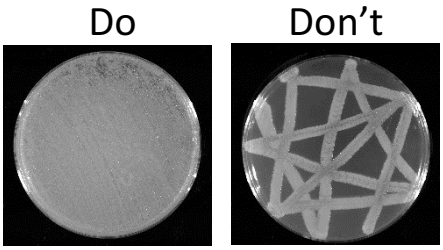
Purpose

To test which model best explains the phenomenon of antibiotic resistance, we will observe bacterial growth over the course of 5 days.

Materials Day 1

1. An agar plate culture of bacteria (provided by instructor)
2. Sterile sticks and swabs
3. A micro centrifuge tube with 300 µL Luria Broth (LB) media
4. A test tube with 3mL LB media
5. Pipette - p200 set to dispense 100 µL and sterile pipette tips
6. 1 LB agar plate
7. Tweezers
8. Ethanol
9. Antibiotic disks and blank disks

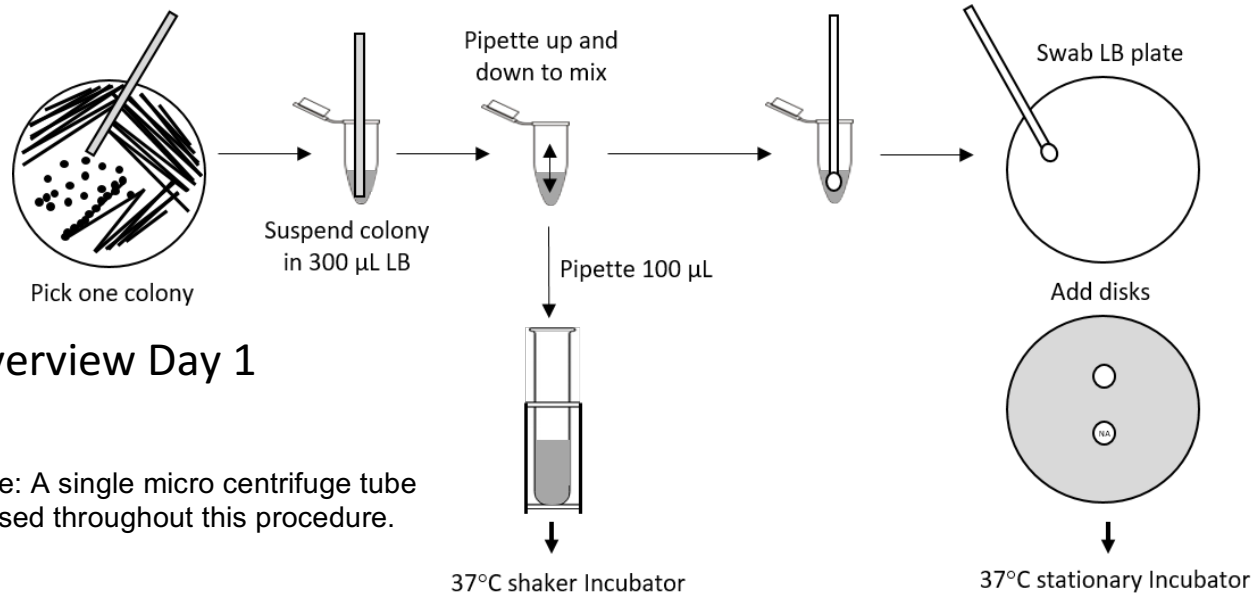
Example 1



Procedure Day 1

1. Clean benchtop and wash hands
2. Label the outside edge of the LB plate on the bottom (the side with agar in it) and the lid of the test tube with 3 mL of media with:
   a. your initials **b.** your class **c**. day 1.
3. From a culture of bacteria that has been grown on a plate, pick one colony and suspend it in 300 µL of liquid LB media using a sterile stick.
4. Using the p200 pipette attach a sterile tip and pipette up and down several times to mix the liquid, then transfer 100 µL from the micro centrifuge tube to the test tube with 3 mL of LB media.
5. Use the swab to soak up the liquid from the micro centrifuge tube, then streak the entire surface of the plate with the swab**. IMPORTANT! Make sure to cover the entire surface of the plate swabbing back and forth several times in multiple directions.** See example 1
6. Use the tweezers to put one antibiotic disk and one blank disk onto the LB plate. Tap the top of the disks lightly so they stay in place. **NOTE:** Do not put the disks too close to the edge of the plate.
7. Incubate plates, lid facing down in the 28°C stationary incubator for 1 day.
8. Incubate the test tube in the 28°C shaking incubator.
9. Clean benchtop and wash hands

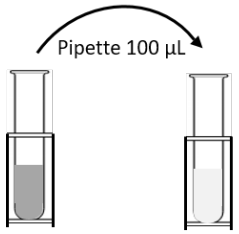
Overview Day 1



Note: A single micro centrifuge tube is used throughout this procedure.

