Evolution of Bacterial Resistance to Antibiotics

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Abstract

Groups of students first conduct a prescribed hands-on simulation of the effects of partial in comparison to complete antibiotic treatments on the evolution of resistance in bacteria. Secondly, students develop their own hypothesis concerning the evolution of antibiotic resistant bacteria and design and conduct a simulation to evaluate their hypothesis. Both simulations use a variety of common implements (e.g. forceps, pliers, spoons) to represent antibiotics with specific antibiotic properties and use a variety of household objects (marbles, beads, bolts, blocks) to represent bacteria with variable resistance to different antibiotics.

Instructor's Introduction

This exercise includes two simulation experiments. In the first experiment, students are given a hypothesis and they are given explicit methods. They must determine the predicted result of the hypothesis, conduct the simulation, collect the observed results, and use their results to evaluate the hypothesis. In this first simulation, students compare the evolutionary consequences of a series of four partial treatments with an antibiotic to one full treatment. In the second simulation, each student group proposes their own hypothesis, and designs and conducts a simulation in order to evaluate their hypothesis. The first experiment provides them with a sense of the limits and the potential of this type of simulation, and by doing so, prepares them to design a simulation to test their own hypothesis.

In addition to developing students' understanding of the use of simulation experiments, this exercise allows students to work with important components of evolutionary theory; it also provides a compelling example of the relevance of the study of evolution. The simulations will help students distinguish the interacting roles of environmental selection pressures, an individual's fitness, and the evolution of a population.

The purpose of this exercise is to improve students' understanding of the following concepts:

Scientific Process, specifically hypothesis formation, design of simulation experiments, elucidation of predicted results of the hypothesis, forming a conclusion, and consideration their work in light of scientific literature to develop the implications of their conclusions.

Adaptive Evolution in Response to Natural Selection, specifically the interaction between individuals and environmental selection pressures, the role of heritable variation among individuals, and the evolution of populations.

This experiment is designed for a non-majors introductory biology course. Once the items have been purchased, the experiment can be set up in ½ hour. Because it requires so little prep work, it can be held in reserve in case a problem arises with another exercise (e.g. a shipment of living organisms doesn't arrive on time). If students are given the opportunity to develop, evaluate, and discuss their own hypotheses in the second experiment the activity takes 2 ½ hours.

Student Outline

The widespread use of antibiotics to fight bacterial pathogens has saved millions of lives in the past 60 years. Traditionally, the term **antibiotic** refers to naturally produced substances, often derived from fungi, that can be used to kill bacterial pathogens. More recently, many of these substances are chemically enhanced; and so antibiotic more generally refers to any medicine that kills bacterial pathogens. However, the ability of antibiotics to kill populations of bacterial pathogens is declining due to the evolution of bacterial resistance. This evolution of resistance to antibiotics, which is primarily due to their misuse, threatens us with uncontrolled outbreaks of infectious diseases.

Over at least the last eight years, there have been multiple unsuccessful attempts to pass laws in the United States that address the problem of bacterial resistance to antibiotics. Currently, three different bills have been introduced to Congress. The most comprehensive of these is the Strategies to Address Antimicrobial Resistance Act sponsored by Rep. Jim Matheson of Utah. It states that the 6 most common forms of resistant bacteria are costing hospitals in the U.S. $1.9 billion per year. This increased cost is passed on to patients and is reflected in skyrocketing health insurance

premiums. Increased resistance of bacterial pathogens to antibiotics also results in prolonged suffering of patients, as they wait for an antibiotic to begin to help them fight off an infection. The Strategies to Address Antimicrobial Resistance Act calls for the establishment of an Antimicrobial Resistance Advisory Board, a Task Force, and the development of at least 10 Surveillance and Research Network sites. This serious public health issue will be more effectively addressed as medical professionals, farmers, and the general public become more cognizant of the principles of evolution in response to natural selection. Additionally, understanding, and therefore taking more seriously, recommendations on the appropriate use of antibiotics involves learning about the unique, and subtle, methods by which microbes transfer bits of genetic information—including genes that infer resistance to antibiotics.

