Topic 8: Body Systems – 8b. Immune System
8b1. Antibiotics and Disinfectants

Resources:

Miller, K., Levine J. (2004). *Biology.* Boston, MA: Pearson Prentice Hall.

Gilroy, W. Researchers analyze how new anti-MRSA antibiotics function [Internet]. Office of News and Information, University of Notre Dame. 27 July 2008. Available from: http://newsinfo.nd.edu/news/9563-researchers-analyze-how-new-anti-mrsa-antibiotics-function

Building on:

Antibiotics and *disinfectants* are used to kill and prevent the *growth of bacteria.* The means by which they work can differ widely. Many disinfectants work in a way that will not only harm *prokaryotic cells* like bacteria, but they will harm *eukaryotic cells* like you. For that reason, some disinfectants can only be used on *abiotic* structures like tables, door knobs, etc. Other disinfectants can be used *topically,* on our skin, but not in our bodies. A few disinfectants can be used in our bodies in limited amounts. Antibiotics work on prokaryotic bacteria. Different antibiotics kill prokaryotes different ways, so specific antibiotics are more suitable for certain bacterial infections.

Many bacteria have *mutated* to form *antibiotic resistant strains.* This has forced the health care industry to find new forms of antibiotics and to alter older antibiotics to maintain effectiveness. Many of these newer antibiotics produce *side effects* like diarrhea and stomach cramping.

Links to Chemistry and Physics:

Enzyme: competitive and non-competitive inhibitors
Molecular interaction of membranes
Disruption of chemical pathways

Stories:

There has been a lot of publicity about antibiotic resistant bacteria in the last couple of years, but the majority of the attention is on MRSA (methicillin-resistant *Staphylococcus aureus*). MRSA was first noticed in the United Kingdom back in 1961. Until the mid-1980s, it was primarily confined to hospitals, prisons, and nursing homes. Recently, MRSA has been seen in the general population with greater frequency. It is estimated that MRSA is responsible for about 20,000 deaths in the United States each year.

Most strains of *Staphylococcus aureus* are sensitive to antibiotics from the penicillin family; methicillin is one of these. The antibiotics work by disrupting the formation of cross-links in the bacterial cell wall that give the cell wall strength and allow bacteria to live in a hypotonic environment without swelling and rupture. To catalyze the formation of these cross-

links, the bacteria use an enzyme known at PBP2a. PBP2a is also compatible with the penicillin-type antibiotics, which attach to the enzyme and inactivate it, preventing the formation of the cross-walls.

Shahriar Mobashery of Notre Dame University discovered that in MRSA the PBP2a enzyme can exist in two forms, open and closed. The closed form in resistant to the antibiotics and the enzyme only opens when it is needed. Using this knowledge a new class of antibiotics, called Cerexa antibiotics, are being developed. These antibiotics fool the closed form of PBP2a into opening and then the antibiotic attaches to the enzyme and inactivate it permanently.

A full paper explaining his research was written by Shahriar Mobashery and published in the July 16, 2008 issue of *the Journal of the American Chemical Society.*

Materials for the Lab:

- Sterile agar Petri dishes
- Permanent makers
- Bunsen burners
- Inoculating loops
- Antibiotic discs (These can be ordered through biological supply company.)
- Bacteria (Use a common bacteria like *Bacillus subtilis,* which can be ordered from a biological supply company.)
- Circles of filter paper (Cut filter paper with a hole puncher.)
- Dishes with iodine
- Bleach (Dilute 50/50.)
- Hydrogen peroxide (from the grocery)
- Dish detergent (like Dawn)
- Incubator (Can be incubated at room temperature.)
- Rulers

Instructions for the Teacher:

It is a good idea to demonstrate sterile technique for the class before they begin. Show them how to flame the inoculating loop and how to spread the bacteria without digging into the agar. Impress on the students that when they spread the bacteria, they won't see much, but given time, the bacterial will reproduce and become visible.

The paper circles don't need to be sterile; after all, you are going to be dipping them into a substance that you hope will inhibit bacterial growth. Since there will be some unknown bacteria on the paper, it is a good idea to tape the dishes shut with Scotch tape (just a small piece on each side of the dish) to prevent the dishes from opening after the incubation period.

Dispose of the dishes by autoclaving them first, if possible. If not, wrap them in a couple of plastic trash bags before you place them in the garbage.

This lab can be modified to test different kinds of antibiotics and no disinfectants or only disinfectants and no antibiotics. The antibiotics should be stored in the refrigerator when not being used and extras can be used the next year.

Antibiotic/Disinfectant Lab

The war against bacteria can take many forms. Advertised products like disinfectants, mouthwashes, and even hand soaps may help rid a person of bacteria. More commonly, it is an antibiotic prescribed by a physician that will kill bacteria. In this lab, you will compare the stopping power of antibiotics and disinfectants.

Procedure:

1. Obtain a sterile Petri dish and use a permanent marker to label the bottom of your dish with your name and period number in tiny letters.
2. Next, use the marker to divide the bottom of the dish into four equal sections; number the sections 1-4.
3. Using the sterile technique demonstrated in class, streak the entire plate with the designated bacteria sample.
4. When the plate has been inoculated with your assigned bacteria, use forceps to place a disc with antibiotic or disinfectant into a specific quadrant of your plate.
5. Use the forceps to lightly tap the disc into place.
6. Re-flame the forceps between trials. You need to use one antibiotic disc, one disinfectant substance, and the third is your choice.
7. When finished, place your dishes in the designated area.
8. After 24-48 hours of incubation, observe your plate for clear zones around the disc.
9. Measure the diameter of each zone in mm and record this on the Class Data Table and your Evidence Table. These open areas are called zones of inhibition.

Hypothesis: Which of your substances do you think will stop bacterial growth the best? Why?

Evidence:

1. You must construct an Evidence Table that will represent your data and the cumulative data from the class. It should indicate each type of substance used to inhibit bacterial growth and the average diameter of the zone of inhibition from the class data as well as your individual findings.
2. A drawing of your dish after incubation with the substances used in each quadrant labeled

Analysis:

1. What do the clear areas indicate?

2. Which substance did the best job controlling the bacteria?

3. What is the appearance of the control disc?

4. What evidence do you have that the inhibition of bacterial growth is due to the chemicals on the discs rather than the discs themselves?

5. Based on the class evidence, what would be the best substance to use on a wound?

6. Write a conclusion, an error analysis, and a next logical question.