Day 2 Subculture bacteria (Teacher Only)

1. Transfer 100 µL from the student's test tube on day 1 to a new test tube with 3 mL of fresh media.
2. Remove day 1 plates from the incubator and store in the fridge to stop bacterial growth.



This subculture step provides the bacteria with fresh food so they can stay actively growing

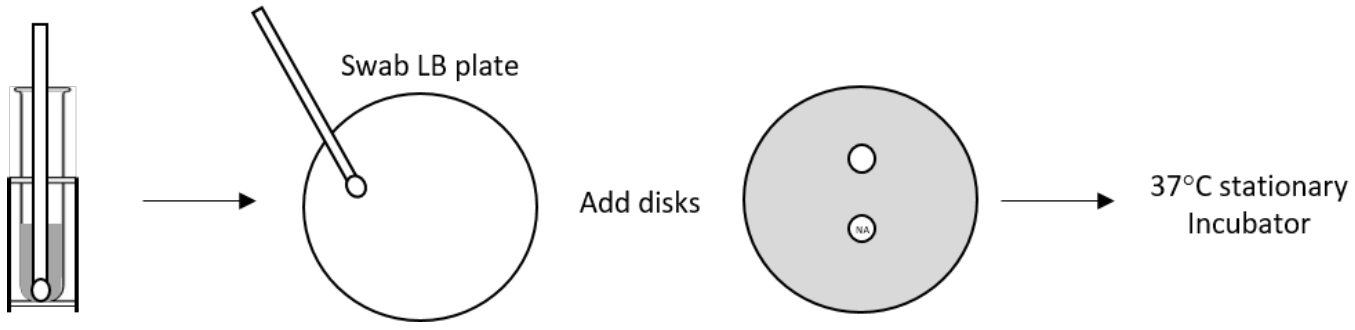
Materials Day 3

1. Your test tube liquid bacterial culture that was started on day 1
2. Sterile sticks and swabs
3. Tweezers
4. Ethanol
5. 1 LB agar plate
6. Antibiotic disks and blank disks

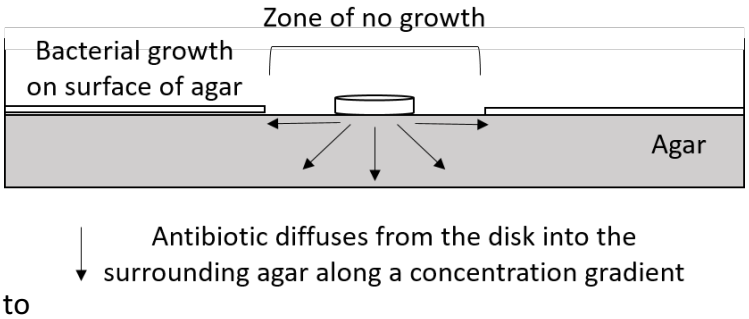
Procedure Day 3

1. Clean benchtop and wash hands
2. Label the outside edge of the LB plate on the bottom (the side with agar in it) and the lid of the 3 mL test tube with:
   a. your initials **b.** your class **c**. day 3.
3. Use the swab to mix the bacteria in the test tube. Touch the bottom of the tube, then swab the plate using the same procedure from day 1.
   IMPORTANT! Make sure to cover the entire surface of the plate swabbing back and forth several times in multiple directions.
4. Use the tweezers to put one antibiotic disk and one blank disk onto the LB plate. Tap the top of the disks lightly so they stay in place.
   NOTE: Do not put the disks too close to the edge of the plate.
5. Incubate plates, lid facing down in the 28°C stationary incubator for 1 day.
6. Clean benchtop and wash hands

Overview Day 3



Antibiotic Disk Diffusion Assay



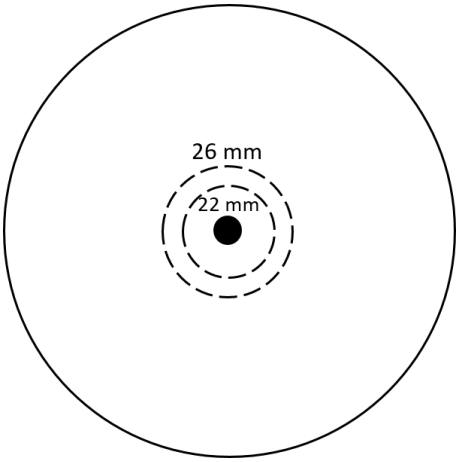
How the test works: A paper disk is infused with an antibiotic. When the disk is placed on an agar plate, the antibiotic spreads out (diffuses) through the agar all around the disk. So, the concentration of antibiotic is strongest closest to the disk, and gets less and less as you get further from the disk. In the image to the right this is illustrated with blue dye instead of antibiotics.

