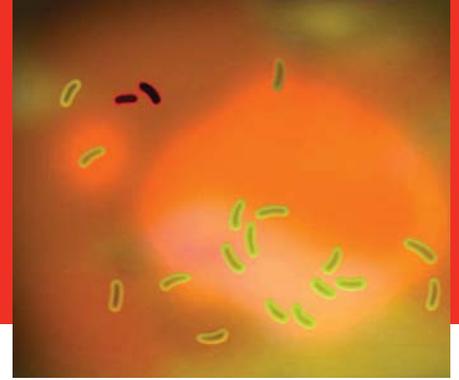# Rise of the Superbugs

For half a century, antibiotics have given us a powerful way to treat infections that once were life threatening. Yet, the growing number of antibiotic-resistant bacteria is putting this golden era of medicine at risk. Now, we find ourselves in a race to prevent bacterial infections from once again becoming one of humanity's major killers.

ACTIVITY AT A GLANCE

PURPOSE: To show how populations of bacteria become resistant to antibiotics via the process of natural selection and human misuse

OVERVIEW: Students follow the story of a teen who develops an infection related to injuries resulting from a bicycle accident. They analyze graphs of cultures that doctors grew to track how the infecting bacteria responded to antibiotics. The graphs show that, over six days, the teen's population of bacteria becomes increasingly resistant to two antibiotics. Students deduce that the teen picked up some bacteria resistant to the initial dose of antibiotics at the hospital. Those bacteria then grew to dangerous levels. Students conclude the activity by making a recommendation regarding the next course of action.

LEVEL: Grades 9–12

TIME: 2 class periods

CORE CONCEPTS

- Bacteria can be resistant to different kinds of antibiotics.

- After being exposed to antibiotics, a population of bacteria can become resistant to antibiotics through the process of natural selection.

MATERIALS

- Student sheet for each student
- Student sheet for Going Further activity (optional)

STANDARDS CONNECTION

Life Science

- 5–8: Reproduction and Heredity; Populations and Ecosystems; Diversity and Adaptations of Organisms
- 9–12: The Cell; Biological Evolution; and Matter, Energy, and Organization in Living Systems

Health

- Standard 1: Comprehend concepts related to health promotion and disease prevention.
- Standard 3: Practice health-enhancing behaviors and reduce health risks.
- Standard 4: Analyze the influence of culture, technology, and other factors on health.

PROGRAM CONNECTION

Before the advent of antibiotics, bacterial infections could be life threatening. With the discovery of antibiotics and the development of methods to mass-produce them, many bacterial infections became easily treatable. But the bacteria have fought back. Any population of organisms faced with a challenge to its survival has the potential to adapt via the process of natural selection. For example, many insects have become resistant to insecticides and many

continued

weeds have become resistant to herbicides. Similarly, some types of bacteria have responded to the increasing presence of antibiotics by becoming resistant to them. So bacterial infections may once again become life threatening.

This program follows the case of an American teenager and his doctor battling against an antibiotic-resistant bacterial infection. Antibiotic resistance is then placed in a public health context by examining the large-scale fight against antibiotic-resistant tuberculosis infection in Peru. The program points out that even when treatments are available, the delivery of those treatments presents yet another set of challenges.

This activity examines one process by which strains of antibiotic-resistant bacteria can arise.

BEFORE WATCHING

- What is an antibiotic? What types of diseases do they treat? Have you ever taken antibiotics? If so, which kind(s)?

  An antibiotic is a substance that controls the growth of bacteria, either by killing them or inhibiting their ability to reproduce. They are used to treat bacterial infections, such as bacterial pneumonia, staph, and ear infections. Examples include bacitracin, cephradine, ciprofloxacin (cipro), erythromycin, nystatin, penicillin, and tetracycline.

- What do you think it means for a disease to be resistant to a drug, such as an antibiotic? List some issues related to treating a disease caused by bacteria that are resistant to antibiotics.

  Issues include: Not being able to control the infection, putting the patient at serious risk; giving resistant bacteria the opportunity to pass their resistance on to other bacteria in the patient's body; and an increased risk of spreading the resistant bacteria to others in the community.

FOR MORE INFORMATION

pbs.org/rxforsurvival

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AFTER WATCHING

- How is the emergence of antibiotic-resistant bacteria an example of natural selection?

