Dolan
DNA Learning Center
www.dnalc.org

Bacteria and Antibiotics

Background

In 1929, Sir Alexander Fleming discovered the first antibiotic. He was a scientist devoted to finding methods for treating infections. During his research, he noticed that a mold had accidentally contaminated his plates of bacteria. The mold seemed to inhibit (slow down or interfere with) bacterial growth on the plate. He hypothesized that the mold was making something that could kill bacteria. With further experiments, he determined that bacteria were unable to grow in the presence of the mold. He tried to purify the substance, but it was too unstable and would break down easily.

During World War II, two British scientists, Sir Howard Florey and Ernest Chain, picked up where Fleming left off. They succeeded in purifying and producing this new substance for treating bacterial infections. They referred to it as an antibiotic; "anti" meaning against and "biotic" meaning life, or living things. Antibiotics kill living bacteria. The mold that contaminated Fleming's plates was Penicillium; can you guess what they named the first antibiotic? It was called Penicillin. All three scientists won the Nobel Prize for their work in developing this new "wonder drug" that saved millions of lives.

When it was discovered, penicillin was considered a breakthrough in modern medicine. Since the discovery of penicillin, scientists have developed synthetic antibiotics that work better. One example is Amoxicillin. Amoxicillin is a very common synthetic antibiotic that is useful against many bacteria, so it is useful against bacteria that are sensitive to it and when the sensitivity of the bacteria is unidentified. It is also more resistant to damage from stomach acid than some naturally occurring antibiotics. Some of its common uses are in treating infected wounds, bladder infections, ear infections and strep throat.

Two other common synthetic antibiotics are Ampicillin and Kanamycin. Ampicillin is very similar to penicillin but is produced synthetically. It destroys bacterial cells when they are going through mitosis, or the process of cell division. For the cell to divide, it needs to create a cell wall barrier. At this point, Ampicillin destroys the cell by punching holes through the wall, causing the bacterium to lyse, or break apart. Kanamycin is another type of synthetic antibiotic. It destroys bacterial cells by binding to the ribosomes within the cell and disrupting RNA translation. Therefore, it stops protein production and kills the bacterial cells outright. Because Ampicillin only destroys cells going through mitosis, it is considered "weaker" than Kanamycin, which kills newly made cells as well as older cells.

One concern with the distribution and use of antibiotics is that over time, bacteria can become resistant to particular antibiotics. Most bacteria present in hospital settings are resistant to one or more of the antibiotics used to commonly treat infections. Some antibiotics attack bacteria by destroying part of their protective cell wall. But bacteria can fight back by changing the cell wall so the antibiotics can't get in. They also know how to pass on their new defenses to other bacteria, rendering the latest generation of antibiotics useless over time. Some bacterial strains are resistant to all antibiotics on the open market and the infections that they cause are treated using potentially toxic or experimental drugs.

One cause of antibiotic resistant bacteria is misuse of antibiotics by doctors and their patients. Often, patients will ask doctors for antibiotics for a cough, cold, or the flu, which are caused by viral infections and cannot be treated using antibiotics. Another problem arises when patients are prescribed antibiotics to treat bacterial infections and fail to finish the full dose given. If the full dose isn't taken, some bacteria may survive and develop a mutation that protects it from last drug used on it. If even one bacterium survives and reproduces, it passes this mutation on to a new generation of bacteria. If the same antibiotic is used to treat this new infection, it won't have any affect and a different drug will be needed. This is a growing problem in the medical field today.

Bacteria and Antibiotics

Description of Activity

In this one-hour activity, students in grades 5 through 8 have the opportunity to explore the role that antibiotics play in disease treatment. Students will culture bacteria and observe the effect of two separate antibiotics on two different strains of bacteria.

Learning Outcomes

Students will:

- observe the affect of two separate antibiotics on two different strains of bacteria.
- understand the function of different antibiotics.
- learn the history of antibiotics and disease treatment.
- appreciate the growing problem of antibiotic resistance.

Assumptions of Prior Knowledge

Students should understand the function of DNA and be familiar with the structure and reproductive processes of prokaryotic cells.

