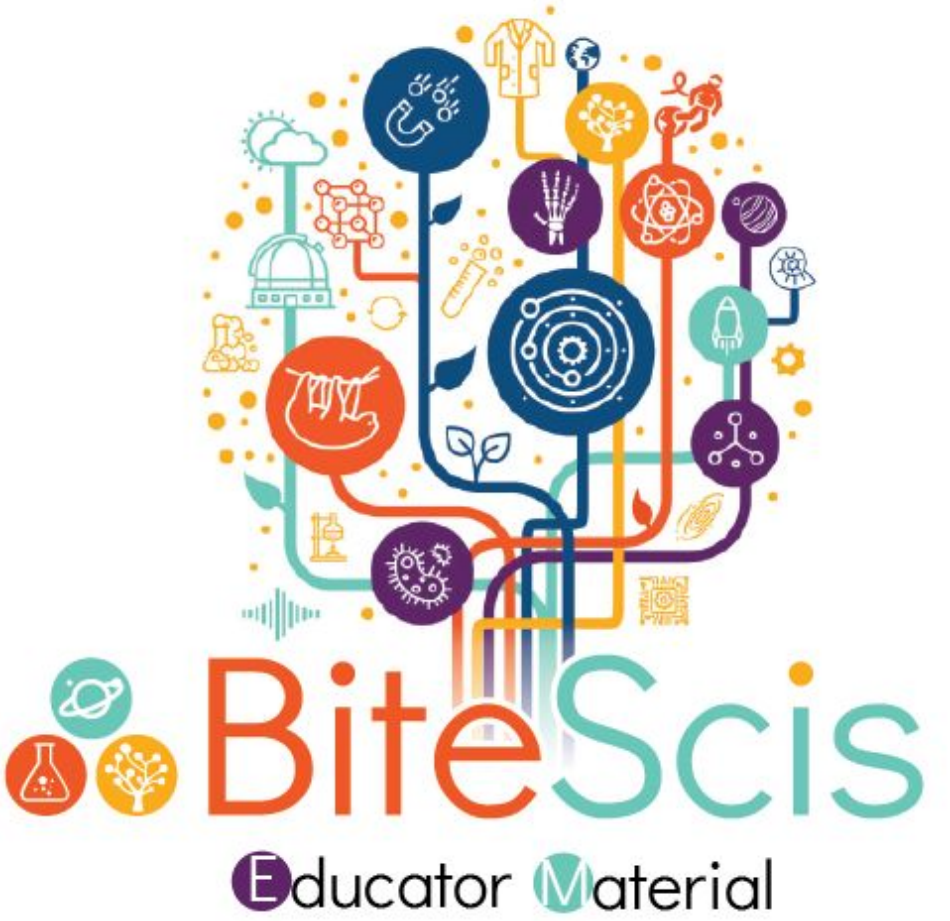

Antibiotic Resistance and Bacterial Evolution

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Purpose

This lesson provides students with an opportunity to engage with research demonstrating evolution in real-time. Students will confront many misconceptions about how evolution works, and will come to understand how evolution affects human health.

Audience

This lesson was designed for use in an introductory high school biology class.

Lesson Objectives

Upon completion of this lesson, students will be able to:

- explain that a population is a group of individuals with different traits and characteristics.
- describe that diversity in a population is the result of accumulated changes in the DNA sequence.
- explain that natural selection identifies individuals with traits best suited for a particular environment.
- argue from evidence why overuse of antibiotics is leading to a public health crisis.

Key Words

antibiotic resistance, mutation, microbiome

Big Question

This lesson addresses the Big Question "*What does it mean to observe*?"

Standard Alignments

- **Science and Engineering Practices**
  - **SP2**. Developing and using models
  - **SP6**. Constructing explanations and designing solutions
  - **SP7**. Engaging in argument from evidence
- **MA Science and Technology/Engineering Standards (2016)**
  - **HS-LS3-4**. Use scientific information to illustrate that many traits of individuals, and the presence of specific alleles in a population, are due to interactions of genetic factors and environmental factors.
  - **HS-LS4-2**. Construct an explanation based on evidence that Darwin's theory of evolution by natural selection occurs in a population when the following conditions are met: (a) more offspring are produced than can be supported by the environment, (b) there is heritable variation among individuals, and (c) some of these variations lead to differential

fitness among individuals as some individuals are better able to compete for limited resources than others.

