Where Will Our Next Antibiotic Come From? Investigating the Effects of Plant Extracts on the Growth of *E. coli*

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Table of Contents:

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In April 2011, the World Health Organization adopted "Combating Drug Resistance and its Global Spread" for its World Health Day theme. With this recent urgent call for global action and after working at an immunology research laboratory through the American Association of Immunologists High School Teachers Summer Research Program in Immunology, I developed science curriculum that develops students' understanding of basic human immune responses, how antimicrobial resistance (AMR) develops and how the discovery of new therapies can alleviate problems associated with AMR. Students will perform internet-based research on antimicrobial resistance public health issues and a hands-on experiment to discover if certain plant extracts have antimicrobial properties. Upon completion of the curriculum, students will not only understand the human immune system response and how antimicrobial resistance presents the world with a looming public health issue, but students will also demonstrate, first hand, the process of drug discovery and research. The ultimate goal of this curriculum is to help students understand how science touches their everyday lives and to provide students with the opportunity to learn about the exciting field immunology.

I. Teacher Section

Intoduction

In early 2011, the World Health Organization chose fighting antimicrobial resistant as the theme for its annual World Health Day. So what is it? Why should students and teachers care about antimicrobial resistance? Antimicrobial resistance occurs when an organism (i.e. bacteria, viruses, and some parasites) becomes resistant to an antimicrobial medicine that had previously been effective at fighting the infection caused by the organism. Resistant organisms are able to survive treatments (e.g. antibiotics, antivirals, and antimalarials). Normal treatment protocols become ineffectual, and infections continue to flourish leading to a prolonged illness and possible death. Currently, there are 440,000 cases world-wide of multi-drug resistant tuberculosis every year causing over 150,000 deaths. Resistance to antimalarial drugs has become common in countries where malaria is endemic. A majority of hospital-acquired infections are caused by an antibiotic resistant form of *Staphylococcus aureus.* Needless to say, antimicrobial resistance is a global concern that is in need of innovative treatments. Without new treatments, the world will see a rise of more drug-resistant "super bugs" creating a worrisome future, especially for our public health.

What are drug companies doing about this problem? Unfortunately, pharmaceutical companies are not putting much effort into creating new antibiotics. Between 2004 and 2007, only 5 new antibiotics were approved by the Food and Drug Administration (Timmerman, 1). Through this curriculum, students will be introduced to the topic of antimicrobial resistance (AMR). They will learn the basics of how our bodies fight off infection via innate and adaptive immunity, why antibiotics are becoming resistant, and finally identify whether certain plant extracts contain antimicrobial properties in an attempt to model how a new drug may be discovered. This series of lessons can be incorporated into general biology, biotechnology and AP biology courses, and the lessons are particularly useful when the class is studying the body's immune system, evolution, genetic mutations, drug discovery, or experimental design.

California Content Standards

The following California Content Standards are addressed in this unit:

Cell Biology

1. The fundamental life processes of plants and animals depend on a variety of chemical reactions that occur in specialized areas of the organism's cells. As a basis for understanding this concept:

c. Students know how prokaryotic cells, eukaryotic cells (including those from plants and animals), and viruses differ in complexity and general structure.

Genetics

2. Mutation and sexual reproduction lead to genetic variation in a population.

4. Genes are a set of instructions encoded in the DNA sequence of each organism that specify the sequence of amino acids in proteins characteristic of that organism. As a basis for understanding this concept:

c. Students know how mutations in the DNA sequence of a gene may or may not affect the expression of the gene or the sequence of amino acids in an encoded protein.

Evolution

7. The frequency of an allele in a gene pool of a population depends on many factors and may be stable or unstable over time. As a basis for understanding this concept:

c. Students know new mutations are constantly being generated in a gene pool.

8. Evolution is the result of genetic changes that occur in constantly changing environments. As a basis for understanding this concept:

a. Students know how natural selection determines the differential survival of groups of organisms

Physiology
10. Organisms have a variety of mechanisms to combat disease. As a basis for under-standing the human immune response:
b. Students know the role of antibodies in the body's response to infection.
d. Students know there are important differences between bacteria and viruses with respect to their requirements for growth and replication, the body's primary defenses against bacterial and viral infections, and effective treatments of these infections.

