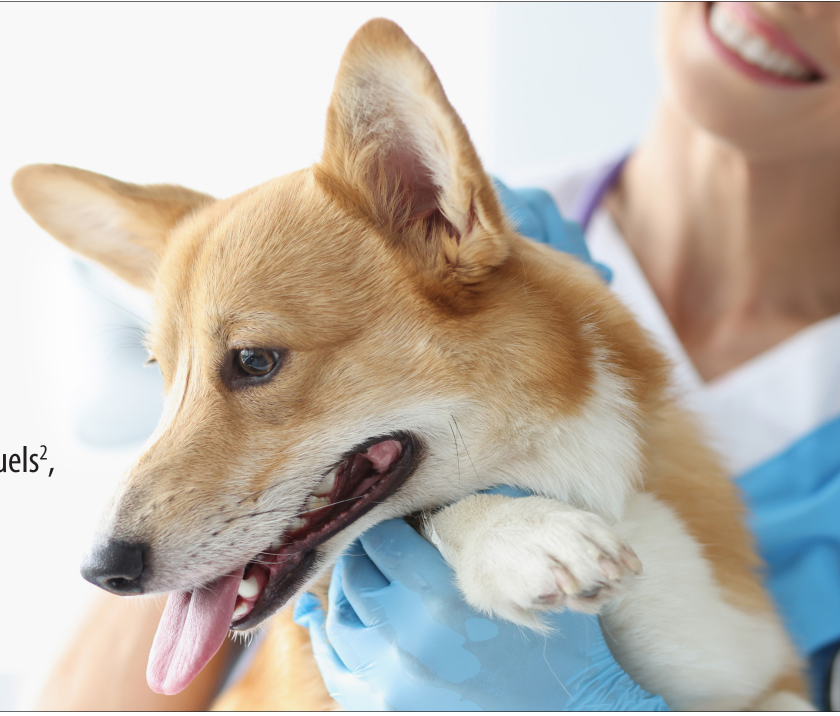
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Beaker Has a UTI: The Molecular Mechanism of Treating Bacterial Infections



by

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Part I – Beaker Goes to the Vet

"She's got blood in her pee!" Kyra exclaimed to the veterinarian. "When I took her outside yesterday morning, she looked like she was in pain when she was peeing, and only a little bit came out. She bugged me all day asking to go out, and every time it was the same. She even had an accident in the house overnight, which isn't like her at all. That's when I noticed the blood and asked to come in today."

Beaker, a five-year-old female spayed corgi, was never very fond of the vet. She was panting by Kyra's side, alert, but nervous.

"Has Beaker been eating and drinking okay?" Dr. Velazquez asked while she gently approached Beaker to begin her physical examination.

"Definitely," Kyra answered sadly. "In fact, she's drinking a lot lately."

Dr. Velazquez felt Beaker's abdomen and took her temperature. Beaker let out a small whimper as the doctor pressed over her belly.

"The most likely cause of Beaker's clinical signs is a lower urinary tract infection," Dr. Velazquez declared. "This infection is caused by bacteria that have overgrown inside her bladder. With your permission, I'd like to take a sample of her urine and look at it under the microscope to assess for the presence of bacteria and inflammatory cells. With the microscope, I can also assess for other causes of her urinary signs, such as kidney issues or urine crystals. I'd also like to run the urine through my analyzer to assess for other causes for her urinary signs."

"Of course!" Kyra immediately responded. "Anything to get her better!"

Dr. Velazquez used a sterile needle and syringe to take a urine sample from Beaker's bladder in a procedure known as cystocentesis. After a few minutes, the veterinarian returned with the results.

"Beaker does indeed have a bacterial urinary tract infection. The most likely culprit is *E. coli,* given the appearance of the bacteria under the microscope. *E. coli* is the most common cause of urinary tract infections in both dogs and people. We're going to put Beaker on a very common antibiotic called amoxicillin to treat this."

E. coli is a type of gram-negative, facultative anaerobic bacteria. Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall that is further surrounded by a lipopolysaccharide membrane. Facultative anaerobes can make ATP via aerobic respiration when oxygen is present, but can switch to fermentation in the absence of oxygen. This type of bacterium is normally found in the intestines of healthy people and animals.

Questions

Answer the questions below. If you use an outside resource, be sure it is credible and provide a citation. Links to several reliable outside resources are the following:

- Merck Veterinary Manual: https://www.merckvetmanual.com
- MedlinePlus: https://medlineplus.gov
- National Human Genome Research Institute: https://www.genome.gov
- Vet Drug List: https://www.vet-ebooks.com/vetdrugslist
- Clark, M.A., M. Douglas, & J. Choi. (2018). *Biology*, 2nd ed. OpenStax: Houston, Texas. https://openstax.org/books/biology-2e/pages/1-introduction

1. Why is it necessary to confirm that the cause of the clinical signs is bacterial before prescribing an antibiotic?

2. How can looking at a sample under the microscope help Dr. Velazquez determine if Beaker's infection is bacterial or viral?