Misconceptions

Students often believe that antibiotics can be used to treat all types of infectious disease and that all strains of bacteria are harmful. In addition, they might not understand the role of vaccines in disease prevention, and may believe that vaccines can be used to treat or cure infectious disease.

Lesson

Materials and Equipment (for groups of two students)

- Petri dishes with Luria Broth agar (2 per group)
- Small tubes of sterile glass beads (2 per group)
- 1 box of toothpicks
- 250ml plastic beakers (1 per group)
- Small tube racks (1 per group)
- 100-1000µl large micropipettors (1 per group)*
- Boxes of large pipette tips (1 box per group)*
- Permanent markers (1 per group)
- Glue sticks (1 per group)
- Masking tape (1 per group)

* If you do not have access to micropipettors, substitute plastic transfer pipettes (droppers).

Reagents

- 250µl aliquots of mm294 *E. coli* in Luria Broth labeled #1 (1 per group)
- 250µl aliquots of Ampicillin Resistant mm294 *E. coli* in Luria Broth + Ampicillin labeled #2 (1 per group)
- Ampicillin "pill" dispensers (1 per group)
- Kanamycin "pill" dispensers (1 per group)

Recipes

Luria-Bertani Agar

- 10g Tryptone
- 5g-yeast extract
- 10g NaCl
- 875µl 4N NaOH (4g NaOH /100ml of dH_2O)
- 15g Bacto-agar
- Mix ingredients into 1 liter of dH_2O.
- Autoclave for 25 minutes.
- Let solution cool to 55°C and pour into sterile Petri plates.

Luria-Bertani (LB) Broth (1L)

- 10g Tryptone
- 5g-yeast extract
- 10g NaCl
- 875µl 4N NaOH (4g NaOH /100ml of dH_2O)
- Mix ingredients into 1 liter of dH_2O.
- Pour into glass bottles (100ml each) and cap loosely.
- Autoclave for 25 minutes and allow to cool. For LB + Amp, add 1ml of Ampicillin (10mg/ml) to 50 ml of LB solution after it has been autoclaved and cooled. Store at 4°C.

Ampicillin 10 mg/ml

- Add 1g of ampicillin sodium salt (m.w. = 371.40) to 100ml of dH_2O in a clean 250 ml flask.
- Stir to dissolve.
- Pass solution through a Nalgene filter for sterilization.
  Store at 4°C (up to 3 months)

Bacteria and Antibiotics

Basic overnight culture

- Use a sterile, medium-size pipette tip to pluck a colony from a fresh plate of desired bacteria.
- Eject tip directly into LB aliquot.
- Incubate tube overnight at 37°C in a SHAKING incubator. Make sure tube caps are loose so that cells can aerate.

Purchasing Information

- Antibiotic pill dispensers (Ampicillin and Kanamycin)- Fisher Scientific
- Glass beads- Fisher Scientific
- Transfer pipettes-VWR International
- Micro-tubes (Assorted colors)-USA Scientific
- Petri dishes- Fisher Scientific
- Miscellaneous chemicals – Fisher Scientific
- mm294/amp resistant mm294 – Carolina Biological Supply Company

Before Class

- Grow overnight cultures of mm294 (in LB broth) and ampicillin resistant mm294 (in LB broth with ampicillin) in a shaking incubator at 37°C (8ml of each)
- Prepare 250µL aliquots of MM294 and ampicillin resistant MM294.
- Photocopy the corresponding student worksheets.
- Distribute the beakers, tube racks, markers, tape, pipettes, and pipette tips to each student station.

