NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Antibiotic Resistance: Can We Ever Win?

by
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Part I – Measuring Resistance

Katelyn was excited to start her summer job in her microbiology professor's research laboratory. She had enjoyed Dr. Johnson's class, and when she saw the flyer recruiting undergraduate lab assistants for the summer, she had jumped at the opportunity. She was looking forward to making new discoveries in the lab.

On her first day, she was supposed to meet with Dr. Johnson to talk about what she would be doing. She knew the lab focused on antibiotic resistance in *Staphylococcus aureus*, especially MRSA (methicillin-resistant *S. aureus*).

She still remembered the scare her family had last year when her little brother, Jimmy, got so sick. He'd been playing in the neighborhood playground and cut his lip when he fell off the jungle gym. Of course he always had cuts and scrapes—he was a five-year-old boy! This time though his lip swelled up and he developed a fever. When her mother took him to the doctor, the pediatrician said the cut was infected and had prescribed cephalothin, an antibiotic related to penicillin, and recommended flushing the cut regularly to help clear up the infection.

Two days later, Jimmy was in the hospital with a fever of 103°F, coughing up blood and having trouble breathing. The emergency room doctors told the family that Jimmy had developed pneumonia. They started him on IV antibiotics, including ceftriaxone and nafcillin, both also relatives of penicillin.

It was lucky for Jimmy that one of the doctors decided to check for MRSA, because that's what it was! MRSA is resistant to most of the penicillin derivatives. Most cases of MRSA are hospital-acquired from patients who are already susceptible to infection, but the ER doctor explained that community-acquired MRSA was becoming more common. The doctor then switched the treatment to vancomycin, a completely different kind of antibiotic, and Jimmy got better quickly after that.

Katelyn had dropped Jimmy off at swimming lessons just before coming to work at the lab. As she waited in the hallway for Dr. Johnson, she hoped that she would be at least a small part of helping other people like Jimmy deal with these scary resistant microbes. She was surprised when the professor burst out of the lab, almost running into her.

"Hi Katelyn, I'm really sorry but I have to run to a meeting right now—they sprung it on me last minute. There are a bunch of plates in the incubator right now that need their zones of inhibition measured. I'll be back in a few hours," Dr. Johnson said as he rushed down the hallway with a stack of folders.

Katelyn dug out her old lab notebook to look up what she was supposed to do. She found the lab where she and her fellow students had examined the antimicrobial properties of antibiotics using the Kirby-Bauer disk diffusion technique. Looking at the plates Dr. Johnson had told her about, she saw they had all been "lawned," or completely coated with microbes to make a thick hazy layer over the agar surface. She could also see paper disks with letters on them, and some of the disks had clear zones around them where the microbe had been inhibited (Fig. 1). Her notebook explained how to measure the zone of inhibition around the disks (Fig. 2).