  This question is explained in the answer to student sheet question 11.

- Discuss what our lives would be like if most bacteria adapted to the presence of antibiotics and became resistant to them.

- Ask why antibiotic-resistant bacteria are such a big problem in hospitals.

  Hospitals treat sick people. As a result, they have a larger number and diversity of disease-causing microorganisms than is typically found in the community. Furthermore, hospitals regularly use medicines that kill disease-causing microorganisms, creating an environment that favors microbes resistant to these medicines. Finally, while the skin is an excellent barrier against microorganisms, it is often broken in hospitals—many patients arrive with open wounds, and it is often punctured by needles and cut with medical implements as well.

- Discuss how human behavior helps resistant strains of bacteria arise. Ask why the overuse and misuse of antibiotics is risky, both for those who overuse or misuse them and for the overall health of the public.

PROCEDURE

1. Distribute the student sheet. Have students read about the teen with the antibiotic-resistant infection on the student sheet and answer question 1.

   It is helpful to assign this reading prior to class and to review the concept of natural selection.

2. Have students work individually, in pairs, in teams, or as a class to complete the student sheet.

   Students take on the role of epidemiologists, identifying antibiotic-resistant infections and recommending the best course of treatment. To do this, they analyze

graphs showing the rate of growth of Eva's bacteria, which were collected at different times during the course of the infection. See the Assessment section for answers to the questions and for key discussion points.

In steps 2–11, students analyze the graphs showing the growth of Eva's population of bacteria, develop an explanation of how her antibiotic resistance developed, and recommend a treatment strategy. A complete explanation should mention that the graphs show when the bacteria acquired resistance to antibiotics A and C and how the proportion of antibiotic-resistant and non-resistant bacteria change over the six days. Students should recommend that Eva be treated with Antibiotic B. The following explanation summarizes the key points.

- *Figure 4 shows that the population of Eva's bacteria had no initial resistance to Antibiotics A, B, or C. Therefore, she acquired her resistance in the hospital.*

- *Eva became infected with bacteria resistant to Antibiotic A during her visit to the hospital on Monday afternoon. Figure 5 shows that the population declines as Antibiotic A kills the susceptible bacteria. But the population of bacteria resistant to Antibiotic A increases in the test tube. By hour nine, their numbers increase dramatically.*

- *Tuesday morning, Eva took the left-over Antibiotic A. This dose killed the remaining bacteria susceptible to Antibiotic A, leaving only a population of bacteria resistant to Antibiotic A. The graph line in Figure 6 shows that, by Tuesday evening, the population of bacteria was completely unaffected by Antibiotic A.*

- *Figure 4 shows that, initially, a few bacteria in the population of Eva's bacteria are resistant to Antibiotic D. Since Eva never takes Antibiotic D, the proportion of these resistant bacteria does not change over the week—they have no particular advantage over the unresistant bacteria. Figures 4–8 show Eva's Antibiotic D-resistant population is the same each time, increasing only slightly after being grown in a test tube for 24 hours.*

- *Figures 6 and 7 show Eva's population of bacteria becoming resistant to Antibiotic C. Since there were no Antibiotic C-resistant bacteria on Tuesday evening, Eva must have acquired them after visiting the hospital on Tuesday evening. Eva started taking Antibiotic C Thursday. By Saturday, Eva's bacterial population is mostly Antibiotic C-resistant because nearly all of the Antibiotic C-susceptible bacteria have been killed.*

GOING FURTHER

Have students analyze the provided electrophoresis gel photograph and identify the points at which Eva's bacteria acquired antibiotic-resistant genes. The handout entitled Identify the Genes Carrying Antibiotic Resistance steps students through this analysis.

Bacteria have both chromosomal DNA and plasmids, which are circular units of DNA. Plasmids can become incorporated into the chromosomal DNA or remain independent. Some genes that confer antibiotic resistance are carried in plasmids. Bacteria in a population can exchange plasmids. This exchange can transfer antibiotic-resistant genes between bacteria, including bacteria of different species. Before beginning this Going Further activity, you may want to review plasmids, restriction enzymes, and gel electrophoresis.

