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 (10⁴) 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:

![Graphs showing bacterial growth](image)

- **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.

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: ________________________________

   Antibiotics and some resistant bacteria: ________________________________

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

   ________________________________

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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.

**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.

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**RISE OF THE SUPERBUGS**

2 | STUDENT SHEET
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.

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 since Tuesday morning. 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?

**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.

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: 

**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.