If there is variation among individuals in a trait that influences their ability to survive and reproduce, **natural selection** favors individuals with more adaptive versions of the trait. If this adaptive characteristic is genetically based, natural selection can result in **evolution**—a change in the frequency of a heritable trait as it passes from one generation to the next. In many microbial populations, individual bacterial cells differ in their resistance to particular antibiotics. This resistance is often a function of proteins that have been coded for by bacterial DNA. Therefore, variation in resistance to antibiotics is heritable and can evolve in response to natural selection. Also, notice that evolution in response to natural selection is a response of a population to an interaction between individuals and their environment; if the particular antibiotic is not present in the environment, resistance to it does not increase bacterial survival and reproduction, and so the frequency of resistant individuals does not increase in the population.

The evolution of bacteria resistant to antibiotics depends on genetic variation between individual bacteria that influences their level of resistance. New alleles are ultimately generated through mutation. Sexual reproduction recombines alleles existing in the parents to produce offspring with unique genotypes. Bacteria do not have sex. They reproduce through binary fission that divides a bacterial cell to produce two offspring that are genetically identical to the parental cell and to each other. However bacteria can generate new alleles, such as an allele associated with resistance to antibiotics through mutations that occur during binary fission. Mutations occur approximately once every 250,000 bacteria cell divisions. However, because of their rapid generation time (20 minutes under ideal conditions) and their enormous population sizes (a bacterial colony in a Petri dish contains approximately 1 million individuals) bacterial populations are constantly generating genetic diversity which can then be acted upon by natural selection to result in evolution. Unlike organisms composed of eukaryotic cells, bacteria can also move small amounts of DNA from one individual to another through horizontal gene transfer. This is not a form a reproduction; it is simply movement of DNA between individuals of the same species in a bacterial population, or even between individuals of different species in a bacterial community.

There are two general concepts that underlie many specific policy recommendations aimed at reducing the evolution of resistance to antibiotics. First, many bacterial species are necessary, and often beneficial, components of microbial ecology. For example, bacteria that live on our skin, as well as in mucous membranes and intestinal tracts, are a part of our initial line of defense against infection by harmful pathogens. Antibiotics should be judiciously used to target virulent pathogenic bacteria, not to futilely attempt to sterilize our external and internal environment. Secondly, resistant genes move rapidly between microbial populations due to horizontal gene transfer, and so, the prevalence of resistant bacteria is a community and environmental characteristic. For example, individuals working in or even simply living near farms, daycare centers, and hospitals that are using

large quantities of antibiotics have been found to harbor increased levels of resistant bacteria (Levy 1998).

There are a variety of public health policy, agricultural practices, and individual behaviors that can inhibit the evolution of bacterial pathogens resistant to antibiotics and that can therefore reduce the cost of health care and reduce patient suffering while doctors search for an effective antibiotic to knock down their illness, and that can save lives. This exercise is designed to help you understand the biological theory behind the following recommendations (Johnson 2001).

- When using an antibiotic, complete the full treatment, even after symptoms lessen (unless there are complications).
- As a patient, do not request, and as a doctor, do not prescribe, antibiotics in response to a cold, flu, or other viral illnesses. This simply increases selection for resistant bacteria and does not affect the virus.
- Use antibiotic ointments on cuts and abrasions, but avoid antimicrobial hand creams, soaps, and laundry detergents.
- Reduce the use of antibiotics in cattle feed and as sprays on fruit trees.

Experiment One: many partial or one complete antibiotic treatment

Student groups will use various tools, such as forceps and spoons, to represent antibiotics. You will use these ‘‘antibiotics’’ to grab objects representing bacteria, a set of objects of a variety of shapes and colors. Each antibiotic will easily pick up some bacteria; other bacteria will be more resistant, harder to pick up. The objects will be sitting on trays; each tray represents an individual host.

In the first stage of the simulation, you will address the questions: Why should individuals continue an antibiotic treatment after their symptoms are gone? Isn’t this simply over-using antibiotics and selecting for resistance to antibiotics? You will address these questions by testing the following hypothesis. Initially, the use of an antibiotic kills off the most susceptible bacteria and eventually the density of the bacterial pathogen is low enough that disease symptoms begin to disappear. However, the remaining few bacteria are somewhat resistant to the antibiotic, and if treatment is not continued until they are also killed off, they will form a new, more resistant, population.

The independent variable will be the timing of antibiotic treatments; the dependent variable will be the final frequency of resistant bacteria in the individual. Read through the following methods and, before you begin, record the predicted result of the hypothesis.