ASSESSMENT

Students' responses to the questions on the student sheet should incorporate the points discussed in the answers (included in this section). In addition, consider the following when assessing student work:

- Supported the team by contributing to the discussion, listening to others' ideas, working together to analyze the graphs, and helping the team develop a consensus.

- Understood how data are presented on a graph and interpreted graphs correctly.

- Could articulate when the bacteria acquired resistance to antibiotics A and C and how the proportion of antibiotic-resistant and non-resistant bacteria changed over the six days.

- Wrote thoughtful responses to student sheet questions and supported conclusions with data presented in the graphs.

- Explained how the development of antibiotic resistant-bacteria illustrates the process of natural selection.

- Correctly recommended that doctors treat Eva with antibiotic B.

- Demonstrated an understanding of how our use of antibiotics promotes the development of antibiotic resistant-bacteria and that these bacteria pose a serious threat to global health.

1. (a) What might explain why Eva's infection is not responding to treatment by antibiotics?

Some bacteria may be resistant to the antibiotics.

(b) What information about the infection would you want in order to find a way to treat it?

It would be good to understand what kinds of bacteria are present and whether they are resistant to antibiotics.

3. Explain how the graphs in Figures 1–3 describe the bacterial growth over the 24 hours.

No antibiotics: *The number of bacteria increases quickly.*

Antibiotics and susceptible bacteria: *The number of bacteria declines quickly as the antibiotic kills them.*

Antibiotics and some resistant bacteria: *First, the number of bacteria falls as the antibiotic kills the susceptible bacteria. Then, because the resistant bacteria have been reproducing, a point is reached where the number of susceptible bacteria being killed equals the increase of the resistant ones. As the population of resistant bacteria continues to grow, the curve stops dropping and begins to rise.*

4. How will Dr. Hincapie use the three standard graphs?

These standard graphs show the pattern of growth in bacteria whose resistance to antibiotics is already known. Knowing these growth patterns, he can analyze the growth patterns from Eva's bacteria in order to identify antibiotic-resistant and antibiotic-susceptible bacteria.

5. Were antibiotic-resistant bacteria present in the tissue samples taken when Eva first arrived at the hospital on Monday? (Figure 4) How can you tell?

Yes. The slight rise starting around hour 12 shows that there were a small number of bacteria resistant to Antibiotic D in the population of Eva's bacteria when she first arrived.

6. By Saturday, which antibiotics were the bacteria resistant to?

Antibiotics A, C, and D.

7. At what point did the population of bacteria show resistance to:

Antibiotic A – *When Eva was at the hospital on Monday afternoon, she picked up some bacteria resistant to Antibiotic A. By taking the leftover antibiotics on Tuesday morning, she killed most of the bacteria susceptible to Antibiotic A, allowing those resistant to it to flourish. The population of resistant bacteria increased dramatically over the week.*

Antibiotic B – *No bacteria were resistant.*

Antibiotic C – *When she returned to the hospital on Thursday evening.*

Antibiotic D – *Eva had some bacteria resistant to Antibiotic D even before her accident.*

8. Explain the difference in the growth rates of bacteria grown in the presence of Antibiotic A on Monday versus on Tuesday.

Eva had no bacteria resistant to Antibiotic A on Monday afternoon. (Figure 4) Yet, after her visit to the hospital, Eva had acquired some bacteria resistant to Antibiotic A. Figure 5 shows the population of bacteria decreasing at first as the susceptible bacteria die, but then grows as the resistant bacteria increase in number. On Tuesday morning, Eva took some leftover Antibiotic A. Figure 6 shows that, by Tuesday, all the surviving bacteria are resistant to Antibiotic A. (Figures 7 and 8)

9. Explain the difference in growth rate of the bacteria resistant to Antibiotic C from Thursday to Saturday.

As of Tuesday, Eva has no bacteria resistant to Antibiotic C. However, Figure 7 shows that on Thursday, there are a few bacteria resistant to Antibiotic C—Eva must have picked some up when she visited the hospital on Tuesday. Figure 7 shows the population decreasing until hour 20, indicating that most bacteria are still susceptible to Antibiotic C. However, Figure 8 shows that by Saturday the proportion of Antibiotic C-resistant bacteria has increased. Since Thursday evening, Antibiotic C has killed most susceptible bacteria. Figure 8 shows the bacterial population decreasing at first, as the remaining susceptible bacteria are killed. Then the number of Antibiotic C-resistant bacteria increases rapidly.