Predictions

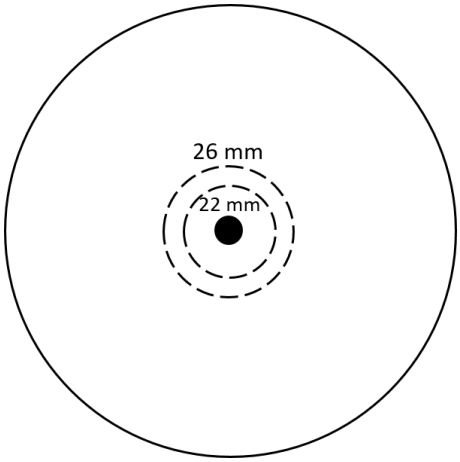
Using the chart below, make predictions on what each plate should look like based on the conditions given

Antibiotic	Disc Code	Susceptible ≥ mm	Intermediate mm	Resistant ≤ mm
Nalidixic Acid	NA	26	23-25	22

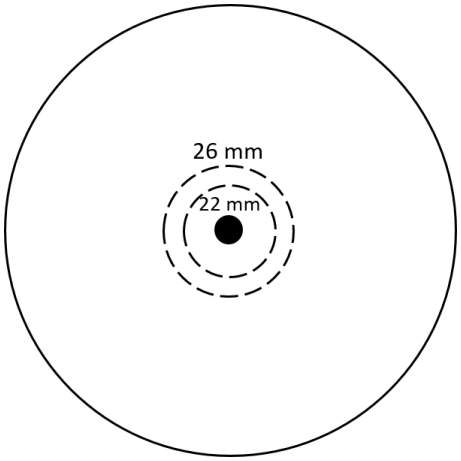
Draw where the bacteria will grow if a disk with **no antibiotic** is placed on the plate



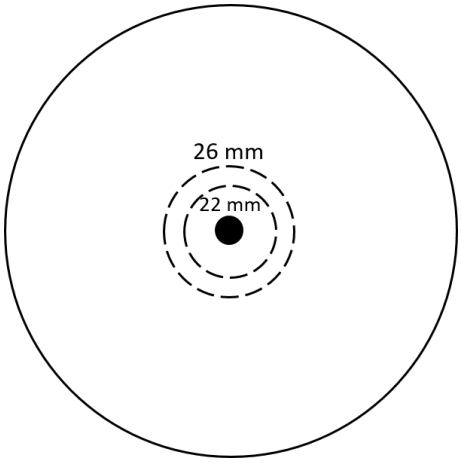
Draw where the bacteria will grow if a population of **susceptible** bacteria is swabbed onto the plate and the disk has antibiotics



Draw where the bacteria will grow if a population of **intermediate** bacteria is swabbed onto the plate and the disk has antibiotics



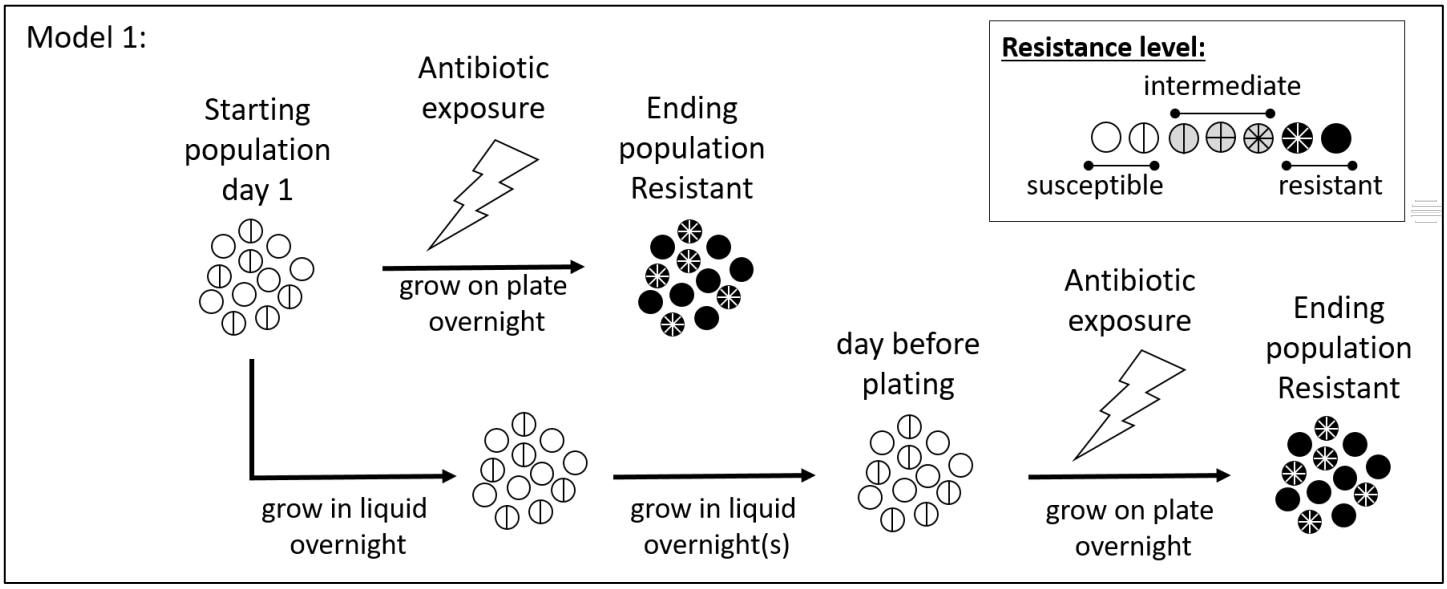
Draw where the bacteria will grow if a population of **resistant** bacteria is swabbed onto the plate and the disk has antibiotics