- **HS-LS4-4**. Research and communicate information about key features of viruses and bacteria to explain their ability to adapt and reproduce in a wide variety of environments.

- **NGSS Standards (2013)**
  - **HS-LS4-2.** Construct an explanation based on evidence that the process of evolution primarily results from four factors: (1) the potential for a species to increase in number, (2) the heritable genetic variation of individuals in a species due to mutation and sexual reproduction, (3) competition for limited resources, and (4) the proliferation of those organisms that are better able to survive and reproduce in the environment.
  - **HS-LS4-4.** Construct an explanation based on evidence for how natural selection leads to adaptation of populations.
- **Common Core Math/Language Arts Standards**
  - **CCSS.ELA-LITERACY.RST.9-10.1.** Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.
  - **CCSS.ELA-LITERACY.RST.11-12.1.** Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.

Misconceptions Addressed

- This lesson addresses many common misconceptions about evolution, including:
  - Evolution gives organisms what they need to survive. (Question 10)
  - Evolution occurs because organisms want it to, or wills it to. (Question 10)
  - Natural selection ensures only the fittest (the strongest, fastest, fiercest) survive. (Question 9)
  - Evolution is about progress. (Question 9)
- Further information about student misconceptions on this topic can be found here.

Primary Sources

Bite "Antibiotic Resistance and Bacterial Evolution" *based on*:
Baym, Michael, Tami D. Lieberman, Eric D. Kelsic, Remy Chait, Rotem Gross, Idan Yelin, and Roy Kishony. 2016. "Spatiotemporal microbial evolution on antibiotic landscapes." *Science* 353(6304): 1147–1151. doi: 10.1126/science.aag0822

- **MEGA-Plate Video**
  Harvard Medical School. "The Evolution of Bacteria on a 'Mega-Plate' Petri Dish (Kishony Lab)." YouTube, YouTube, 9 Sept. 2016

- **Contagion Video**
  Travis, John. "Don't Call It Viral Marketing: The Story Behind Contagion Microbial Billboard." Science | AAAS, 11 Dec. 2017. Accessed September 13, 2018.

- **Write-Up from Science News for Students**
  Hamers, Laurel. "Scientists Watch Germs Evolve into Superbugs." Science News for Students. November 09, 2016. Accessed September 13, 2018.

- **Misconceptions**
  Anderson, Diane I., Kathleen M. Fisher, and Gregory J. Norman. 2002. "Development and Evaluation of the Conceptual Inventory of Natural Selection." *Journal of Research In Science and Teaching* 39(10): 952–978. doi: 10.1002/tea.10053

Materials

- Copies of the Student Handout and Science Bite for each student
- Means to show the figures in the student handout in color
- Means to show the videos
- For optional extension (Day 2): Sets of Bacterial Invasion! game board, game cards (standard or advanced depending on level of class) and player pieces

Time

This lesson should take approximately one to two 50-minute class periods.

Student Prior Knowledge

Students should be familiar with bacteria, including how they reproduce and form colonies. They should also know some basic information about the growing problem of antibiotic resistance before starting the lesson, enough to be able to describe it accurately in their own words. In addition, students should have a general understanding of how DNA encodes genetic information and that mutations to DNA can result in new traits. Finally, students should be familiar with the evolutionary mechanism of natural selection and the concept of fitness.

Instructions and Teacher Tips

- **General Procedure**
  - At the beginning of the lesson, even before introducing the topic, show students the *Contagion* billboard video.
    - Ask students if they have seen the movie *Contagion* and gather first impressions of what they saw.
    - Ask students what was on the plate. Why didn't it show up right away?

- Discuss our current antibiotic crisis. Facts about the crisis can be found on the CDC and WHO websites.