3. Describe the structure of an *E. coli* cell. What features does it possess that are different from a eukaryotic cell? What features are similar?

4. What metabolic processes used to generate ATP are the same between bacteria and eukaryotic cells? Which are different?

Part II – What Is Happening in Beaker's Body?

Luckily Beaker's infection was isolated to her lower urinary tract. If the infection had spread to the kidneys, Beaker would have shown a fever. Dogs normally have a temperature between 100.5 and 102.5 degrees Fahrenheit. Body temperature is one aspect of organismal homeostasis and is controlled by the hypothalamus. Certain types of bacterial infections result in the release of signaling molecules via the immune system, which travel through the blood and signal the hypothalamus to start releasing chemicals that increase metabolic rate and vascular constriction, causing shivering and fever. Beaker's immune system was trying to fight the infection, but there were too many *E. coli* in her bladder for her cells to fight the bacteria on their own. The antibiotic the doctor prescribed to Beaker is called a bactericide, meaning it kills the bacteria, thereby helping Beaker's immune system to clear the infection.

Questions

Answer the following questions. If you use an outside resource, be sure it is credible and provide a citation.

1. Using your knowledge of endocrine signaling and the function of the kidneys, explain why a fever is more likely in a kidney infection compared to a bladder infection.

2. Which of the following cell types would you expect to be activated by the UTI? For any cell you select, indicate if it is a part of the innate or adaptive immune system.
   - Epithelial cells
   - Hepatocytes
   - Macrophages
   - Cytotoxic T cells
   - Osteoclasts
   - Neutrophils
   - Muscle fibers
   - Neurons
   - Memory B cells
   - Helper T cells
   - Astrocytes
3. If Beaker's urinary tract *E. coli* infection is treated with the antibiotic and cleared effectively, do you expect her to be immune to another *E.coli*-based UTI? Why or why not?

4. When the prostaglandin PGE-2 is produced at the site of infection, it induces a fever response in the hypothalamus via binding of a G-protein coupled receptor (GPCR) called E2 in the neuronal cells. Activated E2 in turn signals the expression of other downstream targets that help turn on a fever response. Using your knowledge of GPCRs, suggest a mechanism for stopping or suppressing a fever using small molecule drugs.

5. If Beaker had a significant kidney infection that compromised the function of her kidneys, list two aspects of body homeostasis other than temperature that could be affected. Why?

Part III – Why Amoxicillin?

Dr. Velazquez stepped out of the room to get Beaker's medicine.

Kyra believed Dr. Velazquez wanted Beaker to get better, but she also realized that she had no idea what amoxicillin did or how it worked. She started researching amoxicillin, its structure, and its mechanism of action.

She found the structure of amoxicillin and learned that it was a penicillin derivative (Figures 1 & 2). It contains an additional amino group as compared to penicillin and is called a beta-lactam. Beta lactam antibiotics bind to and inhibit the action of proteins in bacterial cells that cross link peptides during cell wall synthesis; as a result, the cell wall cannot be completed, which triggers autolysis of the cell.

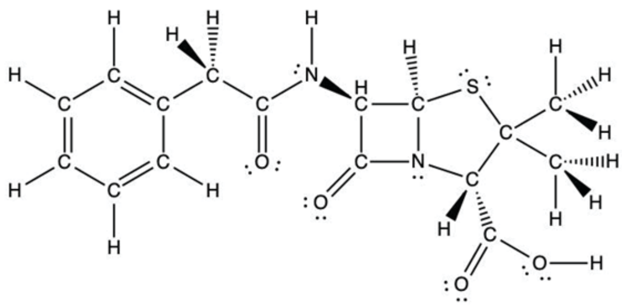


Figure 1. Structure of a typical penicillin. Filled triangles (◄) are coming towards you while lined triangles (⦙⦙⦙) are going away from you.

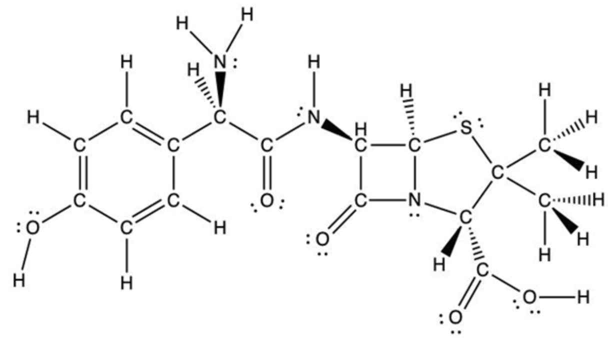


Figure 2. Structure of amoxicillin. Filled triangles (◄) are coming towards you while lined triangles (⦙⦙⦙) are going away from you.