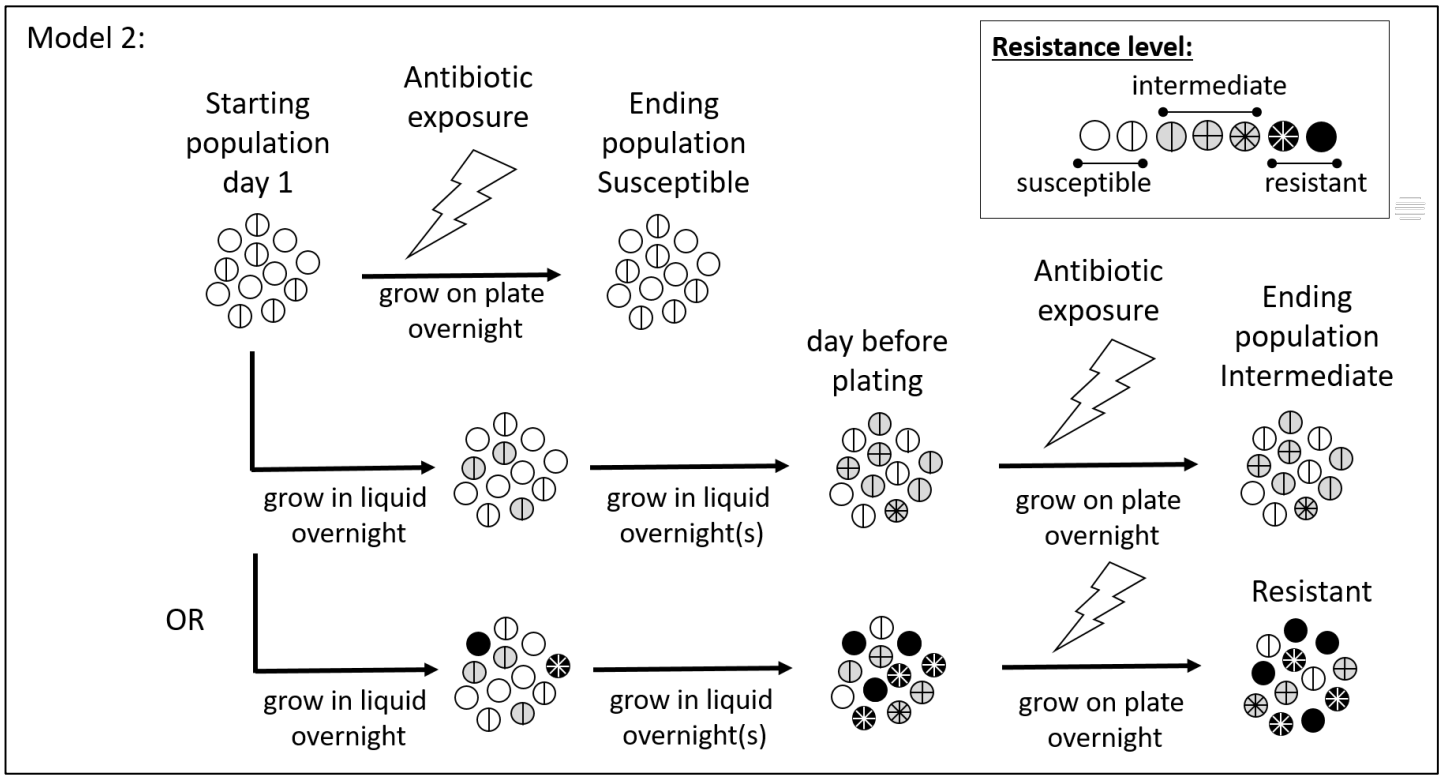


Antibiotic Resistance Lab

Purpose:
We will use *E. coli*, a harmless rod-shaped bacterium, in place of the dangerous MRSA (Methicillin-resistant *Staphylococcus aureus*) to understand how antibiotic resistance occurs.

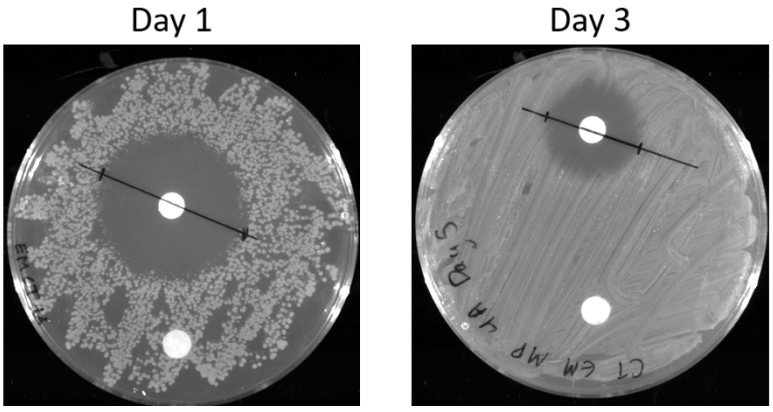
Our research and observations about MRSA raise an interesting question:
How do bacteria become resistant to antibiotics?
Here are two possible model explanations:





Data collection and analysis Day 5

How to measure the zone of inhibition:



1. Draw a line through the center of the zone with a marker
2. Mark the edges of the zone **NOTE: especially for the day 3 plates, the edge of the zone may have a faint inner ring of growth. If this occurs, use the inner ring as the edge of the zone of no growth.**
3. Use a ruler to measure the diameter of the zone in millimeters along the line you drew
4. Repeat this procedure for your day 3 plate
5. Record your data in the table below
6. Compare your results to the chart below to determine the resistance level

Record your data here

Antibiotic	Day 1 Plate		Day 3 Plate	
	Diameter (mm)	Resistance Level	Diameter (mm)	Resistance Level
Nalidixic Acid				

Antibiotic	Disc Code	Susceptible ≥ mm	Intermediate mm	Resistant ≤ mm
Nalidixic Acid	NA	26	23-25	22

What do you observe about the blank disk? Why did we use a blank disk?

Share your data with the class. (What data should we share?) Record the class data in the space below.

Directions: Use the chart below to outline your argument about which model best answers the question: *How and why do antibiotics become useless?*

Claim:	
Evidence:	**Reasoning (Analysis + Synthesis + Evaluation):**