Science Background

This unit of study is designed to help high school students discover the facts of antimicrobial resistance, study the function of the human immune system, and carry out an experiment to find out if ordinary plant extracts may be our defense against resistant bacteria. Prior to beginning this unit, students should understand the concept that antibiotics aren't always effective in treating infections. Student should have a basic knowledge of immunity (Note: This doesn't have to be in depth knowledge, just an awareness of the fact that we are surrounded by microbes, and yet we aren't sick often). You will be providing the student with a lecture regarding human immunity to help fill in their understanding. An excellent resource to introduce the concept of multi-drug resistance is a PBS video on Teacher's domain (http://www.teachersdomain.org/resource/tdc02.sci.life.evo.whymatters/ If this link does not work, you can go directly to www.teachersdomain.org and search for multi-drug resistance in order to locate the video.)

Student Outcomes

The concepts in this unit are antimicrobial resistance as a public health concern, an introduction to the human immune system, and an investigation lab that will allow students to see if certain plants (of their choosing) possess any antimicrobial properties. Students will complete a web-based research project, take pre- and post quizzes, complete a lecture worksheet, and perform all lab procedures and analyze their results.

Learning Objectives

Students will:

1. Know how antimicrobial resistance (AMR) develops.
2. List the causes of AMR.
3. Identify multinational task forces and other agencies committed to stopping AMR.
4. Work cooperatively with other classmates to create an oral presentation highlighting key points of AMR.
5. List several ways to prevent or lessen AMR.
6. List and describe the two main defense mechanisms of the human immune response: Innate immunity and acquired immunity.
7. List the major components of the immune system.
8. Perform a lab investigation as to the antimicrobial effects of various plant extracts on E. coli.
9. Compile class results for the lab experiment and prepare a graphic representation of the class results.
10. Identify if a plant has antibacterial properties.
11. Communicate their knowledge of AMR orally in a class presentation of their experiment.
12. Describe how a scientist may discover a new drug.

Time Requirements

This unit will require up to 10, 55-minute class periods to complete. If the students have home-access to the Internet, the computer WebQuest may be performed as a combination of class work and home work. The WebQuest should take 2 class periods to complete. The immune system lecture is one class period, and the investigation lab requires five days to complete. Do not feel as if you have to perform every portion of this unit. If all you want to do is the investigative lab, you can do so. Make the lessons work for you and your students.

Advance Preparation

Teachers should make certain that they have access to a computer lab if they are going to complete the WebQuest. The WebQuest can be found at http://zunal.com/process.php?w=157039; however, if you are unable to access this website, the information is provided in the Appendix. You will need to obtain E. coli to make the bacterial culture. You can obtain E. coli through Sargent-Welch, Catalog No. WLBPAP9751 (~$55.00). Note: If you have contact with a college biology lab, you can always ask if they can provide you with an aliquiot of bacterial culture to avoid any expense. Sterile packs of petri dishes can also be purchase through Sargent-Welch (Cat. No. WLS26028) or any other science supply provider. Please note that when students are gathering their plants, have them choose plants that are known to be non-toxic to humans (this is why I chose herbal plants to make extracts. These plants are common, non-toxic, and easy to find. Also, many stores sell herbal extract drops in the health food section. I purchased several and posed the question: How do you know that the extract works (what is efficacy)? These could be used to save time, however; making an extract is a better. You could even use the store extracts and compare results to the student extracts if you want to expand the investigation.

Materials and Equipment

Computers with Internet access

Lab Activity:

Making Plant Extracts Material

Balance, weight boats and lab scoop
Mortar and pestle (or mini food processor)
11 cm filter paper
Funnel
125 media bottle or flask
shaker or spin plate/spin magnets
tap water
5 g of herbs (leaves only) (i.e. rosemary, lavender, oregano, basil, thyme, etc.)

CAUTION! Do not use any plants that are known to be poisonous! MAKE CERTAIN THAT ALL PLANTS HAVE BEEN IDENTIFIED – if you don't know what it is, don't use it. WEAR GOGGLES AND GLOVES WHEN HANDLING PLANT MATERIAL.

Media Preparation and Sterilization Materials

Tabletop balance	glass rods	lab marker pens
Weight boats	magnetic stir bars	LB broth base
Lab scoops	hot plate with stirrer	250 mL beaker
LB agar base	hot hands protector	125 mL media bottle
1 L media bottle	500 mL media bottle	sterilizer/autoclave

Pouring Agar into Petri plate using Sterile Technique Materials

Petri plates	Bunsen burner or laminar flow hood
Prepared, sterilized agar	
10% bleach solution	

Using Plant Extracts for Antimicrobial Assay Materials

Prepared Plant Extracts	1 mL Pipette	Incubator 37°C
Glass spreader	forceps	E. coli broth
Filter paper disks 5mm diameter	Neosporin ointment	Prepared agar plates

Student Prior Knowledge and Skills

Student should have a solid background in basic laboratory procedures and techniques. They should also be completely knowledgeable with laboratory safety and measurement procedures. Students should also have a fundamental understanding of evolution (adaptations), genetic (mutations), and how diseases are communicable.