10. How is the growth of the bacteria resistant to Antibiotics A, C, and D an example of natural selection?

Environmental conditions determine which individuals in a population are the fittest. In this case, the environment favored bacteria that were resistant to Antibiotics A, C, and D; all others were killed. Since only resistant bacteria continued to breed, soon the entire population derived from these initial bacteria and were resistant. Thus, the environment selected for resistant bacteria, illustrating the process of natural selection.

11. What advice about the next antibiotic to try can Dr. Hincapie give to Eva's doctors based on these results?

Treat Eva with Antibiotic B.

RELATED *RX FOR SURVIVAL* WEB SITE FEATURES (see pbs.org/rxforsurvival)

Why Global Health Matters: Learn why we should all be involved in global health initiatives.

Deadly Diseases: Learn about some of the diseases that are humanity's most feared killers.

Global Health Champions: Learn about men and women who have profoundly changed global health outcomes and saved lives in many parts of the world.

Get Involved: Find meaningful ways to take action.

Dispatches from the Field: Hear first-person accounts from people on the frontlines of health care.

LINKS

Alliance for the Prudent Use of Antibiotics
tufts.edu/med/apua
> Learn about the efforts to promote the responsible use of antibiotics at home and abroad.

Antibiotics: The Untold Story
prairiepublic.org/features/healthworks/antibiotics/index.htm
> Examine our dependence on antibiotics as the most common treatment for illness.

Evolving Ideas: Why Does Evolution Matter Now?
pbs.org/wgbh/evolution/library/11/2/e_s_6.html
> Relates how evolving bacterial resistance helps us understand disease treatment and prevention.

Evolution of Antibiotic Resistance
pbs.org/wgbh/evolution/library/10/4/l_104_03.html
> See resistant bacteria survive, divide, and multiply in this animated NOVA feature.

National Library of Medicine
nlm.nih.gov/medlineplus/antibiotics.html
> The NLM's Antibiotics page contains detailed information and recent articles on antibiotics.

BOOKS

Antibiotics: Actions, Origins, Resistance Christopher Walsh. Cambridge: Harvard University Press, 2003.
> Describes how antibiotics combat infection and disease at the molecular level.

The Coming Plague: Newly Emerging Diseases In a World Out of Balance Laurie Garrett. New York: Farrar, Straus & Giroux, 1994.
> Tracks diseases that travel the world, which are spreading faster and farther than ever before.

The Other End of the Microscope: The Bacteria Tell Their Own Story Elmer Koneman. Washington, DC: American Society for Microbiology Press, 2002.
> Tells the story of some bacteria upset at their continued mistreatment at the hands of humans.

Revenge Of The Microbes: How Bacterial Resistance is Undermining the Antibiotic Miracle Abigail A. Salyers, Dixie D. Whitt. Washington, DC: American Society for Microbiology Press, 2005.
> Details the consequences of turning one of our best weapons against disease into a powerful enemy.

Infections and Inequalities: The Modern Plagues Paul Farmer. Berkeley: University of California Press, 2001.
> Examines how economic disparities are often indicators of who will be treated and survive.
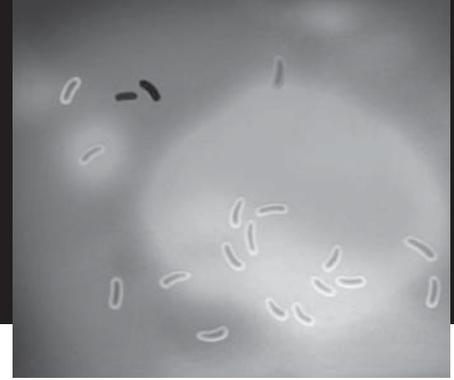

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Rise of the Superbugs

Stephen Schudlich ©WGBH Educational Foundation

On Monday, Eva went to the emergency room following a fall from her bike. Fortunately, her only broken bone was a finger. But she suffered scrapes and cuts, including some deep cuts on her legs. After spending several hours in the emergency room having her wounds cleaned, stitched, and bandaged, Eva returned home.