Evidence Continued:	**Reasoning (Analysis + Synthesis + Evaluation) Continued:**

Category		**4**	**3**	**2**	**1**
Description		Clearly and thoroughly integrates specific, relevant, and accurate evidence, creating a strong foundation for the argument.	Integrates specific, relevant, and accurate evidence, creating a foundation for the argument.	Integrates limited and/or general evidence; may lack relevance and/or accuracy; creating a weak foundation for the argument.	Attempts to integrate evidence, but is insufficient in creating a foundation for the argument.
Reasoning	**Analysis**	Clearly and efficiently breaks down and elaborates on meaning and significance of each piece of evidence.	Breaks down and elaborates on meaning and significance of each piece of evidence.	Breaks down evidence but provides limited meaning and significance.	Breaks down evidence in a confusing or incomplete manner.
	Synthesis	Clearly and efficiently connects the evidence and analysis to the thesis to develop the implications and significance.	Connects the evidence and analysis to the thesis to develop the implications and significance.	Attempts to connect the evidence and analysis to the thesis, but implications and significance are limited.	There is little to no development of implications and significance of evidence and analysis to the thesis.
	Evaluation	Clearly supports argument by making a reasoned judgment based upon thorough examination of multiple perspectives.	Supports argument by making a reasoned judgment based upon examination of multiple perspectives.	Attempts to support argument by making a judgment based upon limited examination of perspectives.	Does not make sufficient judgment to support the argument. Little to no consideration of other perspectives.