Exploring Antibiotic Resistance using Gel Electrophoresis

Student Materials

Exploring Antibiotic Resistance using Gel Electrophoresis

Background Reading:

Antibiotics are powerful drugs that can kill bacteria that cause infections like strep throat, salmonella and pneumonia. There is no doubt that antibiotics have saved millions of lives. Before the scientist Alex Fleming discovered the first antibiotic in 1945, people frequently died from a simple cut or infection.

- *What kind of organisms do antibiotics kill?* _____

Today, the world faces an antibiotic crisis. Many kinds of bacteria have evolved resistance to different kinds of antibiotics making it harder and harder for doctors to treat infections caused by bacteria. Large numbers of people now die from diseases that antibiotics once cured. Worldwide deaths from antibiotic-resistant infections are predicted to hit 10 million a year by 2050[1].

Bacteria, not people or animals, become antibiotic-resistant. **Antibiotic resistance** happens when a bacterial cell gets a new piece of DNA that protects it from the drug. For example, the DNA for the ampicillin resistance gene, amp^R, codes for a protein that breaks ampicillin down before it can kill the cell. If the gene for antibiotic resistance is found on the bacterium's chromosome, the gene will be passed on when the bacterium reproduces.

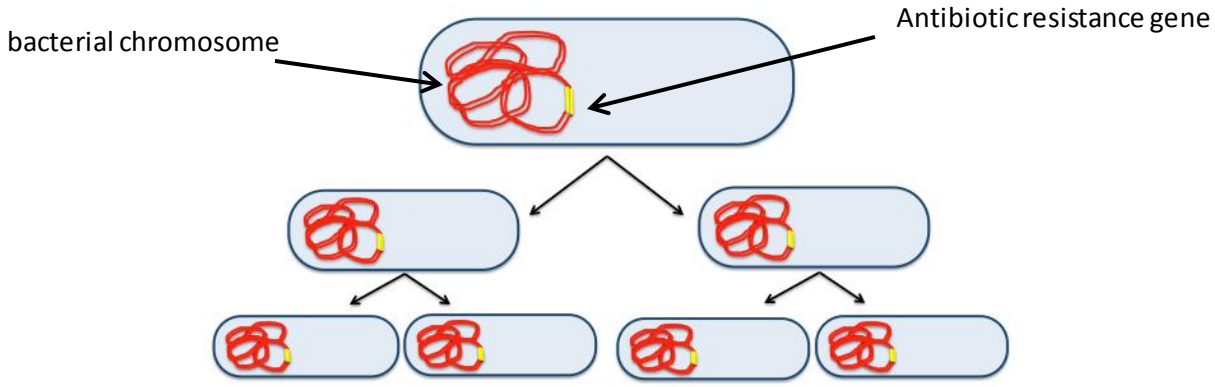


Figure 1. This model of a bacteria cell shows a gene for antibiotic resistance that is found on the bacterial chromosome. The gene is passed on to future generations of bacteria when the cell reproduces.

- *What does it mean to say that a cell is "resistant" to an antibiotic?* _____

_____

- *Why are doctors worried about antibiotic resistance?* _____

_____

_____

[1] "A possible solution to the world's antibiotic crisis could lie in dirt" 2018 Newslea https://newsela.com/read/new-antibiotic-dirt/id/40536/ adapted from Washington Post

Most often, the gene for antibiotic resistance is found on a plasmid (Figure 2). **Plasmids** are tiny circular pieces of DNA that contain only a few extra genes. Plasmids can easily be passed between different kinds of bacteria. If a plasmid contains a gene for antibiotic resistance it can be passed on to new types of bacteria. Plasmids are also copied when a cell reproduces. Because of plasmids, antibiotic resistance can spread very quickly.

