MICROBIAL DISCOVERY LESSON

Modeling the Rise of Antibiotic Resistance

Author Information

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Description

This lesson begins with a brief video setting the stage for the topic and open-ended discussion questions designed to elicit student ideas. Working in pairs, students then model what happens to the gut microbiome when exposed to multiple rounds of antibiotic treatment. They answer a series of questions that guides them toward the understanding that the rise of antibiotic resistant bacteria within the population is an example of evolution by natural selection. Students extend their understanding by predicting and then modeling a variation of the original scenario.

Learning Objectives

1. Describe how antibiotic resistance in bacterial populations demonstrates natural selection.

2. Explain why standing trait variation, heritability, and a link to reproductive success are necessary for evolution by natural selection within a population.

3. Distinguish between evolution and natural selection.

Total Activity Time
This activity takes approximately 190-250 minutes (4-6, 45 min class periods), depending on if pieces are done as homework or not.

Grade Band
9-12

NGSS
HS-LS-4-3; HS-LS-4-4

Topics
Antibiotic Resistance; Evolution; Human Microbiome; Natural Selection

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Engage

1. Show "The Rise of Superbugs" from PBS Digital Studios' *It's Ok to be Smart* series up to the 3:19 mark (when Joe says, "So, how does resistance work?"). Then discuss the following questions with the class to gauge students' conceptions about antibiotic resistance. Listen to student ideas without commenting on their validity.

2. Ask for a show of hands, how many students have either taken an antibiotic themselves or known someone who has? What was it for? Did it work?
   a. Note that students may not understand the differences between antibiotics, drugs for non-infectious conditions, and vaccines. If time allows, a brief discussion of these classes of medicine may be useful.

3. In the video, Joe references the bacteria that live in and on us. How do you think these microbes, which make up your microbiome, are affected when you take an antibiotic?

4. When we say "antibiotic resistance," who or what is resistant to the antibiotics? How do we know?

5. Sparky and Bailey are bacteria that represent two extremes of antibiotic sensitivity—Sparky cannot survive any exposure to penicillin at all, while Bailey can survive exposure to twice the concentration of penicillin used to treat most infections. In a healthy person who has never taken penicillin, is Sparky or Bailey most likely to survive and reproduce? Why?

10 min

6. Present the following scenario and ask students to vote on the statement they agree with most. Ask one person who voted for each statement to explain their reasoning.

   "A population of bacteria live in the soil on a farm. The farmer begins using antibiotics to fatten the animals, gradually exposing the soil bacteria to them through runoff and animal waste. Which statement about how the bacteria cope with their changing environment is most accurate?"

   a. The bacteria mutate in response to the antibiotic exposure. Through mutation, some individuals develop the ability to survive at higher antibiotic concentrations. These individuals reproduce while the other bacteria die. Because their offspring inherit the ability to survive at higher antibiotic concentrations, the entire population evolves to be more tolerant of antibiotics.

   b. Because of genetic variation, there are already individuals in the population of bacteria that can tolerate increased exposure to antibiotics. These individuals

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survive better than their peers and produce more offspring. Because their off-spring inherit the ability to survive at higher antibiotic concentrations, the entire population evolves to be more tolerant of antibiotics.

c. All of the bacteria adjust their cell machinery so that they can survive exposure to antibiotics. When the bacteria reproduce, their offspring inherit this adjustment and the entire population evolves to be more tolerant of antibiotics.

Explore

5 min

1. Students will work in pairs for this activity. Distribute one set of student materials to each pair.

2. Once they have their microbiomes, students should use their divided trays to sort and count the number of individuals of each species in their starting population (i.e., the number of pieces of pasta for each shape). Record this information in the first column of the Data Tables sheet (Appendix I). Students should also record the total number of plain, orange, and green microbes.

3. Have students dump the counted microbes into their bowls and mix by hand before returning them to the zippered baggie.

4. Present students with the following scenario: "You received a nasty scratch on your hand that became infected, so your doctor prescribed a course of oral antibiotics, specifically methicillin (which is related to penicillin). How do you think the methicillin will affect other bacteria that live in and on your body as part of your microbiome?" [Antibiotics are non-specific and kill both "bad" bacteria, such as those that infected your hand, and "good" bacteria, such as those in your gut microbiome.]

10 min

5. Ask students to simulate the effect of taking methicillin on their gut microbiome by removing individual green and plain pieces of pasta (regardless of species) **one at a time** for 40 seconds from their baggie.

   a. Students should use their bowls as discard containers.

   b. One person should time the other during the antibiotic "treatment."

   c. After treatment, record the number of individuals of each species, as well as the total number of plain, orange, and green individuals, that remain. These microbes have survived methicillin treatment.

   d. After counting, be sure that students thoroughly mix their surviving microbes and add them back to their zippered baggie.