Questions

Answer the following questions. If you use an outside resource, be sure it is credible and provide a citation.

1. Compare the structure of amoxicillin and penicillin. Draw a box around the areas of the molecules that are different.

2. Describe the polarity of amoxicillin. Draw partial charges on the area of the molecule that are polar in character.

3. Why would using an antibiotic that targets the cell wall be a good choice for treating a bacterial infection in dogs?

4. The beta-lactam ring is the same in both penicillin and amoxicillin (as well as in other effective penicillin derivatives). The beta-lactam ring is shown highlighted in red in Figure 3. This part of the molecule is important in binding to the enzyme involved in cell wall crosslinking. Do you think the antibacterial efficacy of a molecule that looks like penicillin without the beta-lactam ring would increase, decrease, or stay the same? Why?

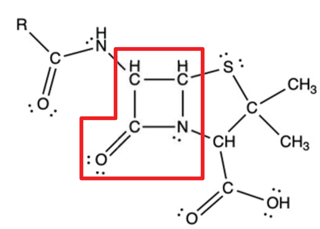


Figure 3. Beta lactam ring.

Part IV – What Do You Mean It May Not Work?

"There is a small chance that the antibiotics we prescribed to Beaker may not work," Dr. Velazquez said cautiously as she returned with the medication. "We have seen an uptick in the number of antibiotic resistant infections in veterinary medicine over the last few years. Physicians have also observed this problem in human medicine. We are going to give Beaker amoxicillin. If we don't see her get better, we will add clavulanate to help fight any antibiotic resistant bacteria. Based on Beaker's size and weight, you need to give her one capsule twice a day for five days. She should start feeling more like herself in about two days. After you start giving her these, please watch for side effects such as decreased appetite, vomiting, or diarrhea. The best way to reduce the chance of these side effects is to give the medication with a meal. If you see any side effects, or if her clinical signs don't improve or get worse, give me a call."

After taking Beaker home and giving her the first dose of medicine, Kyra decided to research the cause of *E. coli* resistance to amoxicillin. What did Dr. Velazquez mean by "antibiotic resistant bacteria," anyway? And for that matter, how could we counteract that?

In her research, Kyra determined that some bacteria have developed resistance to penicillin derivatives by acquisition of the gene encoding the enzyme beta-lactamase. Beta-lactamase cuts the beta-lactam ring of penicillin derivatives, thereby preventing them from binding to the penicillin binding protein. The ribbon structure of an example monomer of beta-lactamase is shown in Figure 4.

Clavulanate or clavulanic acid is a beta-lactamase inhibitor that binds to the active site of the beta-lactamase enzyme (see Figure 5). Beta lactamase irreversibly binds to clavulanate via a serine side chain. After binding, clavulanic acid undergoes a structural rearrangement that binds even more tightly to the enzyme. The inactive, clavulanate-bound enzyme is ultimately degraded by the cell.

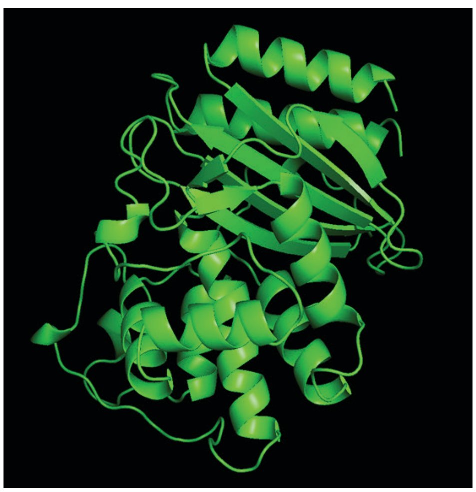


Figure 4. Ribbon structure of beta-lactamase.

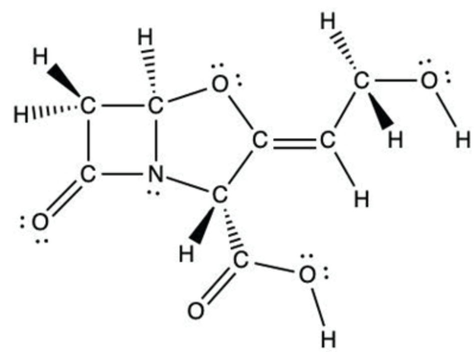


Figure 5. Structure of clavulanate.