Student Expectations

Students are expected to learn about Antimicrobial Resistance: how it occurs, what are some of the consequences of AMR, and how it is affecting our world. They will also learn an introduction to the human immune system. Students will also be expected to organize their experiments, run it according to instructions, and analyze the results. It is important that students are able to both verbally and visually communicate their understanding of their laboratory experiment results.

Assessments:

Students are assessed on their ability to master learning objectives for each section of this unit. Assessments include: completion of WebQuest tasks, accuracy in answering content questions (answers for student questions are found in the appendix), communicate their laboratory results both orally and visually in the form of a table or chart prepared by the student, and formative assessments, which are pre- and post-test imbedded in the WebQuest.

References:

http://www.cdc.gov/getsmart/antibiotic-use/antibiotic-resistance-faqs.html#a
http://www.who.int/mediacentre/factsheets/fs194/en/
http://www.cdc.gov/drugresistance/index.html
http://wwwn.cdc.gov/dls/master/ams/intro/open.swf
http://www.niaid.nih.gov/topics/antimicrobialresistance/understanding/pages/default.aspx

Murphy, Kenneth, et al. *Janeway's Immunobiology, seventh edition*. New York and London: Garland Science. 2008. Print.

I. Student Section

Antimicrobial Resistance: Why are antibiotics becoming ineffective?

Welcome!

Have you ever looked at sneeze in slow motion (at some point in your life, you need to, but not this second)? Or maybe you've grown cultures of bacteria taken from door handles or toilet seats? If you have, then you've seen just how surrounded we are by microbes ... those tiny, unseen creatures that at times can make us miserably sick. If you haven't, you still know how easy it is to get an infected cut if you don't wash it properly or catch a cold when your little brother sneezes all over you. Bacteria are all around us, on us and in us too. Some bugs (a cute name scientists call bacteria) are helpful and even essential--like bacteria in our intestines that help us digest food. But others, well other bacteria are responsible for causing huge gaping wounds that won't heal and can eat away our flesh, for spreading sexually -transmitted diseases, like gonorrhea, that won't get better with medical treatment, or for causing infections that can flat out kill you. It all sounds so scary, but, what about antibiotics? Wasn't penicillin the miracle drug that saved thousands and thousands of lives? The short answer is yes, antibiotics are life-saving medications that when used correctly can speed us along to a rapid recovery. But here's the situation, some bacteria are now becoming resistant to antibiotics. What does that mean? Well, that's what this WebQuest is about.

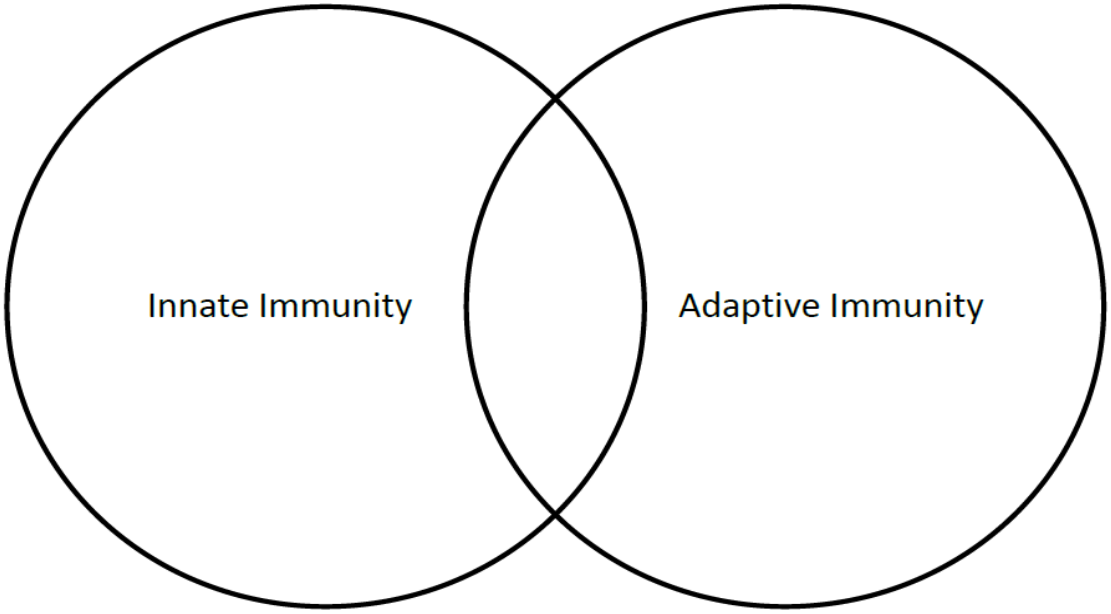
Go to: http://zunal.com/webquest.php?w=157039