5 min

6. Bring the class together to inform them: "Your infected cut was successfully treated with methicillin and you are no longer taking antibiotics. What happens to the microbes in your microbiome that survived treatment?" [They grow and reproduce asexually through binary fission, where each surviving individual divides into two identical daughter cells.]

7. To mimic growth after the removal of the antibiotic, have students repopulate their empty microbiome space by doubling the surviving microbes using the discard pile and leftover pasta containers. The total number of each species and col-

or should be recorded in the "After Reproduction" column and the microbiome should again be thoroughly mixed before proceeding.

8. "This time, you go to the doctor with hives and press her to prescribe methicillin since it worked so well for your infected scratch." Have students do another 40 second round of methicillin treatment and again record the number of individuals of each species, as well as the total number of plain, orange, and green individuals, that remain.

9. Again have students repopulate the empty microbiome space by doubling the surviving microbes using the discard pile and/or any leftover pasta. The total number of each species and color should be recorded in the "After Reproduction" column.

10. For students' initial microbiome and each round of methicillin treatment, ask them to calculate the proportion of each species within the population (# of individuals of species X/total microbes) and the proportion of plain, orange, and green individuals (# of individuals of color Y/total microbes). They should record their proportions in the Analysis Tables (Appendix II).

11. Have students plot a line graph for their microbiome that shows species proportions initially, after one round of treatment, and after two rounds of treatment.
    a. The Y axis should be "Percentage of the Total Population" and the X axis should be "Rounds of Treatment with Methicillin." Each species of microbe should be represented with a separate data series/line.
    b. Graphs can be created in Excel or by hand on graph paper.

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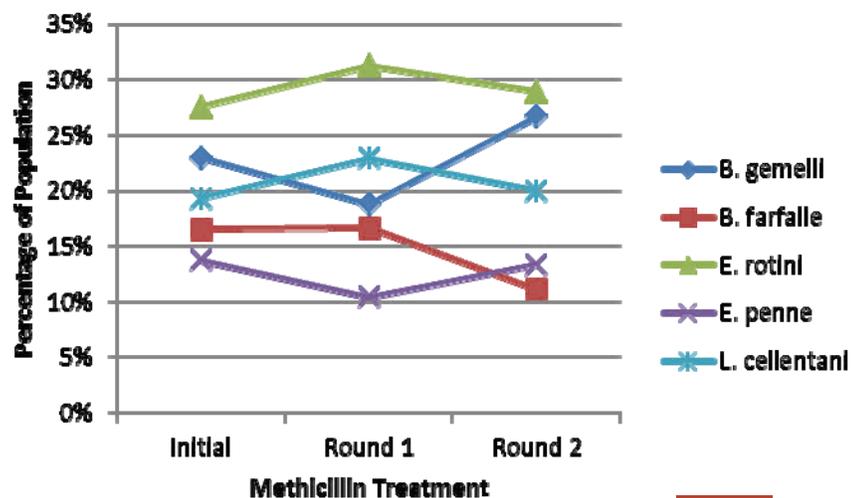
Explore

10 min or HW

12. Students should create a similar line graph showing the proportion of plain, orange, and green individuals over the rounds of methicillin treatment.

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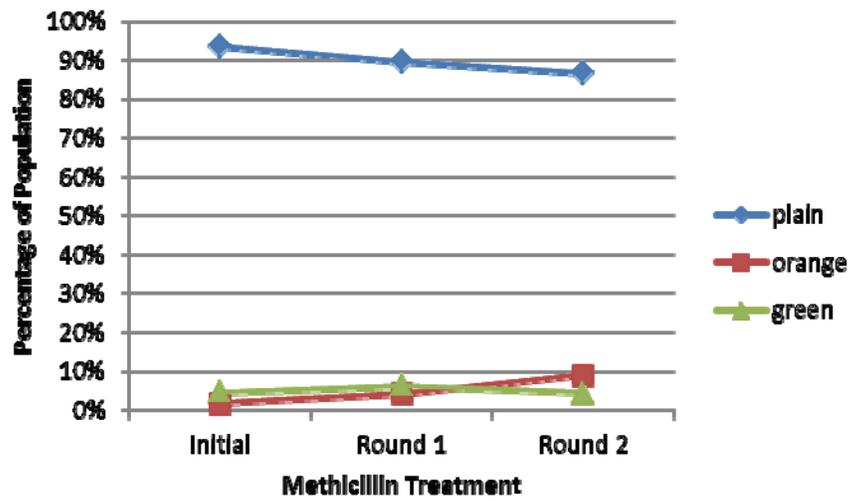
Explain

