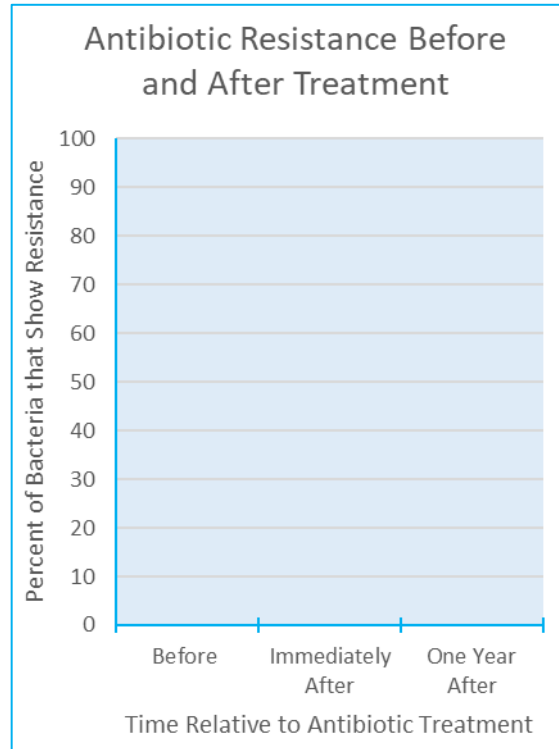


Model 2: Antibiotic resistance does not exist in the population. Use of antibiotics causes most bacteria to die. The ones that survive must adapt and change, making them resistant and allowing them to proliferate.

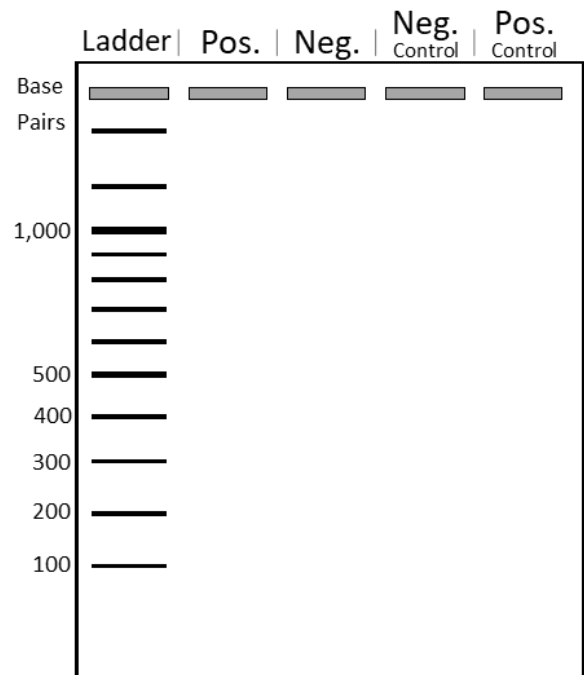


8. On the graph to the right, imagine that a farmer notices a possible disease developing in her herd. To combat the disease, she administers a course of antibiotics. If bacteria were sampled from the soil on this farm, predict what the percent of antibiotic resistant bacteria will be before, immediately after, and one year after treatment. You are not expected to know exact numbers, just try to predict generally when resistance will be high or low.

9. Explain why you drew what you did on the graph.



10. Before running your electrophoresis gel, predict your possible results. Draw bands on the gel where you expect to see them. A DNA ladder indicating DNA fragment size is provided in the first lane.





Post lab

1. Was either environmental sample positive for the bla_{NDM-1} gene?

2. In this lab, we are interested in whether carbapenem resistance is present in *two* different environmental samples. Explain then why you performed *four* PCR reactions.

3. The reason for performing this experiment was to test for the presence of a single gene bla_{NDM-1}. Why then do our positive results have two bands on the gel? What is the point of the second band?

4. Use of carbapenems are restricted to hospital settings in the United States. Why would carbapenem resistant bacteria be found in an environmental setting?

CER Table

Fill in the table based on your results from the lab.

Question:

Can you determine if either environmental sample was positive for the bla_{NDM-1} gene?

<p>Claim</p> <p>Make a clear statement that answers the above question.</p>	
<p>Evidence</p> <p>Provide data from the lab that supports your claim.</p>	
<p>Reasoning</p> <p>Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.</p>	



CER Table Rubric

For evaluating student CER tables.

	4	3	2	1
CLAIM <i>A statement that answers the original question/ problem.</i>	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE <i>Data from the experiment that supports the claim.</i> <i>Data needs to be <u>relevant</u> and <u>sufficient</u> to support the claim.</i>	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING <i>Explain why your evidence supports your claim. This must include scientific principles/knowledge that you have about the topic to show why the data counts as evidence.</i>	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

Rubric Score	3	4	5	6	7	8	9	10	11	12
Equivalent Grade	55	60	65	70	75	80	85	90	95	100

We recommend that teachers use the following scale when assessing this assignment using the rubric.

Teachers should feel free to adjust this scale to their expectations.