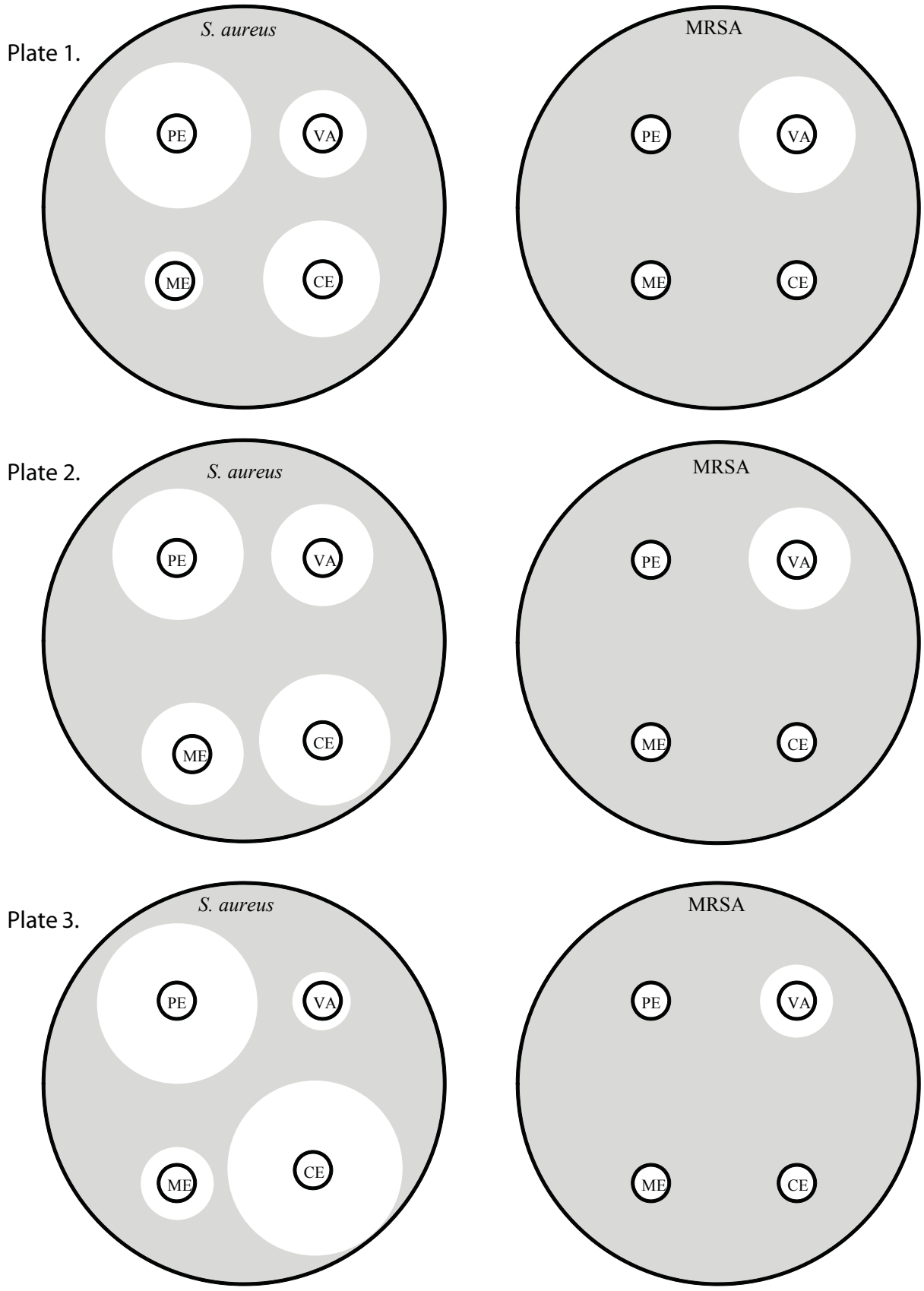


Figure 1. Agar plates of *S. aureus* or MRSA lawns with antibiotic disks placed on them.

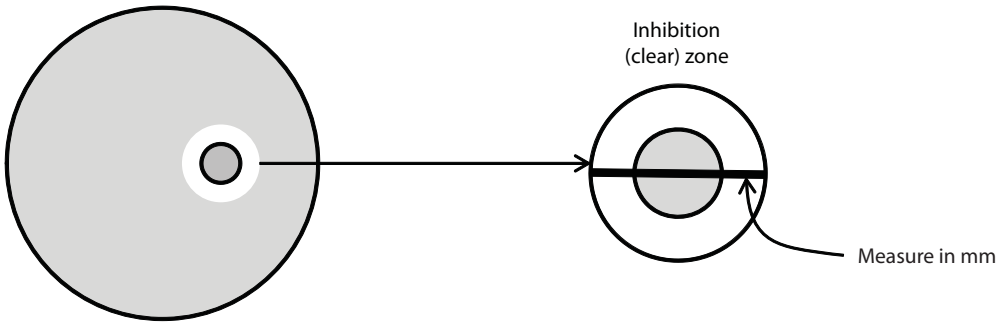


Figure 2. Katelyn's diagram of how to measure a zone of inhibition from her microbiology lab notebook.

Exercise1

Measure the zones of inhibition for each antibiotic on the plates shown in Figure 1 and note the measurements in the spaces in Table 1 below. (*Note:* The Kirby-Bauer method is standardized so that no zone of inhibition is scored as a 0, and all others include the disk as part of the zone.)

Key: PE = penicillin, ME = methicillin, CE = cephalothin, and VA = vancomycin

Plate		*S. aureus*	MRSA
1	PE		
	ME		
	CE		
	VA		
2	PE		
	ME		
	CE		
	VA		
3	PE		
	ME		
	CE		
	VA		

An average, or mean ($\overline{x}$), is a measure of central tendency in the data, or what value occurs in the middle of the data set. The mean is calculated by adding up all the values for a given set of data, then dividing by the sample size (n).

Average $$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Standard deviation measures the spread of the data—as in how variable the data set is. The standard deviation (s) is calculated by the following:

Standard deviation $s = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$

Standard error measures the difference between the sample you have taken and the whole population of values. The standard error (SE) is calculated as follows:

Standard error $SE = \frac{s}{\sqrt{n}}$

Exercise 2

In Table 2 below calculate and record the averages and standard errors for each antibiotic in *S. aureus* and MRSA.