- Have the students read the Science Bite and answer the first six questions on the Student Handout.
- Have students watch a video of the MEGA plate on their own if you have the ability, or show it to the class as a group. The video was made by the authors of the study.
- After the students watch the MEGA-plate video, have them answer the remaining questions on the Student Handout.
- Finish with a discussion of the Big Question (see below).
- Optional Day 2: Modeling of MEGA-plate with "Bacterial Invasion!" board game activity.
  - Before the class begins, determine how many groups will play the game and at what academic level. We would recommend using the "standard" game cards (that have just directions on them) with standard-level classes and the "advanced" games cards (that have additional vocabulary, relevant gene names and rationales on them) with honors or AP classes. These playing cards were developed using the real genes and the associated effects identified by Baym, *et al.* 2016 and provide an opportunity for students to deepen their understanding of mechanisms of antibiotic resistance.
  - Print out and if necessary color the bacteria pieces and board for each group. Cut out, and if possible laminate the board, game cards, and bacterial pieces so that you can use them in repeated classes. The game board can be printed on 11×17 paper, or you can tape or glue two 8.5×11's together. Store game pieces and cards in separate plastic bags to keep things organised.
  - Create groups with 3 or 4 students in each.
  - Go over the rules of the game with the class. Taking turns having students read the rules aloud to the class works well to make sure everyone understands. Emphasize that some things carry over from turn to turn. Once you get a high antibiotic resistance level, for example, that can't be taken away if a lower level is drawn. Players should keep their cards in front of them so that they can track what their highest antibiotic resistance level is, whether they are skipping every other turn, and moving one space or two spaces per turn. However, how each player moves, whether up/down left/right or diagonally does not carry over from turn to turn, but determined by each card that is drawn.
  - Pass out game boards, game cards and player pieces to each group. Allow students to play the game. This should take about 20 minutes.
  - While they are playing, circulate and ask questions to make sure they understand what they're modeling in the game. For example, you could ask how there can be mutations that don't affect the resistance level (the mutation either didn't change the phenotype of the bacterium at all, or changed it in a way that didn't have an effect on its ability to withstand antibiotics).

- After completing the game, have students go back and review their responses to the questions in the student handout, paying particular attention to Questions 5, 7, 8 and 9. Have the students add to or clarify their responses using a different color pen/pencil from their original response to track their learning.
- Review student answers as a class. Track student learning by allowing them to add or clarify their responses using a third color pen/pencil.

- **Tips, Extensions, and Variations**
  - Students may misinterpret the color scale in Figures 1 and 2 in the Student Document to be a *y*-axis. Make sure that they understand that the colors correspond to the colors of the dots in the figure, and that it does not mean that colonies toward the top have higher resistance than those at the bottom.
  - Students may be curious about *Contagion*. If time permits, you might want to show the movie trailer. A synopsis is here. It is interesting to note the movie is about an emerging viral pandemic but the billboard is constructed from bacteria and fungi. This can be a launching point for a discussion about how antibiotics work, why they are ineffective against viruses and how overprescription can lead to antibiotic resistance.
  - Model what is happening in the MEGA plate with a quick classroom model. Have all students stand up bunch together on one side of the room. Tell them that in the environment a step ahead, only people with something like glasses can survive and get to step forward. Then, a step ahead, only students wearing red can survive, etc. You can change the characteristics, of course, but the idea that should be reinforced is that the characteristic that enables passage to the next environment already has to exist in the population. Students don't get to "evolve glasses so that they can move"; that's not how evolution works. You should also reinforce that even if students have the trait that enables growth two environments (steps) ahead, it doesn't matter if they don't have the characteristic that enables growth one step ahead. Some questions to ask:
    - What does stepping forward model?
    - How can you model the "trapped" colony explored in Question 8?
    - How could the model be improved?
  - If you are running short of time and students haven't finished responding to the questions, consider assigning unfinished questions as homework and reviewing their responses at the beginning of the next class. Beware, however, that the educator document (with answers) is available online.