Methods

Work with a group of 3 or 4 students during this experiment. One member of your group, or your instructor, will be the timer. The remaining members of your group will be antibiotics, rapidly trying to kill off bacteria. In the first stage of this experiment, you will simulate the behavior of someone who takes an antibiotic until the symptoms disappear and the saves the rest until next time they get sick, and then takes them again. You will work through 4 cycles of illness and periods of feeling fine.

1**.** Select 25 each of four different strains of bacteria (a total of 100 bacteria) and record each type in Table 1.

Table 1. Results of Short treatment Simulation

	Bacterial Strains				
Descriptions of bacterial strains (shape, color, size)					Total
Initial Population	25	25	25	25	100
Number of each type surviving after Antibiotic treatment 1					
Percent of total survivors composed of each type after treatment 1					100
Number of each type added during reproduction					
Number of each type surviving after Antibiotic treatment 2					
Percent of total survivors composed of each type after treatment 2					100
Number of each type added during reproduction					
Number of each type surviving after Antibiotic treatment 3					
Percent of total survivors composed of each type after treatment 3					100
Number of each type added during reproduction					
Number of each type surviving after Antibiotic treatment 4					
Percent of total survivors composed of each type after treatment 4					100

2. Scatter the bacteria into the individual.

3. Each member of your antibiotic group should use the same type of antibiotic, the same type of tool. When the timer says ‘‘Go!,’’ use the tool to pick up bacteria as quickly as you can and place them in a cup. After 15 seconds your timer will announce ‘‘Stop!’’ What member of your group is the most effective antibiotic?

4. Count the number of each type of bacteria remaining in the individual after this antibiotic treatment and record them in Table 1.

5. Calculate the percent of the remaining population composed of each type of bacteria and record your results in Table 1.
6. Assume that now that the antibiotic treatment has been stopped (because bacterial populations were low enough to reduce symptoms), the bacteria can reproduce offspring similar to themselves to bring the population back up to a total of 100. For example, if eight of the remaining bacteria were round, and if this represents 28% of the remaining population, you would sprinkle 20 more round bacteria into the tub (for a total of 28 round bacteria). You would reproduce each bacterial type in this fashion, and you should end up with a total of 100 bacteria in the individual.
7**.** Repeat Steps three through six until you have filled Table 1.

You have now completed the first stage of the experiment by simulating someone who is repeatedly taking short treatments of an antibiotic. You will now simulate someone who uses the same amount of antibiotic for the entire duration of one complete treatment, rather than four partial treatments.
1**.** Select 25 of each of the same four strains of bacteria you used in the first treatment; record the description of each type in Table 2.
2**.** Scatter the bacteria into the individual.
3. Each member of your antibiotic group should pick the same type of antibiotic as in the last treatment. Last time you killed bacteria in four 15-second intervals; this time you will kill bacteria in a single 1-minute interval. Once again, your TA will be the timer for this 1 minute treatment with antibiotics.
4. Count the number of each type of bacteria remaining after this antibiotic treatment and record them in Table 2.
5**.** Calculate the percent of the remaining population composed of each type of bacteria and record your results in Table 2.

Table 2. Results of Full Treatment Simulation

	Bacterial Strains				
Descriptions of bacterial strains (shape, color, size)					Total
Initial Population	25	25	25	25	100
Number of each type surviving after antibiotic treatment					
Percent of total survivors composed of each type after treatment					100

Experiment Two: developing and evaluating your own hypothesis

In this section, your group will propose a hypothesis, design an experiment, deduce the prediction of the hypothesis, and conduct your experiment. Consider the following information as your group brainstorms ideas about antibiotics that you could formalize into a hypothesis and test with a simulation experiment. What other variables influence the evolution of bacterial resistance to antibiotics that your group could evaluate with this type of simulation experiment?

Levy (1998) coined the phrase ‘‘My resistance is your resistance’’ to emphasize the importance of managing resistance at a community and environmental level, rather than simply at the level of individual patients. This concept has been supported by observations of the movement of resistant bacteria from one individual to another in hospitals, daycare centers, and even between species on farms.

Immediately after an antibiotic treatment, individuals are susceptible to infection by opportunistic bacteria, because their resident bacterial population densities are low. This is one reason individuals receiving treatments in hospitals often acquire new infections.