Tuesday morning, one of the deeper cuts on Eva's legs was red and felt warm. She had a few pills of an antibiotic left over from her bout with strep throat that previous winter. Thinking it might help to prevent infection, she took them according to the prescription instructions.

Throughout Tuesday, the cut on Eva's leg became increasingly red, swollen, and painful. Eva felt awful and returned to the hospital on Tuesday night. Her cut had become infected. The doctors cleaned and restitched her leg and prescribed a daily dose of Antibiotic A, a stronger version of the same antibiotic Eva had taken at home just that morning.

By Thursday, Eva's infection had spread to the point where it was too painful to walk. In addition, Eva felt ill. She returned to the hospital and this time was admitted. The doctors immediately administered a different kind of antibiotic, Antibiotic C, directly into Eva's bloodstream through an intravenous tube.

Friday, Eva felt better, and her leg became less painful and swollen. But on Saturday, it was clear that Eva had taken a turn for the worse. The infection on her leg continued to spread, and she had become feverish. The medical staff involved with Eva's case held a meeting to plan the next steps in Eva's treatment.

1. Answer the following questions:

   (a) What might explain why Eva's infection is not responding to treatment by antibiotics?

   _____

   _____

   (b) What information about the infection would you want in order to find a way to treat it?

   _____

   _____

   _____

DID YOU KNOW?

Streptococci, the bacteria that cause sore throats and tonsillitis, are usually present in the body. These bacteria cause no harm until the immune system is weakened in some way, such as by a virus or malnutrition.

2. Read the following story about a doctor who tested the bacteria causing Eva's infection.

Dr. Hincapie, a conscientious intern interested in sports injuries, had followed Eva's case since she arrived at the emergency room after her bike crash on Monday. On each visit, he had taken samples of fluid and tissue from Eva's wounds. He wanted to analyze them to see how the cells in her immune system changed as she healed.

As Eva's condition worsened, he realized that the samples he had collected might hold clues as to why her infection was not healing and what new treatments might work. Dr. Hincapie thought that bacteria that were resistant to the antibiotics she had been given might be causing Eva's worsening condition. To test this idea, he grew cultures of bacteria from each of Eva's visits. (**FIGURES 4–8**) Dr. Hincapie compared the resulting graphs to standard graphs. (**FIGURES 1–3**) To make a standard graph, researchers grow 10,000 (10^4) bacteria in a test tube under known conditions. They measure the growth over 24 hours and graph the results. The standard graphs Dr. Hincapie used in his comparisons are:
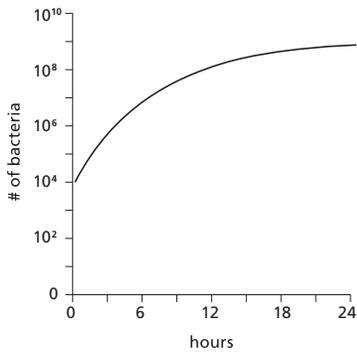

FIGURE 1. No antibiotics. *The test tube contains bacterial-growth media, which allows the bacteria used in testing to undergo unlimited growth.*
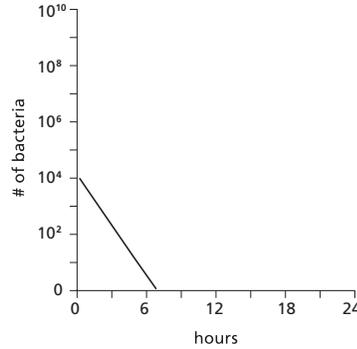
FIGURE 2. Antibiotics and susceptible bacteria. *The test tube contains bacterial growth media and antibiotics known to kill the bacteria used in the test.*
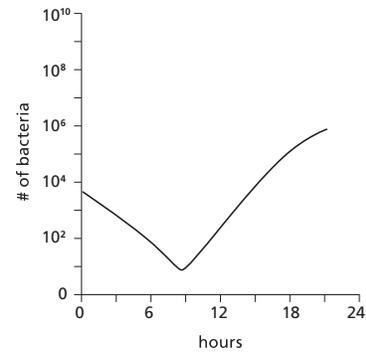
FIGURE 3. Antibiotics and a population with some resistant bacteria. *The test tube contains bacterial growth media and antibiotics to which some of the bacteria are resistant.*

> In any population, the individuals are not identical to each other—just look around the classroom! There is always variation in a population. In bacteria, this includes variation in resistance to antibiotics. Some bacteria may be resistant to antibiotics while others are susceptible. While antibiotics kill most bacteria, some will be resistant and survive. Because the conditions determine which individuals in a population are the most fit to survive, this is an example of natural selection.