1. Have students individually answer the Analysis Questions in Appendix III.

10 min

2. Pick five student pairs at random to display their species and color graphs up on the board. Have each of the pairs briefly discuss how their microbial population changed over time (Analysis Questions #1 and #2).
   a. Note trends each group mentions on the board next to their graphs. Ask the class "Are there any trends that are consistent across all five microbiomes?"
   b. With two rounds of antibiotic, students may or may not see a clear indication that *E. rotini* as a species is taking over the population. However, they should consistently see the proportion of plain and green microbes decrease, while orange microbes increase in the population.

15 min

3. As a class, discuss the questions below.
   a. "The orange *E. rotini* contain an extra gene called *mecA*. What do you think the *mecA* gene does? What evidence do you have?" [Based on the fact that methicillin killed only plain and green microbes, *mecA* most likely makes microbes resistant to methicillin.]
   b. "The green *E. rotini* contain an extra gene called *mcr-1.* What do think it does? What evidence do you have?" [This is a bit of a trick question—the green microbes are treated exactly the same as the plain microbes in this scenario, so there is no indication of what the *mcr-1* gene does.]
   c. "In this scenario, an environmental condition (methicillin exposure) disrupted your microbiome and led individuals with the *mecA* gene to become more common in the population over time. What biological process does this model?" [This models evolution through natural selection.]
      i. Note that it is important to distinguish between evolution (the "what") and natural selection (the "how"). Evolution is a change in population traits over time. This change can be the result of natural selection, or the result of other mechanisms like migration or genetic drift.

15 min

3. Further break down the model by having students think-pair-share to identify the conditions necessary for natural selection in the model.
   a. "Natural selection within a population only occurs under certain conditions. First, individuals within the population have to show standing variation in a trait. In our model, what trait was variable between individual microbes?" [Their ability to tolerate methicillin was the variable trait.]

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Explain

b. "Second, that trait variation has to be heritable, meaning passed from parent to child. Is variability in the trait we've identified heritable? How?" [Yes, through presence or absence of the *mecA* gene, which is passed from mother to daughter cell.]

c. "Third, the success of an individual in reproducing and contributing offspring to the next generation has to be influenced by the variable, heritable trait. The factor linking reproductive success to the variable trait is called the selective pressure. What selective pressure caused individual microbes to experience different reproductive success? How?" [Methicillin exposure was the selective pressure and it caused individuals with lower tolerance (i.e., those without the *mecA* gene) to have lower reproductive success by killing them before reproduction.]

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Elaborate

10 min

1. Give each student a copy of the Elaborate Questions (Appendix IV). Read question 1 aloud and have students write individual responses: "What if your initial microbiome did not have any microbes with the *mecA* gene (i.e. no orange *E. rotini*)? What do you think would happen if it were exposed to the same rounds of methicillin treatment? Will the population change over time? Explain your reasoning."

45 min

2. Have students reconstitute their initial microbiomes, replacing 1/2 of orange *E. rotini* with plain *E. rotini* and 1/2 with green *E. rotini.* Students should repeat the methicillin treatment scenario and record their data on a second set of Data Tables (Appendix I) and Analysis Tables (Appendix II).

10 min or HW

3. Once students have made their graphs, have them work with their partner to answer the other Elaborate Questions (Appendix IV).

20 min

4. Discuss the results of this variation of the scenario as a class.

    a. Guide students toward understanding that their new population (most likely) did evolve, but **not** by natural selection. Natural selection could not occur because there was no longer any variation in the individual microbes' ability to tolerate methicillin. Therefore, surviving methicillin treatment to reproduce was random and not linked to any trait inherited by future generations. [This variation actually demonstrates evolution by genetic drift.]

    b. Be sure to also ask whether the *E.rotini* changed in any way in response to the methicillin. Did they mutate to get the *mecA* gene or alter themselves in any other way? Draw parallels to the fact that individual students also can't spontaneously develop traits or abilities in order to survive.

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Evaluate

20 min or HW

1. Have students revisit and reflect on their vote on the soil bacteria scenario at the beginning of the lesson. Do they agree with their original vote? How does their experience with antibiotic resistance in the gut microbiome support or refute each of the three options?

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Background

What are antibiotics?