In addition to reducing the evolution of resistant bacteria by avoiding unnecessary use of antibiotics and by encouraging correct use of antibiotics, individuals and populations can consider using more than one type of antibiotic. Resistance to one type of antibiotic often does not provide the bacteria with resistance to a different antibiotic. If you were in charge of a department in a hospital, how could you use this concept to reduce the spread of resistant bacterial pathogens?

List factors that may influence the evolution of resistance and that you could use as an independent variable in a simulation experiment.

1.

2.

3.

4.

5.

After your group has chosen the factor that you find the most interesting, formalize your statement proposing the effect of this factor on resistance into a hypothesis. Remember, hypotheses are written as tentative proposals, not as questions.

Describe, in detail, your group’s plan for conducting the experiment. Specifically describe how you plan to manipulate the independent variable and how you plan to measure the dependent variable.

Manipulation of Independent Variable:

Measurement of Dependent Variable:

The next step in your experiment is to describe the result that should occur if the hypothesis is true, and if the experiment is fairly conducted.

Before conducting your experiment, ask your instructor to check your proposed hypothesis, methods, and prediction. After your experiment is approved, draw a results table in the space below that matches the structure of your experiment. Conduct your experiment and record your results.

Table 3. Results of Your Group's Experiment

Discussion Questions

1. Which type of bacteria was most resistant in your first experiment? How did the final percent of this resistant bacteria compare when antibiotics were applied in four short treatments versus one full treatment?

2. Describe the methods of your second experiment.

3. Write a discussion of your second experiment. In this discussion, you should explain your hypothesis and why it is important. Also, explain how your hypothesis led to a prediction. Use a comparison of predicted and observed results to evaluate the hypothesis. If you had problems with the methods of your experiment, discuss whether or not they could have influenced your conclusion. Compare your conclusion to information that is presented in magazines and journals. You should refer to least two articles in this discussion and list them in a References section.

Materials

In our laboratories students work in six groups with three to four students in each group. Each group uses the following materials.

- Plastic Tub approximately 10 cm deep, 1 m long, and 0.5 m wide. The dimensions of the tub can be determined by what is available. It simply needs to be large enough for three students to reach into as they are using "antibiotics" to grab "bacteria".
- Stop Watch
- Variety of Tools to be used to represent "antibiotics". Ideally, there are enough of each tool so that all students could be using the same tool, and enough different types of tools (approximately six) to provide groups with choices when they are using different tools. We have used spoons of various sizes, forceps of various shapes and sizes, clamps that are designed to hold test tubes, cloths pins, and different types of pliers. Use your imagination and what is available around your department or at a local store.
- Variety of Objects to be used to represent "bacteria". Each group should have at least 100 of at least four different types. Look for a variety of objects that will differ in which tools can be used to readily pick them up, such as: small beads, large beads, round beads, square beads, and paper clips. Look for objects that will differ in which tools are most efficient at picking them up.
- Plastic Beakers Each student will need a plastic beaker to hold the "bacteria" that they collect.
- Simple Calculators. Each student group will need at least one.

Notes for the Instructor

Instructors need to strike a balance between encouraging students to competitively play the simulations and encouraging students to realize that the simulation are simply a tool being used to help them understand some serious biology. If students are not engaged in the competition of grabbing objects as quickly as possible, if they are slowly picking up objects in a disinterested fashion, then the match between the shape of the tool and the shape of the object may have no effect on the rate at which the objects are picked up. So, let them have some fun, encourage them to compete. On the other hand, students also need to be engaged in the more serious topic of bacterial resistance to antibiotics. If this topic is well covered in lecture prior to the laboratory activity, they should understand the importance of the topic; if not, laboratory instructors may need to precede the activity with a discussion of the importance of bacterial resistance to antibiotics.

We provide our laboratory instructors with the following background literature (Austin *et al.* 1999, Bonhoeffer 1997, Lawson 2008, Normark and Normark 2002, Novick 2008, Olson 2000, Steinman *et al.* 2003, Taylor and Leitman 2002, Weinstein 2001).

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About the Authors

Amy Marion earned her B.S. at Marywood College (now Marywood University) where she conducted her senior thesis on the transformation of *Bacillus*. At University of Vermont, she earned her Ph.D. conducting research in fungal molecular genetics. After moving to New Mexico, Dr. Marion taught at the Albuquerque Technical Vocational Institute and at the University of New Mexico. She now works in the New Mexico State University Department of Biology as Laboratory Coordinator and Director of the Biology Advising Center.

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