During Class

- Provide the appropriate handouts.
- Review the history of antibiotics and disease treatment. Focus on Alexander Fleming and his discovery of the antibiotic properties of *Penecillium* mold. Also discuss Howard Florey and Ernst Chain, the scientists that helped to purify and distribute Penicillin in the 1940's. They all went on to win a Nobel Prize in 1945.
- Discuss what happens when the doctor thinks an individual might have "strep" throat. The doctor performs a throat culture. What does that mean?
- If the culture is positive for strep, the doctor then prescribes an antibiotic. Why is it so important to follow the directions on the bottle? Misuse of antibiotics can lead to antibiotic resistant strains of bacteria.
- Explain to students that Ampicillin is a type of synthetic antibiotic. It kills bacterial cells by preventing the formation of new cell walls. For this reason, it only kills cells that are actively dividing. Kanamycin is another synthetic antibiotic derived from bacteria called *Streptomyces kanamyceticus*. It kills bacterial cells by binding to the ribosome within the cell disrupting protein production.
- Explain that the goal of this laboratory is to culture bacteria just like the doctor does, and to determine if the bacteria are Ampicillin resistant. Make sure all of the students understand that the mm294 strain of *E. coli* is harmless. It is not the strain responsible for food poisoning.
- For the experiment, divide students into groups of two. Direct students to follow the instructions on their handouts.
- Before you begin the experiment, discuss sterile technique with the students. Explain that the agar inside the petri plates in sterile until the plate is opened and certain steps will be taken to prevent contamination.
- When handing out the plates to each group, place them on the table upside down. Tell the students not to open them. The plates should have both partners' initials, the date and the name of the bacteria they are adding (#**1** or **#2**). A line should be drawn down the middle of the plate with an A on one side of the line and a K on the other. Make sure that when the students label their dishes they write along the edge of the plate so that the writing doesn't obstruct the view of the bacteria when it grows. It is helpful to make a diagram on a chalkboard of what the labeled plates should look like.
- Demonstrate how to plate the bacteria using sterile technique. Students must clam shell plates when opening, and close them again right away to prevent contamination. The glass beads are sterile as well, and can be poured directly into a petri plate and gently shaken to spread the bacteria over the surface of the agar.
- Once the bacteria are plated, students can use toothpicks and a small bit of glue to transfer one ampicillin and one kanamycin pill onto the appropriate side of the dish.

Ampicillin will be placed on the side of the dish labeled A, and Kanamycin on the side labeled K.
- Tape both plates together with masking tape, and incubate upside down in an incubator at 37°C overnight. If there is no incubator, plates can be left upside down at room temperature for 24-48 hours.
- On the handout, make predictions about how the bacteria will be affected by the presence of ampicillin and kanamycin. Ask the students how they will know if the bacteria on their plates are resistant to ampicillin.

After-Care Information for Bacteria Plates

- To dispose of the plates, pour a 10% bleach solution directly onto the agar surface and soak for half hour. Dispose of plates in any trash receptacle.

Analysis and Discussion

- The following day, students can observe their results. The bacteria should grow in a lawn, except where they were killed by the antibiotics. Usually an area of inhibited growth, called a "halo", presents around the edge of the antibiotic pill. If the bacteria are resistant, there should be no halo. Ask the students which strain, #1 or #2, is resistant.
- Make correlations between types of antibiotics and expected results. If one antibiotic produces a larger halo, what might that mean?
- Consider the growing problem of antibiotic resistance. Encourage students to discuss ways that they can help prevent it.

Further Explorations

Research how bacteria have been used as a model organism in genetics research using the timeline at:
www.dnai.org/timeline/index.html < Jacob and Monod
www.dnai.org/timeline/index.html < Meselson and Stahl
www.dnai.org/timeline/index.html < Cohen and Boyer

Students can research various types of bacteria and discuss differences between them, such as symbiotic relationships with the host, harmless versus harmful and where certain strains prefer to live or infect.

Have students pretend to be a doctor writing a letter to a patient that is continuously asking for antibiotics for her cold. In the letter, have them explain why antibiotics won't help the patient and could contribute to antibiotic resistance.

Investigate and discuss the use of viruses called bacteriophage to treat infections caused by antibiotic resistant strains of bacteria.

Resources

Websites

www.dnai.org
The Dolan DNA Learning Center's Internet site.
Information on the past, present and future of DNA science.

www.dnaftb.org
The Dolan DNA Learning Center's Internet site.
An online textbook, with chapters about specific topics in genetics from inheritance to genetic engineering.

Film

"Modern Marvels: Antibiotics" Wonder (2000), A & E Home Video
ASIN: 0767014057

"Understanding: Bacteria"
Discovery Channel School (1997), Discovery Communications Inc.
http://school.discovery.com

Books

Cell Wars. Dr. Fran Balkwill. Carolrhoda Books, Inc., 1993.

Germ Zappers. Fran Balkwill and Mic Rolph. Cold Spring Harbor Laboratory Press, 2002.