Background Information and Research Details

- Before this study, most of our knowledge about the evolution of resistance was based on laboratory setups with well-mixed environments. This study's set up is unique. The antibiotic-gradient and large size allows growth to be observed and recorded with a camera.
- The two different antibiotics were used in the study each disrupt bacterial reproduction in different ways:

- Trimethoprim (TMP) prevents bacterial DNA replication by inhibiting an enzyme that synthesizes the base, T (thymidine). If the enzyme isn't functioning, the bacteria are unable to produce the building blocks necessary to replicate their DNA.
- Ciprofloxacin prevents bacterial cell division by inhibiting enzymes that help DNA stay unzipped during replication.

- To prevent contamination with other bacteria or fungi, additional antibiotics were added to the plates: kanamycin to inhibit general bacterial growth and cycloheximide to prevent fungi from growing. This allows the researchers to focus specifically on internal competition among the starting population of bacteria.
- Some of the initial starting population of bacteria happened to have mutations that caused them to be resistant to the antibiotic, just by chance. Those bacteria survive antibiotic exposure and passed on resistance to their clones. For humans, passing a trait from generation to generation is the only way an individual can get an adaptive trait—but bacteria are special! They can also transfer genes from one individual in a colony to another, a process known as horizontal gene transfer. Horizontal gene transfer is thought to be an incredibly important mechanism of bacterial evolution and antibiotic resistance. For more information on horizontal gene transfer, check out this resource from the University of Utah.
- The researchers used two types of media in the MEGA plate:
  - solid agar, which helps keep the boundaries of the plate separate; and
  - swim agar, which contains less sugar and is therefore semi-solid. The swim agar allows the bacteria to move across the top layer of the plate.
- *E. coli* can propel themselves through the "swim agar" in the top layer of the MEGA-plate using flagella. These flagella extend off the back of the bacterial cell and rotate, allowing them to move forward, much like the propeller on a boat.
- Researchers sequenced the entire *E. coli* genome (which contains about 5 million base pairs) and compared the sequence of the initial population to each of the populations along the way. Differences in DNA sequence allowed the researchers to identify mutations.
  - Some mutations that the researchers found are "non-synonymous mutations" (these include both missense and nonsense mutations). Non-synonymous mutations change the codon sequence of the protein. Synonymous (silent) mutations do not change the sequence of the protein and don't cause phenotypic changes.
  - The most common mutations associated with TMP resistance were in the bacterial gene folA, which encodes dihydrofolate reductase (DHFR), the protein that TMP normally inhibits. The mutations the researchers identified change DHFR so that TMP can no longer bind to and inhibit this enzyme.
  - Mutations that increased TMP resistance were often also associated with a decreased growth rate. The researchers observed that the resistant colonies often subsequently developed compensatory mutations that restored more typical growth rates.

- The scientists observed that sometimes the most highly resistant mutants were trapped behind more sensitive mutants and spatially constrained by them.
- Bacteria were unable to adapt directly from zero to the highest concentration of either drug, The researchers hypothesize that this is because the mutations that cause the greatest resistance to TMP often have a high impact on growth rate.

Big Question Discussion

This lesson should get students thinking about the Big Question "*What does it mean to observe?*" In particular, how does experimental design define or limit what you can observe? If you choose to delve into the Big Question, consider the following ideas:

- Ask the class "How did the scientists in this study design the experiment so that they could observe evolution?" Students should key in on: 1) the use of bacteria (fast growing), 2) the MEGA-plate (size, environment-antibiotics), and 3) video (progression over time). These factors defined the parameters and allowed them to observe changes in the population over time.
- As a summarizer, ask students "How do we know that existing variation in populations allows for evolution rather than the environment determining which mutations should arise? Design an experiment using bacteria and antibiotics to test this hypothesis."
- Review student responses and share how Esther and Joshua Lederberg used replica-plating to demonstrate that genetic variants pre-existed in a bacteria population prior to exposure to antibiotic. A summary of the Lederberg's experiment can be found here.

Answers

1. Think back to the video from the beginning of class about the *Contagion* billboard. Write down three observations about this billboard.