Antibiotics are chemicals made by microbes to slow the growth of or kill **bacteria**. We may think of antibiotics as medicine prescribed to fight off a current bacterial infection or prevent an infection after surgery. However, antibiotics are originally microbial weapons. Life as a microbe is tough. Conditions constantly change. Food and water are scarce and limit reproduction and growth. In a race for finite food and space, speed of growth and reproduction are essential to win. Fungi, archaea, and even some bacteria, have been producing antibiotics for millennia to out-compete other bacteria for food and a place to live.
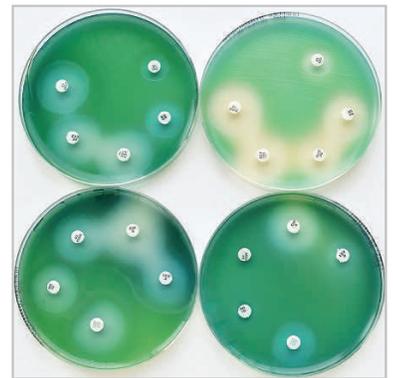
Alexander Flemming co-opted penicillin – an antibiotic naturally produced by mold – for human needs in the 1940's. While cleaning out old bacterial dishes, Flemming noticed that the bacteria were dead around where a *Penicillium* mold had grown. Throughout the centuries, healers worldwide had placed mold scraped from cheeses, soy beans, and even horses' saddles onto bandaged wounds to speed healing. Yet Flemming was the first to isolate the antibiotic compound, penicillin, for medical use. Since then, scientists have identified numerous antibiotics and produced purified forms of them in high concentrations. Antibiotics have saved countless lives from bacterial infections.

How do they work?

As weapons made by one microbe to slow or kill another microbe, antibiotics target differences in microbial proteins or other structures essential for cell function. The antibiotic-producing microbes often protect themselves by targeting cell structures that their competitors have and they themselves lack. Similar to how Superman is weakened by kryptonite, while his arch enemy, Lex Luthor, is not, the penicillin made by *Penicillium* fungi prevents certain bacteria from making the peptidoglycan needed for their cell walls. The chitinous cell walls of fungi are structurally very different and are not affected by penicillin. Methicillin, the antibiotic used in this lesson, is a synthetic derivative of penicillin, but works in the same way. These structural differences between microbes make good targets for competition for limited resources. The diversity of microbes in our world fighting for limited nutrients and space has led to a vast array of microbially toxic

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Background

antibiotic substances.

How do microbes fight back?

With any new weapon on the battlefield comes a defensive maneuver. The battle between microbes has been going on for eons, allowing them to not only evolve antibiotics, but also techniques to stave off antibiotic attacks. At its simplest, variation in the charge or shape of the protein or structure an antibiotic targets can make the antibiotic ineffective. Individual bacteria with versions of the protein the antibiotic can no longer bind survive exposure and divide, while their sensitive relatives do not. This is how bacteria with the *mecA* gene survive treatment with penicillin and its derivatives. *MecA* encodes a resistant variant of the protein penicillin targets. Bacteria with this gene can continue building their cell walls, impervious to the antibiotic.

More sophisticated antibiotic resistant bacteria secrete chemicals to inactivate the antibiotic before it reaches the cell or cellular target. Other resistant bacteria pump the antibiotics out of the bacterial cell before the chemical toxin hits its target. Some, such as colistin-resistant bacteria that carry the *mcr-1* gene, even make proteins that then modify the antibiotic's target so that it is no longer affected. Resistant microbes thus have a "better body armor" that they pass on to their offspring.

Why is antibiotic resistance increasing?

In nature, antibiotics are used briefly on a local "battle" scale to allow one type of microbe to gain an advantage. These natural battlefields of antibiotic resistance are rarely encountered by human pathogens, and do not endanger human health. It is our over-zealous use of antibiotics that is the problem. In our hospitals and doctor's offices, antibiotics are routinely used "just in case" or mis-prescribed for viral illnesses, like the common cold and many ear infections. In large-scale, agricultural feedlots, low doses of antibiotics are added to feed to promote rapid animal growth. Antibiotic products are used routinely in our schools and homes. These practices expose higher numbers of bacteria to antibiotics routinely, increasing the chances that a resistant bacterium will be exposed and will survive to produce offspring with little or no microbial competition. To compound the situation, the density of people or other animals living close together increases the opportunity for antibiotic resistant bacteria to spread between individuals.