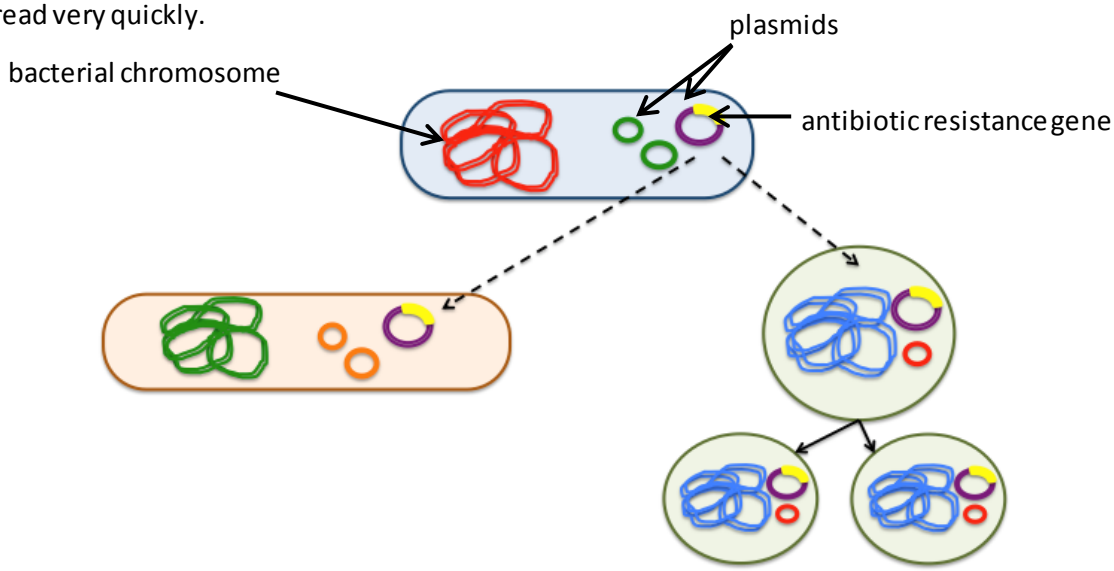


Figure 2. This model of bacteria cells shows a bacterial chromosome on the left and small plasmids on the right. Plasmids can be passed around between cells but chromosomes stay with the cell. Both plasmids and chromosomes are copied when a cell reproduces.

- *Why is antibiotic resistance spreading so quickly?* _____

_____

The world needs to change the way it uses antibiotics to reduce the threat of antibiotic resistance. People need to try to reduce the use of antibiotics by stopping the spread of infections. Everyone should get vaccinations, wash their hands carefully, prepare food safely and stay away from others when they are sick. Antibiotics should only be used if a doctor prescribes them.

- *Name three different ways that you can help slow the spread of antibiotic resistance.* _____

_____

_____

Exploring Antibiotic Resistance using Gel Electrophoresis

Introduction:

Imagine that you are a doctor and that you are treating a family of three for strep throat. You know that *Streptococcus* bacteria causes strep throat. You prescribed the standard antibiotic (amoxicillin) to treat the infection. But amoxicillin isn't working. The family is still sick. You need to find a medicine that will kill the bacteria causing the infection. You've have decided to test bacteria from each family member to see if the bacteria causing the infection are resistant to antibiotics.

In today's lab you will be testing the bacteria from your three patients to find out if the bacteria have the genes that make them resistant to the antibiotics – amoxicillin, erythromycin or kanamycin. Before your experiment, lab technicians already collected DNA from bacteria samples and used PCR (polymerase chain reaction) to make billions of copies of the DNA.

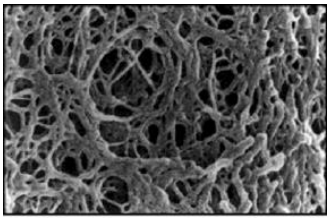


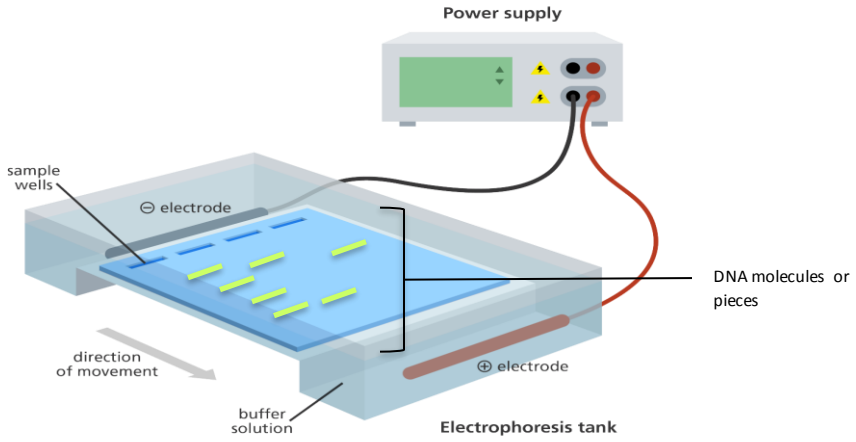
Figure 3. Scanning electron microscope image of an agarose gel. ©Source unknown

You will be using gel electrophoresis to study the DNA of the bacteria. **Gel electrophoresis** is a method used to separate charged molecules like DNA and proteins by their size. The gel in gel electrophoresis looks and feels a lot like JELL-O™. But if you were to study an electrophoresis gel under a high-powered microscope you would see that it is made of a mesh of sugar molecules (see **Figure 3**). The sugar, called agarose, comes from seaweed.

- *Look at Figure 3. Do you think big molecules or small molecules will move faster through the gel? Why?* _____

_____

To begin, the gel is put in a tank and covered with **electrophoresis buffer** (a salt solution) that controls the pH and conducts electricity. The molecules to be studied are injected into sample wells in the gel, and an electric field is applied (see **Figure 4**).



Adapted from: http://www.yourgenome.org/facts/what-is-gel-electrophoresis

Figure 4: Illustration of gel electrophoresis equipment.