Start on the “Welcome Page,” and follow the directions. Be certain that you complete all of the required tasks.

Lecture Notes Work Sheet

Name:_____

Date:_____



List Key Concepts of the Innate Immune System:	List Key Concepts of the Adaptive Immune System:

Where Will Our Next Antibiotic Come From?

Investigating the Effects of Plant Extracts on the Growth of *E. coli*

Everything living thing on Earth competes for resources for survival such as food, water, habitat and mating. Plants, specifically, compete with other plants for water, space to grow and light for photosynthesis. Over time, plants have adapted traits like broad leaves, thorns, and deep root systems as advantageous adaptations to help ensure survival. Certain plants actually produce toxins that leach into the surrounding soil to inhibit growth of other nearby plants in a process called allelopathy. This property is supported by the production of chemicals that can affect the growth and survival of target organsims.

All organisms, including plants, can be infected by bacteria or viruses. Most multicellular organisms have innate defense systems that will control or eliminate foreign invaders. Such a system exists in humans and is linked and complemented by an adaptive immune system. The innate immunity relies heavily on the local production of antimicrobial agents to fight infection. The medical community has exhausted the variety antibiotics, and we are now confronted with an increasing number of antibiotic-resistant pathogens, one key goal for scientists is to find and isolate novel antimicrobial molecules that use novel mechanisms of action in order to develop new anti-infectious therapies.

The task of isolating molecules with antimicrobial properties is not an easy one. First you have to identify and collect samples that are suspected of containing an antimicrobial molecule. Once the samples are collected, a scientist must process the samples and collect an extract. After this, the extract can be tested to determine its efficacy or ability to kill a variety of microbes. Finally, if antimicrobial activity is found, researchers must associate this activity to a particular molecule, isolate it, and evaluate its toxicity in animal models and then humans.

In the most simple scenario to test a plant extract, extract-soaked filter paper is placed into a growing a bacterial culture in a Petri dish. If the extract contains antimicrobial activity, there will be an area of inhibition of bacterial growth or halo around the disk. The extracts that inhibit bacteria growth will then be purified further and separated to determine a single compound. Examples of these types of compounds would be antiseptics, astringents, antibiotics, and toxins.

NOTE: You will be working in groups of three or four individuals.

DAY 1: MAKING PLANT EXTRACTS

Materials:

Balance, weight boats and lab scoop	125 media bottle or flask
Mortar and pestle (or mini food processor)	shaker or spin plate/spin magnets
11 cm filter paper	tap water
Funnel	5 g of herbs (leaves only) (i.e. rosemary, lavender, oregano, basil, thyme, etc.)

CAUTION! Do not use any plants that are known to be poisonous! MAKE CERTAIN THAT ALL PLANTS HAVE BEEN IDENTIFIED – if you don't know what it is, don't use it. WEAR GOGGLES AND GLOVES WHEN HANDLING PLANT MATERIAL. (herbs work great)

1. Weigh out 5-10 g of plant leaves (avoid any woody stems).
2. Grind up the plant material with the mortar and pestle for at least five minutes until completely homogenization. Add all plant material into flask. (If you are using a mini food processor, put plant material and distilled water into unit and process into a slurry.)
3. Add 100 mL of water to the ground plant material.
4. Add magnetic stir bar to flask and place flask on a stir plate for 30 minutes. (You can also use a shaker.)
5. Remove flask from the stir plate, and let stand overnight to infuse into the tap water.
6. Place filter paper into a funnel. (Fold the filter paper in half, and then half again to form a triangle. Open the paper so it resembles a cone and place it in funnel.)
7. Place the funnel with the filter paper onto a 125 mL media bottle with cap.
8. Pour the liquid and plant material from the flask into the funnel and filter to remove particulate matter. Cap the bottle and place the bottle in a refrigerator until needed.