Questions

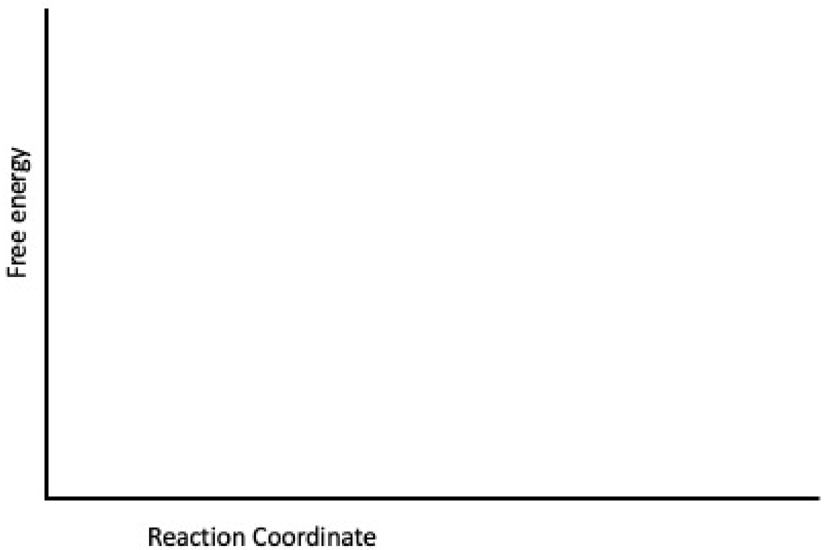
Answer the following questions. If you use an outside resource, be sure it is credible and provide a citation.

1. Draw a flow chart or concept map showing the relationship between amoxicillin, clavulanate, and beta-lactamase. This diagram should depict the products of the reaction catalyzed by beta-lactamase under two conditions: (i) the presence of amoxicillin, and (ii) the presence of amoxicillin *and* clavulanate. For each condition, indicate whether the bacterial cell wall will be crosslinked or not.

2. If an *E. coli* cell containing a gene that confers resistance to amoxicillin is exposed to amoxicillin, which cellular processes must occur and in what order for the cell to survive?

3. Describe the structure of beta-lactamase.

4. Beta lactamase works by catalyzing the ring cleaving reaction of a beta-lactam in an exothermic reaction. Using the axes provided below, draw an energy diagram of this process (E + S ↔ E –TS → E + P) using the following parameters:
   a. Graph free energy as a function of reaction progress.
   b. Include a line depicting both the catalyzed and uncatalyzed reaction on the same diagram.
   c. Label the reactants, products, and transition states for both the catalyzed and uncatalyzed reactions.



5. Speculate as to why upregulating beta-lactamase does not kill the bacteria.

6. What type of bond would you expect clavulanic acid to make with serine in the binding pocket to generate the irreversible binding? Why?

7. Given the structure of clavulanic acid, what type of residues (polar, nonpolar, charged, aromatic) would you expect in the beta-lactamase binding pocket? Why?

8. Do you think bacteria could develop resistance towards clavulanic acid? If so, how? Provide your rationale.

Part V – Beaker's Recovery

After just one day on her medicine, Beaker began to act more like her old self again. She stopped having accidents in the house and seemed to have a bit more energy. After two days, she was jumping around the yard with the neighbor's dogs, and Kyra stopped seeing any blood in her urine. Beaker ended up making a full recovery.

Commonly prescribed antibiotics like amoxicillin are important in both human and veterinary medicine. They are relatively inexpensive and treat a broad spectrum of bacterial infections. Unfortunately, because of how often they are used, both veterinarians and physicians alike have seen a significant increase in the number of antibiotic resistant infections they are treating and have had to make use of other, more potent antibiotics.

Below is a list of antibiotics that may be prescribed for dogs for a variety of bacterial infections. Each of these antibiotics has a different molecular mechanism of action than amoxicillin.

- Gentamicin
- Chloramphenicol
- Sulfamethoxazole
- Tetracycline

Questions

Answer the following questions. When using outside sources, be sure to include a citation.

1. Select one of the antibiotics from the above list. Identify at least two credible sources discussing this molecule. Provide the full citations for these articles.
2. Draw the structure of this molecule and describe its polarity.
3. Describe the range of the different kinds of microorganisms that this antibiotic kills.
4. Describe the cellular mechanism of action for this antibiotic. (For example, we learned in this case about the mechanism of action of amoxicillin. Amoxicillin binds to and inhibits the action of proteins in bacterial cells that cross link peptides during cell wall synthesis; as a result, the cell wall cannot be completed, which triggers autolysis of the cell.)
5. Has bacterial resistance to this antibiotic been observed? If so, describe the cellular mechanism of resistance.
6. Can we combat resistance to this antibiotic? If so, how?
7. Many antimicrobials cause decreased appetite, vomiting, or diarrhea. Speculate as to why this might occur.