Sample answers: 1) The scale (size) was impressive. 2) The plate changed over time as different microbes started to grow on the media. 3) There was great diversity in the number and types of organisms that grew on the plate (students will describe this in terms of size, shape and color of colonies).

2. In your own words, describe why the evolution of resistance to antibiotics is a major problem facing society today.

Bacteria are rapidly evolving resistance to almost all of the antibiotics we know. Multiply antibiotic resistant strains are much more common and deaths from infections are rapidly rising worldwide.

3. The MEGA-plate contains bands of antibiotic at different concentrations. Why didn't the researchers use a set of normal Petri dishes, each containing a different concentration of antibiotic, for their experiments?

No, it wouldn't have worked as well because the large plate the gradient of antibiotic enables the researchers to observe the evolution of the bacteria over time as they move into new environments. If populations were just stated in Petri dishes at different concentrations, the environment stays fixed and evolution could not be observed in the same way.

4. **Connect to the Big Question**. What is the major advantage of studying evolution in bacteria instead of an organism like a human? (Hint: think about what it is possible to observe in each case).

1) Evolution happens across generations, not within an organism. 2) Bacteria replicate quickly, so we can observe evolution in a much shorter time frame because the time from one generation to the next is shorter.

5. The experiment described uses only one type of bacteria, *E. coli*. When grown on the MEGA-plate they all look the same. Are they? Explain your reasoning.

No. The bacteria were clearly different because they grew and divided at different rates and some were able to move into new concentrations of antibiotics and some weren't. There were clear differences among their genes that coded for different phenotypes when presented with different environmental conditions.

6. Many people think that mutations are always bad for the organism. Explain why that is not correct, using the bacteria used in MEGA-plate experiment as an example.

Mutations that enabled certain bacteria to survive in each antibiotic zone had a positive effect.

Observe **Figure 1** below and read the caption.

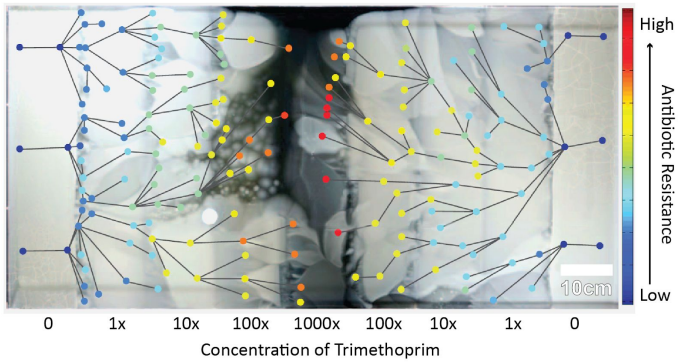

Figure 1. Evolution in the MEGA. Here we are looking down on the MEGA-plate from above 11 days (280 hours) after the start of the experiment. The concentration of the antibiotic trimethoprim increases toward the center of the MEGA plate. The left and right edges of the plate contain no antibiotic, and the center of the plate contains a high dose, 1000-times what would ordinarily prevent the growth of an *E. coli* bacterium. The dots represent colonies of *E. coli*. Each dot is colored based on that colony's level of resistance to trimethoprim. A dot that is dark blue means the colony in that location is not resistant at all, while dark red means the colony is very resistant. Lines connecting the dots indicate the movement of the bacteria across the plate. *Source:* Figure modified from Baym, *et al.* 2016.

Bacteria were introduced to the left and right edges of the plate, essentially performing the experiment twice at the same time. Look carefully at the branching structure depicting how evolution of resistance to trimethoprim occurred. Compare and contrast different pathways that lead to resistance.

7. Do all the bacteria grow at the same rate? Use evidence from the figure to support your conclusion.

No. Some of the patches of bacteria are different sizes and shapes, suggesting that some are growing faster than others.

To confirm their observations, the scientists created a MEGA-plate with a different antibiotic. Observe **Figure 2** below and read the caption.