Microbes Bugs and Wonder Drugs. Fran Balkwill and Mic Rolph. Cambridge University Press, 1995.

Correlations

New York State

NYS Standard 4: Science
The Living Environment

- Living things are both similar to and different from each other and nonliving things.
- Individual organisms and species change over time.
- Human decisions and activities have had a profound impact on the physical and living environment.

National
Content Standard C: Life Sciences
Diversity and Adaptations of Organisms

- Millions of species of animals, plants, and microorganisms are alive today. Although different species might look dissimilar, the unity among organisms becomes apparent from an analysis of internal structures, the similarities of their chemical processes, and the evidence of common ancestry.
- Biological evolution accounts for the diversity of species developed through gradual processes over many generations. Species acquire many of their unique characteristics through biological adaptation, which involves the selection of naturally occurring variations in populations. Biological adaptations include changes in structures, behaviors, or physiology that enhance survival and reproductive success in a particular environment.
- Extinction of a species occurs when the environment changes and the adaptive characteristics of a species are insufficient to allow its survival. Fossils indicate that many organisms that lived long ago are extinct. Extinction of species is common; most of the species that have lived on earth no longer exist.

Content Standard G: History and Nature of Science
History of Science

- Many individuals have contributed to the traditions of science. Studying some of these individuals provides further understanding of scientific inquiry, science as a human endeavor, the nature of science, and the relationships between science and society.

- In historical perspective, different individuals in different cultures have practiced science. In looking at the history of many peoples, one finds that scientists and engineers of high achievement are considered to be among the most valued contributors to their culture.

- Tracing the history of science can show how difficult it was for scientific innovators to break through the accepted ideas of their time to reach the conclusions that we currently take for granted.

AAAS Benchmarks
Chapter 10. Historical Perspectives
Standard I: Discovering Germs

- Throughout history, people have created explanations for disease. Some have held that disease has spiritual causes, but the most persistent biological theory over the centuries was that illness resulted from an imbalance in the body fluids. The introduction of germ theory by Louis Pasteur and others in the 19th century led to the modern belief that many diseases are caused by microorganisms-bacteria, viruses, yeasts, and parasites.

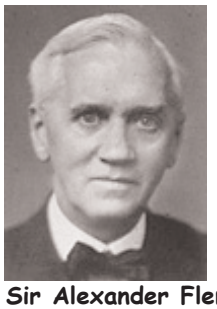
- Pasteur wanted to find out what causes milk and wine to spoil. He demonstrated that spoilage and fermentation occur when microorganisms enter from the air, multiply rapidly, and produce waste products. After showing that spoilage could be avoided by keeping germs out or by destroying them with heat, he investigated animal diseases and showed that microorganisms were involved. Other investigators later showed that specific kinds of germs caused specific diseases.

- Pasteur found that infection by disease organisms-germs-caused the body to build up immunity against subsequent infection by the same organisms. He then demonstrated that it was possible to produce vaccines that would induce the body to build immunity to a disease without actually causing the disease itself.

- Changes in health practices have resulted from the acceptance of the germ theory of disease. Before germ theory, illness was treated by appeals to supernatural powers or by trying to adjust body fluids through induced vomiting, bleeding, or purging. The modern approach emphasizes sanitation, the safe handling of food and water, the pasteurization of milk, quarantine, and aseptic surgical techniques to keep germs out of the body; vaccinations to strengthen the body's immune system against subsequent infection by the same kind of

microorganisms; and antibiotics and other chemicals and processes to destroy microorganisms.

- In medicine, as in other fields of science, discoveries are sometimes made unexpectedly, even by accident. But knowledge and creative insight are usually required to recognize the meaning of the unexpected.



Source: http://nobelprize.org/medicine/laureates/1945/fleming-bio.html

Sir Alexander Fleming

How Do Antibiotics Work?

In 1929, Alexander Fleming discovered the first antibiotic. He was a scientist devoted to finding methods for treating infections. During his research, he noticed that a mold had accidentally contaminated his plates of bacteria. The mold seemed to inhibit (slow down or interfere with) bacterial growth on the plate. He hypothesized that the mold was making something that could kill bacteria. He tried to purify the substance, but it was too unstable and would break down easily.