	S. aureus		MRSA	
	Average	SE	Average	SE
PE				
ME				
CE				
VA				

Exercise 3

GRAPH the data from exercise 2

Questions

1. What do you think the experimental question is?
2. What hypotheses can you come up with to answer the experimental question?
3. If your hypothesis is correct, what would the plates look like (i.e., what predictions would you make for each hypothesis)?
4. Is the experiment you just collected data for an appropriate test of the experimental question you came up with in your answer to Question 1?
5. Which antibiotics where most effective against *S. aureus?* Against MRSA?
6. When comparing the antibiotics effective against both, were there differences in effectiveness?
7. What other questions do the data shown in Figure 1 make you think of?

Part II – Resistance

Among the first antibiotics used on a large scale was penicillin, which was discovered in 1929 by Alexander Fleming. It was finally isolated and synthesized in large quantities in 1943. Penicillin works by interfering with the bacterial cell wall synthesis. Without a cell wall, the bacterial cells cannot maintain their shape in changing osmotic conditions. This puts significant selective pressure on the microbes to evolve, as they cannot survive the osmotic stress. Any microbe that can resist these drugs will survive and reproduce more, making the population of microbes antibiotic resistant.

The specific mechanism of penicillin is the prevention of cell wall synthesis by the β-lactam ring of the antibiotic (Fig. 3), which binds and inhibits an enzyme required by the bacterium in this process.

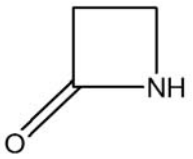


Figure 3. The β-lactam ring common to the penicillin family of antibiotics.

The enzyme is called penicillin-binding protein (PBP), even though it is an enzyme involved in cell wall synthesis. Normally enzymes have names that indicate what they do and end in the suffix *-ase*, like lact*ase*, the enzyme that breaks down lactose. Figure 4 is a representation of PBP and its active site.

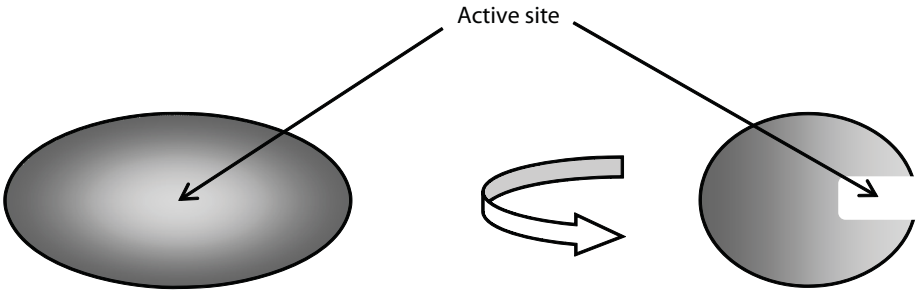


Figure 4. PBP (penicillin-binding protein) active site is a groove allowing formation of cross-links in the bacterial cell wall.

Bacterial cell walls are layered structures, where each layer is made of peptidoglycan, a sugar and protein polymer. Each layer is cross-linked to the next, strengthening the wall and allowing the cell to resist osmotic pressure. The way the enzyme PBP works is to form those cross-bridges by joining strings of amino acids together in the active site, which is a groove in the protein (Fig. 5).

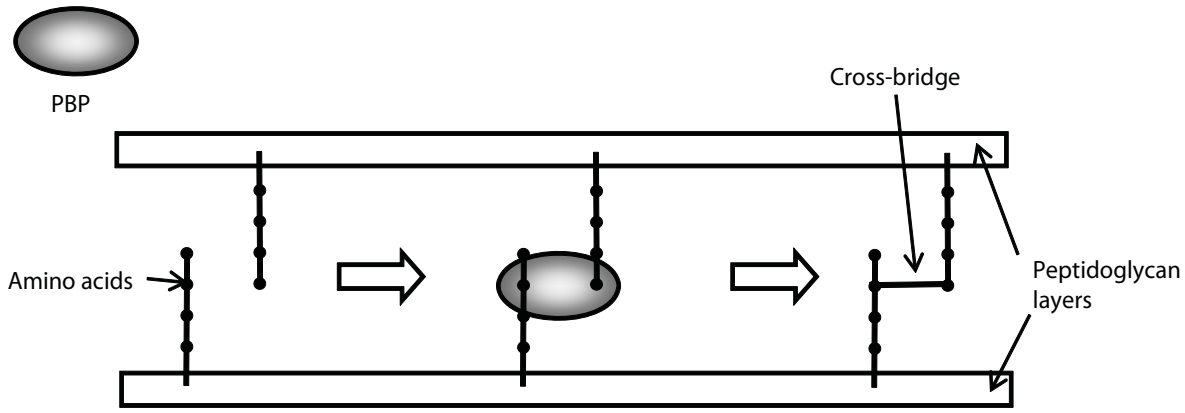


Figure 5. Cross-link formation in bacterial cell walls by PBP (penicillin-binding protein).

