

Why Finish Your Antibiotics ?

Introduction:

Antibiotics and antimicrobials are molecules that kill bacteria. Since the advent of antibiotics in the 1930s, they have become a commonly-used therapy in the treatments of infectious diseases caused by bacteria. There are dozens of different antimicrobials (e.g., penicillin, streptomycin, methicillin, vancomycin) and their use has saved millions of lives.

However, strains of many bacteria have evolved resistance to antibiotics. Doses of antibiotic that used to be strong enough to eliminate a bacterial infection are no longer strong enough.

In 1941, a treatment of 40,000 units of penicillin per day of penicillin was enough to cure pneumonia, now, 24 million units per day may not be enough.

In 1941 all strains of *Staphylococcus aureus* could be treated with penicillin. Now, 95% of the *S. aureus* strains are resistant to penicillin. (Freeman, 2005)

The Center for Disease Control (CDC) has identified the antibiotic resistance of bacteria as “one of the key microbial threats to health in the United States.” They have created an education campaign called “Get Smart: Know When Antibiotics Work.” One of the goals of this campaign is to “decrease the inappropriate use of antimicrobials.” In this campaign, both health care providers and users are being educated about the proper and improper use of antimicrobials, and about how resistance arises in a population of bacteria.

In this lab we will model changes in a population of bacteria over time, as it is subjected to various regimens of antibiotic therapy. The model population contains four strains with varying degrees of resistance to a particular antibiotic.

Methods: We will model the survival, death, and reproduction of one species of bacteria, which has four different strains. The different colors of beans will symbolize the different strains of bacteria.

Each bacterial strain differs from the others in its ability to survive in the presence of a certain antibiotic, i.e. some strains will be more susceptible to the antibiotic than others. Strain 1 will be susceptible on the first day of treatment and each day thereafter, Strain 2 on the second day and each day thereafter, and so forth. (The term ‘strain’ is used with bacteria the same way ‘breed’ is used in dogs, or ‘variety’ is used with apples. All of these terms refer to particular genetic variants within the same species.)

Each group of students will be assigned one of these regimens to follow.

- A. Group A follows doctor’s orders exactly, i.e. takes the antibiotics every day.
- B. Group B stops taking antibiotics early, on the 5th day. (Rationale is that a patient might start feeling better, and stop taking the antibiotics to save the rest for a later date.)
- C. Group C skips the 3rd day of treatment. To make things easy, we are going to assume that the antibiotic concentration falls to zero on this day, so all strains will double. The next day, after the patient begins taking antibiotics again, only Strain 1 is susceptible. Two days after the skipped day, Strain 2 is susceptible, etc. (In other words, on the 4th day, only Strain 1 is susceptible; the next day Strain 2 becomes susceptible again, etc.)
- D. Group D skips the 5th day of treatment. Similar to the directions for Group C, except skip the 5th day, rather than the 3rd day.

Set up your beginning population like this: (each 'cell' represented by 1 bean)

Strain 1: 40 cells Strain 2: 30 cells Strain 3: 20 cells Strain 4: 10 cells.

(In reality, it would take more than 100 cells of bacteria to cause symptoms of a disease; you might think of each bean as representative of 100 million cells, but you do not have to count out 100 million beans)

You will record the population at the end of each day, as the result of the action of both the immune system and the antibiotic therapy.

For each 'day':

1. First model the action of the immune system. All strains are equally susceptible to the actions of the immune system, so remove 10% of the **total** population. To do this, calculate what 10% of the current total population is, then randomly remove that number of beans. (Randomly, because each Strain is as likely to die from the action of the immune system as the next. This may best be done by having someone close their eyes and pick beans from the total mixture.) Do this until 10% of the population has been removed.
2. Second: If the particular bacterial strain is susceptible on this day, remove half of its population. (I.e., the antibiotic is killing half of the bacteria of that strain.) (Remember, Strain 1 is susceptible on Day 1 and each day thereafter, Strain 2 on Day 2 and thereafter, etc. – unless therapy is stopped, in which case, the body clears of antibiotic in our model, and the "susceptibility clock" begins again.)

For example, on Day 1 Strain 1 would be susceptible and its population would be halved.

3. If the particular bacterial strain is not susceptible, double the population of that strain. (Use the extra beans in the extra cups to get new members of the population) On Day 1, the populations of Strains 2, 3, and 4, would each be doubled. On Day 2, only Strains 3 and 4 would be doubled, while both Strains 1 and 2 would be halved.

The actions in Steps 2 and 3 mimic what may happen in the body. For example, Strain 3 may not be killed until the concentration of the antibiotic is relatively high, and perhaps that concentration is not reach until Day 3.

4. Record the population for each strain and the total population on the data sheet (Table 1) after taking Steps 1, 2, and 3.
5. Repeat Steps 1 – 4 for each day, until the population of all strains has fallen to zero, or at the permission of your instructor. (The number of days will vary, depending on which regimen you are following.)

Results:

Once you have your data, you will graph the data in two ways:

On one graph, graph the total population of bacteria for each day of the treatment.

On another graph, graph the frequency (in percent) of the population of each different strain on each day.

Conclusion and Discussion: On another sheet of paper, write the answers to these questions.

1. What happened to the gene frequencies of each strain over the course of the “End Treatment Early” regimen ?
2. Did the population of bacteria change between the beginning and the end of the “End Treatment Early” regimen ? If it did, explain how it changed.
3. Does this model show evolution occurring between the beginning and end of the “End Treatment Early” regimen? Explain your answer.
4. Did selection occur in the “End Treatment Early” regimen?
5. Could selection occur if all the cells were Strain 4 ? Defend your answer.
6. Which Strain became most common in the “End Treatment Early” regime?
7. Did this model show evolution occurring in any of the other regimens?
8. A mutation is a change in the hereditary material of a cell. Did Strain 4 mutate and become resistant in response to the antibiotic ?
9. An organism is immune to an infectious disease when it has the ability to destroy the pathogen that causes the disease. For example, a person immune to measles has an immune system that recognizes and destroys the virus that causes measles. Did any of the modeled patients under the four regimens become immune to antibiotics ? If so, which ones ? Explain.

Bibliography:

Center for Disease Control and Prevention, Department of Health and Human Services. Get Smart, Know When Antibiotics Work. <<http://www.cdc.gov/drugresistance/community>> Accessed Feb. 12, 2007.

Food and Drug Administration, Center for Veterinary Medicine. Animation of Antimicrobial Resistance. < <http://www.fda.gov/cvm/antiresistvideo.htm> > Available: January 11, 2007.

Freeman, Scott. 2005. Biological Science, 2nd Edition. Pearson/Prentice Hall. Antibiotics and the Development of Drug Resistance, p. 604.

Acknowledgements:

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Table 1: Population of 4 Strains of a species of bacterium

In Table 1, use the space above each box's diagonal to record the Strain's population after the immune system has killed some of the bacteria. (Refer to Step 1, on page 2)

Use the space under each box's diagonal to record the population after it has either doubled or halved, depending on whether or not it is susceptible on that day. (Refer to Step 2 and 3, on page 2)

	Strain 1	Strain 2	Strain 3	Strain 4	Total
Begin	40	30	20	10	100
Day 1					
Day 2					
Day 3					
Day 4					
Day 5					
Day 6					
Day 7					
Day 8					
Day 9					
Day 10					
Day 11					
Day 12					
Day 13					
Day 14					