During World War II, two British scientists, Sir Howard Florey and Ernest Chain, picked up where Fleming left off. They succeeded in purifying and producing this new substance for treating bacterial infections. They referred to it as an antibiotic, "anti" meaning against and "biotic" meaning life, or living things. Antibiotics kill living bacteria. The mold that contaminated Fleming's plates was Penicillium. Can you guess what they named the first antibiotic? It was called Penicillin.

All three scientists won the Nobel Prize for their work in developing this new "wonder drug" that saved millions of lives.

Different antibiotics destroy bacteria in different ways. The two antibiotics you will be using are Ampicillin and Kanamycin.

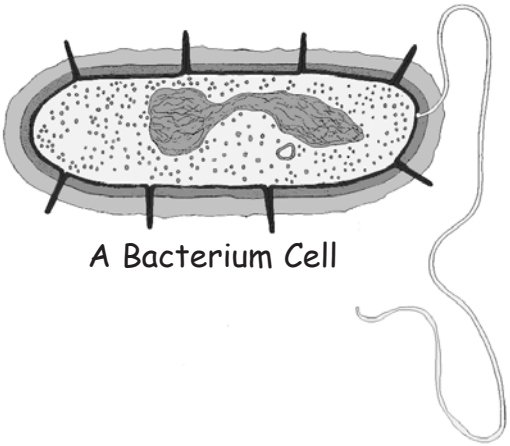
Ampicillin interferes with the formation of new cell membranes. Without a proper cell membrane the cell cannot live. Ampicillin only affects dividing cells; it does not affect cells that already have a cell membrane.

Kanamycin blocks the formation of new proteins in a bacterial cell. Proteins are very important to a cell, without them the cell dies. Kanamycin kills newly made cells as well as older cells.

What kinds of antibiotics have you taken?

Bacteria & Antibiotics

Exploring How Antibiotics Kill Bacteria



A Bacterium Cell

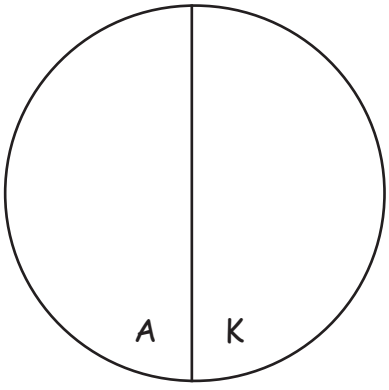
The discovery of antibiotics had an enormous effect upon human health. Before antibiotics were availablepeople regularly died of what are now considered easily treated infections, such as strep throat and bacterial pneumonia. Now, we take antibiotics for these infections and usually feel much better after only a few days.

In this experiment you will observe the effect of two separate antibiotics, **ampicillin** and **kanamycin**, on two different kinds of bacteria.

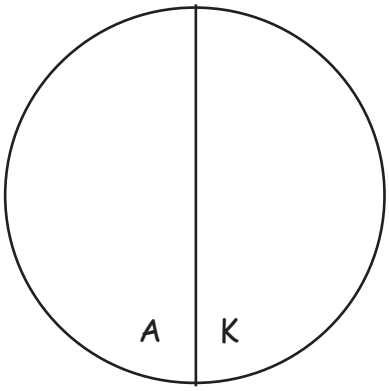
PROCEDURE

1. Label one Petri plate #1 and the other #2. Draw a line down the middle of each plate, label one side "A" for Ampicillin and the other side "K" for Kanamycin. Be sure to label both Petri plates with your initials and your partners initials.
2. Using a sterile spreader, spread the tube of bacteria #1 on plate #1.
3. Using a sterile spreader, spread the tube of bacteria #2 on plate #2.
4. Using a sterile toothpick, carefully remove the Ampicillin pill from the "A" tube and place in the "A" area of plate #1.
5. Using a second sterile toothpick, carefully remove the Kanamycin pill from the "K" tube and place in the "K" area of plate #1.
6. Place an "A" pill and a "K" pill on plate #2. Be sure to use a separate sterile toothpick for each pill.
7. Allow the plates to grow at 37°C (98.6°F) overnight.

What do you predict the results will look like?



mm294



Amp resistant mm294