The PBP takes amino acid residues attached to peptidoglycan layers and forms bridges between them within the active site groove. This cross-linking, or cross-bridging, stabilizes and strengthens the cell wall. β-lactam antibiotics interfere with the PBP enzyme by binding to the active site, blocking the site from the amino acids (Fig. 6).

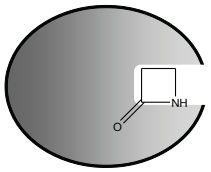
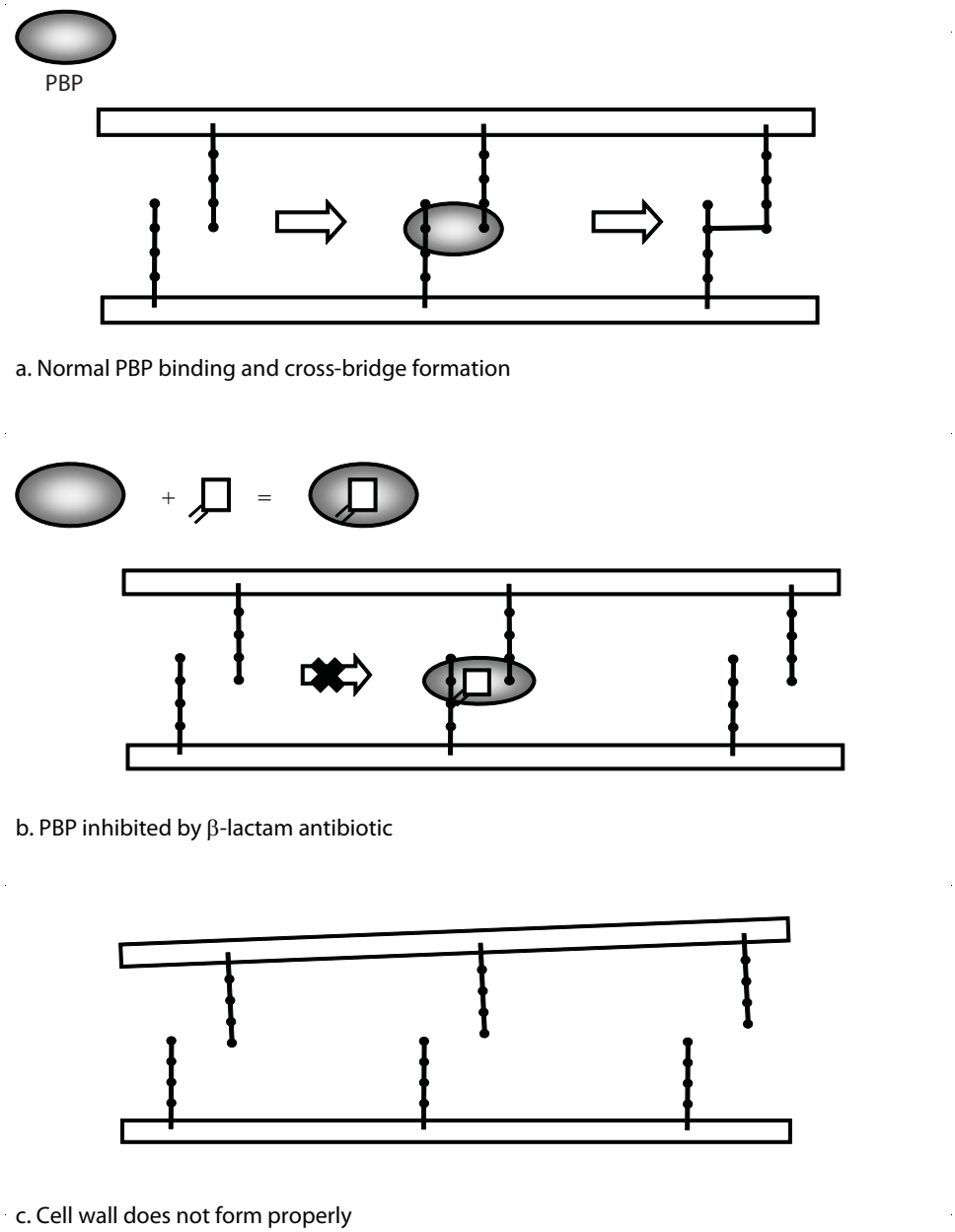


Figure 6. Inhibition of PBP (penicillin-binding protein) by β-lactam blocking the active site.