DAY 2: MEDIA PREPARATION EQUATION and STERILIZATION

Materials:

Tabletop balance	glass rods	lab marker pens
Weight boats	magnetic stir bars	LB broth base
Lab scoops	hot plate with stirrer	250 mL beaker
LB agar base	hot hands protector	125 mL media bottle
1 L media bottle	500 mL media bottle	sterilizer/autoclave

Media Prep Equation:

$$\frac{Mass_1}{Volume_1} = \frac{Mass_2}{Volume_2}$$

M_1 = the mass of LB agar or broth base to use (See label for amount. It will be shown in grams.)
$Volume_1$ = the volume of solvent (dH_2O) for label recipe (usually 1 L)
$Mass_2$ = the mass of media base needed for the desired volume
$Volume_2$ = the desired final volume of media (what you'll need to make your plates)

Example: The information on the Difco™ LB Agar label directs the technician to suspend 35 g of powder into 1 L of purified water and mix. For an experiment, only 300 mL of prepared media are needed. What amount of media base is required to make 300mL of solution?

Step 1 – convert 1 L to 1000 mL
Step 2 – write out formula
Step 3 – make calculation

35 g/1000mL = M_2/150 mL (now solve for M_2)

$$M_2 = \frac{35g \times 150}{1000}$$

M_2 = 5.25 g of media dissolved in 300 mL of dH_2O to make the final volume

Procedure: Part 1 - Preparation of 300 mL of LB Agar for plates

- 300 mL is the maximum amount of agar that can be prepared in a 1 L media bottle.
- Use the media preparation equation to determine the proper amount of media base.

Show Calculation here:

1. Use a clean 1 L media bottle with screw cap. Label with your media type, date, and your initials. (The bottle is to be sterilized.)
2. To mix the media, use a 500 mL glass beaker.
3. Measure out on a scale the amount of LB agar base needed for the 300 mL of volume needed. Pour the LB agar base into the clean 500 mL glass beaker.
4. ***Very slowly***, add 200 mL of distilled water, stirring as it is added. The water should, at first, make a paste. As more water is added, the agar base will become suspended in solution.
5. Add the remaining 100 mL of water for a total of 300 mL of suspended agar.
6. Carefully slide a magnet into the agar solution. Place the beaker on a stirring, hot plate.
7. Heat on high, gently stirring the entire time until JUST before the solution boils. DO NOT LET IT BOIL.
8. Using the hot hands protector, remove the beaker from the hot plate and pour the hot agar suspension into the labeled, clean, 1 L media bottle. ***Very loosely cap the bottle (made sure it jiggles, but remains on the bottle). This will allow pressure to be release during sterilization.***
9. Place the bottle into a pressure sterilizer or autoclave. Bolt down the pressure cooker's top of autoclave's door as directed by your teacher. (**Make certain that the teacher checks your sterilizer. Be sure to follow all of the manufacturer's directions for operation.**)
10. Heat the bottles until the pressure gage reaches 15 PSI.
11. Keep the bottle at 15 to 20 psi for 15 to 20 minutes. Cool the agar to approximately 56°C (cool enough to be able to hold the bottle). If time, continue to Day 2. If you don't have time, it's OK to allow the agar to solidify in the bottle. You can reheat the agar in a microwave at 50% power for approximately 4 minutes in order for it to liquefy. If the bottle won't fit, use a hot water bath at 100° C. Be careful that if you reheat the agar, it becomes completely clear and no opaque fragments are seen in the liquid.

DAY 3: STERILE TECHNIQUE AND POUR PLATES

Materials:

10 Petri plates
Prepared, sterilized agar
10% bleach solution
Bunsen burner or laminar flow hood

1. Disinfect work surface and hands with 10% bleach solution, 70% ethanol solution or a commercial disinfectant.
2. Pouring of the plates should be in an area with little or no air currents.
3. Label the Petri plates on the bottom edge with the media name (LB Agar), initials and date.
4. Place the labeled Petri plates in stacks of three plates high.
5. Remove the bottle cap and "flame" the opening. (Flame means to wave the bottle opening through a flame of a Bunsen burner approximately 3 times)
6. Open the bottom Petri plate lid of the first stack. Pour agar over one-half of the dish's height. Tilt the plate to cover the bottom with agar and put the lid back.
7. Repeat pouring the remaining plates. Stack groups on top of each other.
8. Leave the plates to solidify for at least 15 minutes. Be certain to not disturb the plates.
9. Allow the plates to dry in a clean area for a minimum of 24 hours before using. (Plates are good for approximately two weeks. Be sure to store in a cool, dry place.)