3. Explain how the graphs lines in **FIGURES 1–3** show how the number of bacteria changes over the 24 hours.

No antibiotics: _____

Antibiotics and susceptible bacteria: _____

_____

DID YOU KNOW?

Scottish researcher Alexander Fleming accidentally discovered penicillin in 1928. He observed that a mold growing on one of his Petri dishes had killed all the bacteria growing nearby.

Antibiotics and some resistant bacteria: _____

_____

4. How will Dr. Hincapie use the three standard graphs?

_____

_____

Using the standard graphs (**FIGURES 1–3**), Dr. Hincapie can now analyze the results of the bacterial cultures he grew from Eva's tissues over the course of her infection. Examine **FIGURES 4–8**, and answer questions 5–11.

5. Were antibiotic-resistant bacteria present in the tissue samples taken when Eva first arrived at the hospital on Monday? (**FIGURE 4**) How can you tell?

_____

_____

_____

_____

6. By Saturday, which antibiotics were the bacteria resistant to?

_____

_____

7. At what point did the population of bacteria show resistance to:

   Antibiotic A: _____

   Antibiotic B: _____

   Antibiotic C: _____

   Antibiotic D: _____

8. Explain why the number of bacteria is so different on Monday afternoon (**FIGURE 4**) compared to Tuesday (**FIGURE 6**), after growing for 24 hours in the presence of Antibiotic A.

_____

_____

_____

_____

9. Explain why the number of bacteria is so different on Thursday (**FIGURE 7**) compared to Saturday (**FIGURE 8**), after growing for 24 hours in the presence of Antibiotic C.
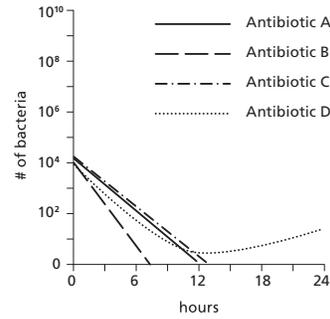
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FIGURE 4.
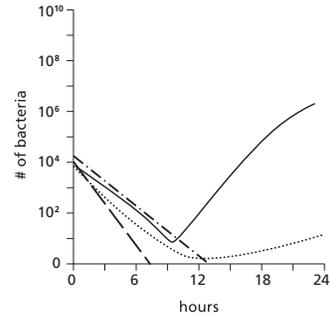*Monday afternoon after arriving at the hospital, before any antibiotics were taken.*

FIGURE 5.
Monday evening just before leaving the hospital, before any antibiotics were taken.
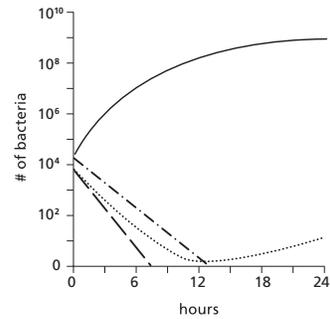
FIGURE 6.
Tuesday night after returning to the hospital. Eva had taken Antibiotic A. The doctors prescribed a new dose of Antibiotic A.
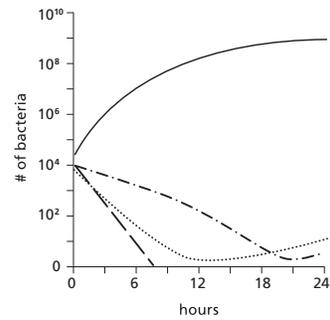
FIGURE 7.
Thursday evening after returning to the hospital a second time and being admitted. Eva had taken Antibiotic A since Tuesday morning. The doctors prescribed Antibiotic C.
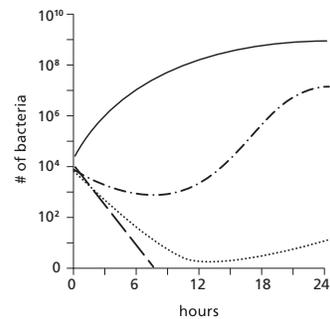
FIGURE 8.
Saturday morning after spending Friday in the hospital and taking Antibiotic A since Tuesday and Antibiotic C since Thursday night.