Molecules with a positive surface charge will move through the gel towards the negatively charged electrode. Molecules with a negative surface charge will move through the gel towards the positively

charged electrode. If the charges of the molecules are the same, then the molecules will move through the gel at different rates based on their size. The smaller pieces or molecules will move faster than larger molecules because they move more easily through the small holes in the gel. Molecules or pieces of DNA appear as small lines or bands on the gel (see **Figure 4**).

- *In what direction does DNA move in the gel? (Hint: DNA is negatively charged)* _____

In addition to running the bacterial DNA from your three patients on the gel, you will also be running DNA from a **control** bacterium that is known to have no antibiotic resistance and a DNA ladder. Lab technicians made the **DNA ladder** by mixing three different standard pieces of DNA. You will use the three bands of the ladder to estimate the size of bands that you see for your patients.

- *Why are you using control bacteria DNA?* _____

_____

- *What is a DNA ladder?* _____

Because the antibiotic resistance genes you are studying are all different sizes (**Table 1**), you can use gel electrophoresis to figure out if a sample of bacteria has one or more of the antibiotic resistant genes.

Table 1. Size DNA fragments of antibiotic resistance gene measured in number of base pairs.

Antibiotic Resistance Gene	Size of gene in base pairs (bp)
amoxicillin resistance (amx^R)	400
kanamycin resistance (kan^R)	600
erythromycin resistance (ery^R)	800

- *Which antibiotic resistance gene fragment will move furthest on the gel?* _____

- *Which antibiotic resistance gene fragment will move the shortest distance on the gel?* _____

Exploring Antibiotic Resistance using Gel Electrophoresis

Student Workstation:		Common Workstation:
3 tubes	bacterial DNA from each patient (Pat 1, Pat 2, Pat 3)	1X electrophoresis buffer
1 tube	Control DNA	UV or blue light source
1 tube	DNA ladder	centrifuge (optional)
1	agarose gel (1.8%) with DNA stain	
1	p20 micropipette and tips	
1	microcentrifuge tube rack	
1	waste container	
1	gel electrophoresis unit with power supply	

Procedure:

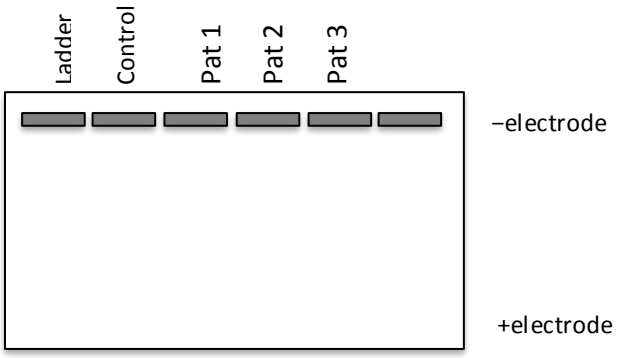
1. Make or get a 1.8% agarose gel and 1X electrophoresis buffer.

2. Put the gel box together.
   - Place the gel tray into the box so that the wells are near the ⊖ electrode
   - Add just enough buffer to cover the gel.
   - Place it on the lab bench where it will run.

3. Using a p20 micropipette, load 10 µL of the ladder into a well in the gel as shown in the diagram below.

4. Using a p20 micropipette and a clean tip, load 10 µL of each sample of bacterial DNA into a separate well of the gel as show in the diagram.

   ⚠ **Reminder: Use a new tip for each sample.**

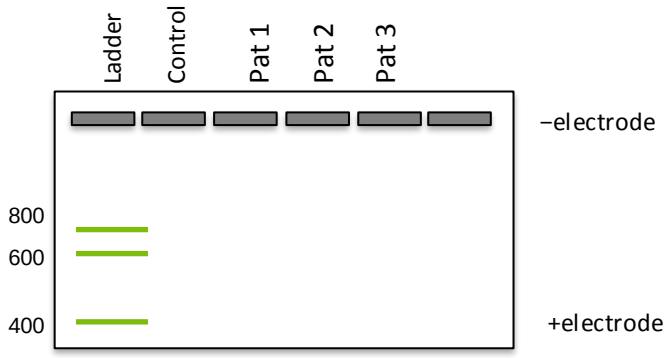


5. Turn on the power ⏻ and run your samples until the purple dye is 2/3 of the way down the gel.

6. Turn on the blue light 💡 and examine your results. 📷 Take a photograph of your gel.

Observations & Conclusions:

1. On the image below, draw what you see after gel electrophoresis:



2. What does any band on the gel represent?

3. Do you see any lanes that do not have a band? Explain what no band means for the bacteria sample.

4. Do you see any lanes with one or more bands? Explain what a band means for the bacteria sample.

5. Based on what your gel, circle the antibiotic resistant gene(s) that each bacteria sample has:

Lane	Bacteria from this patient have what genes?			
Control	amx^R	ery^R	kan^R	none of these
Pat 1	amx^R	ery^R	kan^R	none of these
Pat 2	amx^R	ery^R	kan^R	none of these
Pat 3	amx^R	ery^R	kan^R	none of these

6. Based on your results. What kind(s) of antibiotic will you give to each of your patients next? Why?