DAY 4: SETTING UP ANTIMICROBIAL ASSAY

Materials:

Prepared Plant Extracts	1 mL Pipette	Incubator 37°C
Glass spreader	forceps	E. coli broth
Filter paper disks 5mm diameter	Neosporin ointment	Prepared agar plates

1. Label bottom of plate with the name of plant extract, your name, and date.
2. Obtain an empty Petri plate to use for each type of extract. Place 2 of the paper disks into an empty Petri plate (no agar added). Add 1 mL of filtered plant extract onto the paper discs and allow them to soak up liquid.
3. Using a sterile pipette, add 1mL of the E. coli broth (provided by instructor) to the middle of an agar filled Petri dish.
4. Sterilize glass spreader (using alcohol and allow to dry), and spread out bacterial culture evenly around the plate. Cover plate and allow the culture to soak into the agar for a minimum of 15 minutes.
5. Using sterile forceps (spray with alcohol and allow it to dry), carefully pick up one plant extract soaked disc. Place the disc in the center of the correctly labeled Petri plate. (Forexample, place a disc soaked in rosemary extract in the Petri plate labeled for rosemary extract.)
6. Repeat step 5 twice in order to have 3 replicate Petri plates of each plant extract.
7. For the negative control, add a disc soaked in water into the center of a bacterial culture covered plate. For a positive control, add a disc covered with Neosporin into the center of a bacterial culture covered plate.
8. Allow a few minutes for extracts to soak into agar.
9. Make certain that the discs are adhered to the surface of the agar plate. Cover plate and invert, so the lid is down. Place into a 37°C incubator and incubate for 24 to 48 hours.

DAY 5: ANALYSIS OF ANTIMICROBIAL ASSAY

1. Take plates out of incubator. Examine the plates for a zone of inhibition around the disc.

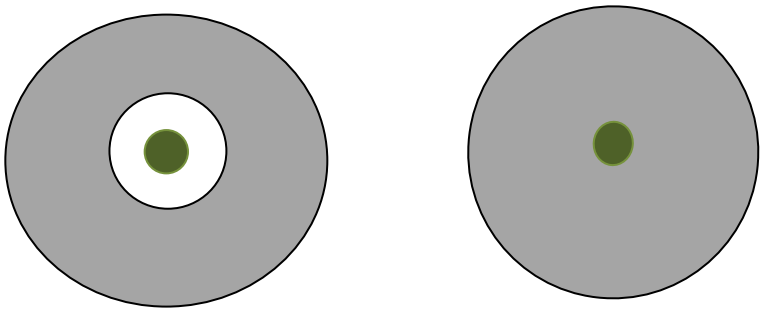


Figure 1 *– Antimicrobial Ring of Inhibition*
Look at the example on the left. The clear area was formed around the disc by an inhibitory substance in the plant extract. The example on the right shows no ring of inhibition; therefore,the plant extract contained no antimicrobial properties.

2. Photograph or draw the plates. Label any region of inhibition of bacterial growth on the picture.
3. Using a ruler, measure and record the size of the ring of inhibition, if present. Average the size of the ring of inhibition of each replicate.
4. Create a data table to collect and present data. Calculate the average size of ring of inhibition for all groups in the class.

Sample Table:

Name of Extract	Ring of Inhibition size for Replicate 1	Ring of Inhibition size for Replicate 2	Ring of Inhibition size for Replicate 3	Average size of ring of inhibition (mm)

5. Based on your data, determine which extract should be considered as a source of potential antimicrobial medicine.

Group 1

Name of Extract	Ring of Inhibition size for Replicate 1	Ring of Inhibition size for Replicate 2	Ring of Inhibition size for Replicate 3	Average size of ring of inhibition in (mm)

Group 2

Name of Extract	Ring of Inhibition size for Replicate 1	Ring of Inhibition size for Replicate 2	Ring of Inhibition size for Replicate 3	Average size of ring of inhibition in (mm)

Group 3

Name of Extract	Ring of Inhibition size for Replicate 1	Ring of Inhibition size for Replicate 2	Ring of Inhibition size for Replicate 3	Average size of ring of inhibition in (mm)

Group 4

Name of Extract	Ring of Inhibition size for Replicate 1	Ring of Inhibition size for Replicate 2	Ring of Inhibition size for Replicate 3	Average size of ring of inhibition in (mm)