Antibiotics have been our miracle cure for bacterial infections since Fleming isolated penicillin. Pathogenic

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Background

bacteria killed easily decades ago now survive ever-higher doses and a wider range of antibiotics. These "super bugs" elude our strongest antibiotics and can potentially push us into an "antibiotic winter" – a time when antibiotics are ineffective. Resistant strains of the bacteria causing tuberculosis, gonorrhea, and many other virulent diseases terrify patients and medical personnel alike. Increased antibiotic resistance is a warning shot in the war between humans and bacteria. We need to rethink how we use these microbial weapons so that they will be effective for generations of people to come.

Preparation

Make Microbiome Baggies

1. Create a communal gut microbiome by combining an equal amount of the following uncooked pastas in a large zippered bag and mix the contents until evenly distributed. For a class of 30, you will need one 16 oz. box of each pasta variety. Each pasta shape represents a different microbial species and each non-plain pasta color represents the presence of an extra gene carried on a plasmid. Green microbes contain *mcr-1* and orange microbes contain *mecA*.
   a. Gemelli —> *Bacteroides gemelli*
   b. Farfalle —> *Bifidobacterium farfalle*
   c. TriColor Rotini (plain, orange, and green) —> *Enterococcus rotini*
   d. Penne —> *Escherichia penne*
   e. Cellantani —> *Lactobacillus cellantani*
   f. See http://mentalfloss.com/article/65049/extensive-guide-pasta-shapes for alternative shapes if needed; it is important that four of the shapes are plain pasta only and the fifth is tricolor, and that each shape is approximately the same size.
2. For each pair of students, fill one snack-sized (approximately 6.5"x3.25") zippered plastic baggie with random handfuls of the communal gut microbiome, making sure that each bag has at least one orange piece of pasta. The baggie should be full, but still able to be closed.

10 min

Assemble Student Materials

1. Make the following copies:

Appendix I—Data Tables	2/pair
Appendix II—Data Analysis Tables	2/pair
Appendix III—Analysis Questions	1/student
Appendix IV—Elaborate Questions	1/student

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Preparation

2. Each student pair will need one microbiome baggie, one shallow paper bowl, one 5-compartment disposable tray (available on amazon.com), and the handouts listed above.
3. Divide leftover communal gut microbiome pasta into several large containers that can be placed around the room for students to replenish their microbiomes between antibiotic rounds.

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Appendix I—Data Tables

For your initial microbiome and each round of antibiotic treatment, indicate the **number of individuals** of each species and color in the population.

Microbe Species	Initial Microbiome	Methicillin Survivors Round 1	After Reproduction	Methicillin Survivors Round 2	After Reproduction
Bacteroides gemelli					
Bifidobacterium farfalle					
Enterococcus rotini					
Escherichia penne					
Lactobacillus cellentani					
Total					

Microbe Color	Initial Microbiome	Methicillin Survivors Round 1	After Reproduction	Methicillin Survivors Round 2	After Reproduction
Plain					
Orange					
Green					

Appendix II— Analysis Tables

For your initial microbiome and each round of antibiotic treatment, indicate each species' and colors' **percentage** of the total population.

Microbe Species	Initial Microbiome	Methicillin Survivors Round 1	After Reproduction	Methicillin Survivors Round 2	After Reproduction
Bacteroides gemelli					
Bifidobacterium farfalle					
Enterococcus rotini					
Escherichia penne					
Lactobacillus cellentani					

Microbe Color	Initial Microbiome	Methicillin Survivors Round 1	After Reproduction	Methicillin Survivors Round 2	After Reproduction
Plain					
Orange					
Green					

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Appendix III—Analysis Questions

1. Did the <u>species </u>composition of your microbiome change over the rounds of antibiotic treatment? If so, how? Did any species die out completely?

2. What about total plain vs. orange vs. green microbes? Did the proportion of microbes of different colors change over the rounds of antibiotic treatment? If so, how?

3. What do you think would happen if you applied a third round of methicillin treatment to your microbiome? Justify your answer.

4. Suppose *Enterococcus rotini* is an opportunistic pathogen. It is usually harmless, but can cause disease if it is not kept in check by your immune system and the other microbes in your microbiome. After the rounds of methicillin treatment you have received, are you more or less likely to experience symptoms caused by *E. rotini* in the future? Why?

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Appendix IV—Elaborate Questions

1. What if your initial microbiome did not have any microbes with the *mecA* gene (i.e., no orange *E. rotini*)? What do you think would happen if it were exposed to the same rounds of methicillin treatment? Will the population change over time? Explain your reasoning.

2. Did your microbiome evolve, or change in composition, over the rounds of antibiotic treatment? If so, how?

3. Did natural selection occur this time? Why or why not?

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