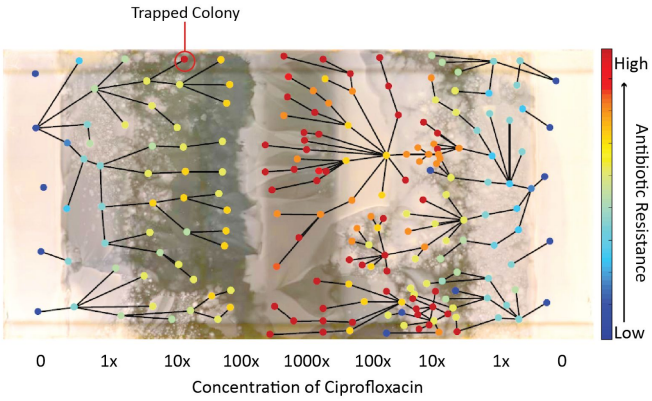

Figure 2. Evolution in the MEGA. Here we are looking down on the MEGA-plate from above after 14 days (340 hours) into the experiment. The set-up of the experiment is similar to the one in Figure 1, only instead of the antibiotic trimethoprim, the antibiotic ciprofloxacin is used. Source: Figure modified from Baym, *et al.* 2016.

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8. The results of this experiment are very different from those presented in Figure 1 with Trimethoprim. Notice that some of the colonies with the highest resistance (dark red) are "trapped" at the 10x antibiotic concentration and never make it to the middle of the plate.

a. What do you suppose is preventing them from moving?

The presence of surrounding bacteria may be blocking movement of the strains from migrating into the higher concentration zones. The scientists demonstrated that this was likely because if they moved those high resistance colonies to the leading edge of the inoculated bacteria, they were able to colonize high MIC zones.

b. Occasionally, bacteria evolve to having a lower antibiotic resistance level. How could that occur?

New mutations in the bacteria could arise that decrease the cells ability to survive in the presence of the antibiotic.

You are on the team of scientists studying the MEGA-plate. You take samples of antibiotic resistant bacteria from the MEGA-plate, and put them onto clean Petri dishes with no antibiotic. You observe that some of the growth rate of some bacteria that evolved antibiotic resistance has slowed down compared to the starting population. Confused by your results, another scientist says "You probably made a mistake and sampled the bacteria without resistance, because natural selection ensures that only the fittest and fastest bacteria survive. Therefore the bacteria that evolved resistance must grow more quickly than the bacteria that didn't."

9. Think about what you have just read.

a. What is wrong with this claim made by the other scientist?

In evolution, "fit" does not mean the same thing as when we say, "that athlete is very fit." The fittest individuals in a certain environment are not necessarily the strongest or the fastest, and fitness is relative and depends upon the environment that the bacteria are in.

b. If the bacteria that evolved resistance grew at a slower rate, then why did they survive?

These bacteria contained a mutation that made them less susceptible to the antibiotic.They were able to grow in the presence of antibiotic while the original (non-resistant) bacteria could not.

c. Based on the evidence from the study, and your understanding of evolution, which of the following claims do you support? Explain your reasoning.

> **Claim 1:** Bacteria with a higher resistance to antibiotics are better than bacteria with a lower resistance to antibiotics in any environment.
>
> **Claim 2:** Bacteria with a higher resistance to antibiotics have an advantage in certain environments over bacterial with a lower resistance to antibiotics.

Sample answer: I support Claim 2. Any bacteria that gets to reproduce is successful. Where there is a higher concentration of the antibiotic, having higher resistance would be an advantage, but in other environments, like in an area where there is a low concentration of antibiotic, having high resistance might not give an advantage and may even give the bacteria a disadvantage, such as slower growth.

10. In the video, the scientist says the following: “When the mutants reach the next boundary, they too have to pause and develop new mutations.” What is wrong with this statement? How would you have said it differently?

Sample answer: Evolution does not happen because the organism wants or needs it to happen. The mutations are already present from the beginning, but it takes time for the single bacteria that contain the mutation to grow and become visible. I would have said “When the mutants reach the next boundary, only those that contain mutations that allow them to grow in the higher antibiotic concentration survive. It looks as though the bacteria pause because the single bacteria must grow and replicate to become visible.”