Group 5

Name of Extract	Ring of Inhibition size for Replicate 1	Ring of Inhibition size for Replicate 2	Ring of Inhibition size for Replicate 3	Average size of ring of inhibition in (mm)

Group 6

Name of Extract	Ring of Inhibition size for Replicate 1	Ring of Inhibition size for Replicate 2	Ring of Inhibition size for Replicate 3	Average size of ring of inhibition in (mm)

Group 7

Name of Extract	Ring of Inhibition size for Replicate 1	Ring of Inhibition size for Replicate 2	Ring of Inhibition size for Replicate 3	Average size of ring of inhibition in (mm)

Class Data

Average Size Ring of Inhibition (mm) for each Group								
Extract Name	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Class Avg.

Student Project

You are a scientist at a large pharmaceutical company. You've been assigned the task of finding a new antimicrobial agent to fight a strain of *E. coli* that has become resistant to our current range of antibiotics. You have worked with your team and believe that you have found a promising candidate.

Your assignment, create a PowerPoint presentation to the head of the company making the recommendation to use a plant extract you've recently discovered to have amazing antimicrobial properties.

What to include in your presentation:

- The name of your plant extract (Find out the scientific name of your plant.)
- Explanation as to why you believe this extract will work
- Data that supports your claim of antimicrobial activity. (Use data from our experiment for this support, i.e. picture of your Petri dishes, ring of inhibition size, etc.)
- Projected time line … How long will it take to make a new drug and bring it to market? (You will need to do a little research on this part. Go to the FDA webpage: http://www.fda.gov/drugs/developmentapprovalprocess/default.htm You can find out what is required for a drug to be developed and brought to market.

Presentation Rubric

Evaluating Student Presentations					
	1	**2**	**3**	**4**	**Total**
Organization	Audience cannot understand presentation, because there is no sequence of information.	Audience has difficulty following presentation, because student jumps around.	Student presents information in logical sequence, which audience can follow.	Student presents information in logical, interesting sequence, which audience can follow.	
Subject Knowledge	Student does not have grasp of information. Student cannot answer questions about subject.	Student is uncomfortable with information and is able to answer only rudimentary questions.	Student is at ease with expected answers to all questions, but fails to elaborate.	Student demonstrates full knowledge (more than required) by answering all class questions with explanations and elaboration.	
Graphics	Student uses superfluous graphics or no graphics	Student occasionally uses graphics that rarely support text and presentation.	Student's graphics relate to text and presentation.	Student's graphics explain and reinforce screen text and presentation.	
Mechanics	Student's presentation has four or more spelling errors and/or grammatical errors.	Presentation has three misspellings and/or grammatical errors.	Presentation has no more than two misspellings and/or grammatical errors.	Presentation has no misspellings or grammatical errors.	
Eye Contact	Student reads all of report with no eye contact.	Student occasionally uses eye contact, but student still reads most of the report.	Student maintains eye contact most of the time but frequently returns to notes.	Student maintains eye contact with audience and seldomly returns to notes.	
Elocution	Student mumbles, incorrectly pronounces terms, and speaks too quietly for students in the back of class to hear.	Student's voice is low. Student incorrectly pronounces terms. Audience members have difficulty hearing presentation.	Student's voice is clear. Student pronounces most words correctly. Most audience members can hear presentation.	Student uses a clear voice and correct, precise pronunciation of terms. All audience members can hear the presentation.	
				Total Points:	

III. Appendix

PowerPoint Lecture

The Human Immune System

Lori Herrington
Chaparral High School

What is immunology?

- Very simply, it is the study of our body's defense against infection and disease.

Even though we are constantly bombarded by microorganisms, we rarely become sick. Why?

Why?

- The reason why we rarely become ill is because our bodies have a built-in defense system. It's called our *immune system*.

So what is an immune response?

- When an invader or *pathogen* enters our body, the responses we make against infection is called an *immune response*.
- Pathogens are microorganisms like bacteria, viruses, fungi, or parasites. Each has the potential to making us ill.

Components of the immune system

- Bone marrow
- Thymus
- Spleen
- Lymph nodes
- Adenoids
- Tonsils
- Peyer's patches
- Appendix
- Lymphatic vessels

Defense Against Infections & Disease

- Our bodies are always ready to defend itself against invasion—the first line of defense is our skin and other bodily secretions (mucus, tears, sweat and saliva)
  - Mucus acts as a trap where microorganisms are swallowed and then digested
  - Tears, sweat and saliva all contain lysozyme, an enzyme that can break down the cell wall of some bacteria.