KEY FOR THE RESPONSE TO FOUR ANTIBIOTICS
Each sample begins with 10,000 (10^4) bacteria. Hour zero represents the bacteria in Eva's blood at the time mentioned in the caption of each figure. The following 24 hours represent the growth occurring in Dr. Hincapie's test tubes. To understand whether or not Eva's bacteria are resistant, see how the population changes over 24 hours.

10. How is the growth of the bacteria resistant to Antibiotics A, C, and D an example of natural selection?

_____

_____

11. Based on the results of his testing, what advice should Dr. Hincapie give to Eva's doctors about the next antibiotic to try?
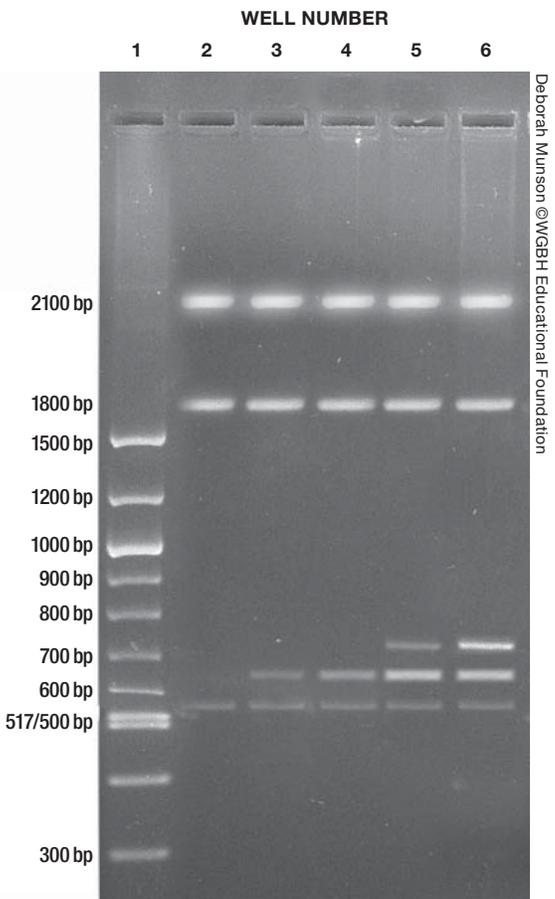
_____

_____

DID YOU KNOW?

Some bacteria exchange genetic material using a tiny tube that connects them together. In this way, a drug-resistant bacterium can pass its resistance on to others.

GOING FURTHER: Identify the Genes Carrying Antibiotic Resistance

In bacteria, the genes for antibiotic resistance are often carried on plasmids (small circles of DNA) rather than in the main bacterial chromosomal DNA. Plasmid DNA can be prepared and viewed using gel electrophoresis.

Dr. Hincapie wanted to determine which gene was responsible for the antibiotic resistance he observed in the bacteria causing Eva's infection. First, he isolated plasmid DNA from each of Eva's original samples. Then, he separated the plasmid DNA samples using gel electrophoresis. Here's a photograph of his gel.

Deborah Munson ©WGBH Educational Foundation

WELL NUMBER	SAMPLE
1	DNA size marker
2	Monday, immediately after arriving at the hospital
3	Monday evening, just before leaving the hospital
4	Tuesday night after returning to the hospital
5	Thursday evening, after being admitted to the hospital
6	Saturday morning

Dr. Hincapie recognized that the two larger pieces of DNA— 2100 base pairs and 1800 base pairs—were from plasmids found in bacteria that cause infections. The brightness of a piece of DNA on a gel reveals two things: larger pieces of DNA are brighter than smaller pieces and larger amounts of DNA appear brighter than smaller amounts. Knowing this, which bands on this gel do you think contain a gene for resistance to:

Antibiotic A: _____

Antibiotic B: _____

Antibiotic C: _____

Antibiotic D: _____

RISE OF THE SUPERBUGS

4 STUDENT SHEET