There are over 80 natural and semi-synthetic forms of β-lactam antibiotics, including cephalothin and methicillin. Vancomycin also interferes with cell wall synthesis, but its mechanism of action is to bind directly to the cell wall components (Figs. 7 and 8).

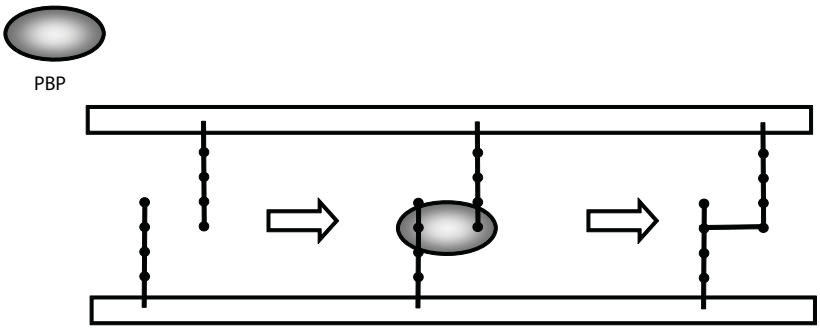


a. Normal PBP binding and cross-bridge formation

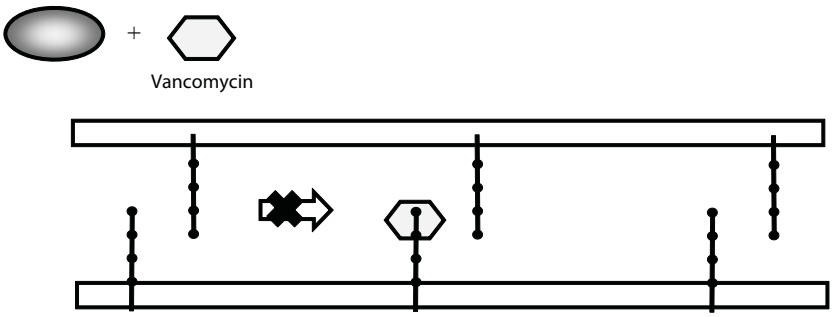
b. PBP inhibited by β-lactam antibiotic

c. Cell wall does not form properly

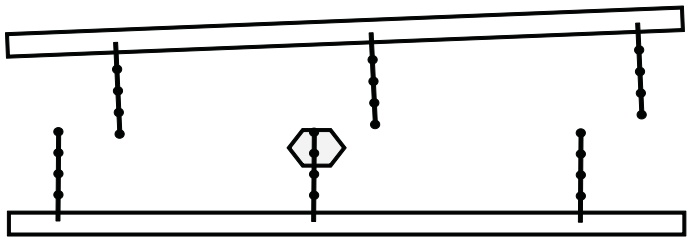
Figure 7. PBP (penicillin-binding protein), the enzyme that allows the bacterial cell wall to form cross-bridges, is inhibited by the β-lactam family of antibiotics. This prevents proper cell wall synthesis and the bacterium will succumb to osmotic stress.



a. Normal PBP binding and cross-bridge formation



b. Vancomycin binds PBP substrate



c. Cell wall does not form properly

Figure 8. Vancomycin inhibits cell wall synthesis a different way by binding PBP's substrates and preventing cross-bridging. This prevents proper cell wall synthesis and the bacterium will succumb to osmotic stress.

The first MRSA case was discovered in 1961 in a British hospital, and was the result of a mutation in the enzyme normally inhibited by the β-lactam ring of methicillin. The site where the antibiotic would bind no longer allowed access to the ring, so the enzyme continued to function normally. The microbe acquired a new gene that, when made into protein, was a different version of PBP, one that couldn't be inhibited by penicillin.

Questions

1. Describe what is happening in Figures 7 and 8 in a complete sentence of your own words.
2. What are the differences in how β-lactam antibiotics and vancomycin work?
3. What other mechanisms might arise to allow resistance to the β-lactam antibiotics?
4. Could resistance arise to vancomycin? Why or why not?