Innate Immunity

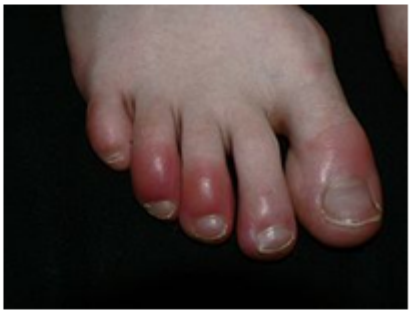
- *Innate immunity* prevents pathogens from entering the body, but, if they do, our bodies eliminate the pathogen before the occurrence of disease or infection.
- *Characteristics of Innate Immunity*
  - Present from birth
  - Non-specific
  - Does not become more effective with more exposure to pathogen

Innate Immunity continued…

- *The first cells to respond after invasion are phagocytic cells, like macrophages or neutrophil*
  - *These cells produce toxic chemicals or are able to ingest & kill microbes.*
- *Phagocytes and other proteins (like opsonin) are located mostly in blood*
  - *Inflammation is the means by which these elements are recruited to the tissue invasion site*

Inflammation

- Inflammation is characterized by four symptoms:
  - Redness
  - Swelling
  - Pain
  - heat



This occurs when damaged tissue and white blood cells (basophils), release histamine. **Histamines,** released from **mast cells,** cause the blood vessels to dilate allowing the tissue to become more permeable to tissue fluid.

More on inflammation…

- Inflammation is localized to the area of infection/tissue injury by the release of substances from micro-organisms or chemicals (chemical mediators) released from cells in tissues.
  - e.g. histamine from **MAST CELLS**: Once the micro-organisms are destroyed, inflammation subsides.

Adaptive Immunity

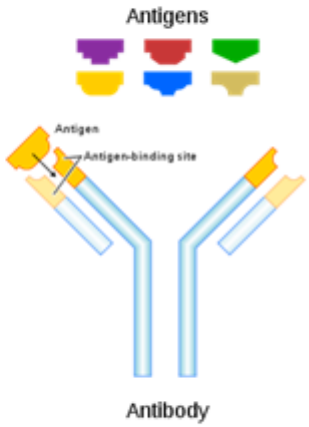
- If an infection continues, then another part of the immune system is mobilized. This is our adaptive or acquired immunity.
- An adaptive response occurs when our body recognizes an antigen (something other than our self) and produces antibodies against it.

More on Adaptive Immunity…

- Adaptive immunity is an antigen-specific defense mechanism and can take several days to become protective.
- Adaptive immunity becomes more effective with more exposures to an antigen.
- There are two major branches of the adaptive immune system:
  - Humoral immunity
  - Cell-mediated immunity

Humoral Immunity

- Involves the production of **antibodies**
  - An antibody is a protein produced by the body when it detects antigens.
  - Each antibody is particular and defends again a specific type of antigen.
  - This response is mediated by B-cells.
  - Humoral Immunity Video clip



Video Clip: http://www.youtube.com/watch?v=L32Na8fGjzA

Cell-Mediated Immune Response

- Cell mediated immunity involves cytotoxic or killer T-cells.

- Cell-mediated Immunity video clip

Video clip: http://www.youtube.com/watch?v=1tBOmG0QMbA

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WebQuest

Note: The following WebQuest is located online (http://www.zunal.com/WebQuest.php?w=157039), and the information is also included below.

Welcome: Antimicrobial Resistance -- Why are antibiotics becoming ineffective?
Description: Have you ever looked at sneeze in slow motion (at some point in your life, you need to, but not this second)? Or maybe you've grown cultures of bacteria taken from door handles or toilet seats? If you have, then you've seen just how surrounded we are by microbes ... those tiny, unseen creatures that at times can make us miserably sick. If you haven't, you still know how easy it is to get an infected cut if you don't wash it properly or catch a cold when your little brother sneezes all over you. Bacteria are all around us, on us and in us too. Some bugs (a cute name scientists call bacteria) are helpful and even essential--like bacteria in our intestines that help us digest food. But others, well other bacteria are responsible for causing huge gaping wounds that won't heal and can eat away our flesh, for spreading sexually -transmitted diseases, like gonorrhea, that won't get better with medical treatment, or for causing infections that can flat out kill you. It all sounds so scary, but, what about antibiotics? Wasn't penicillin the miracle drug that saved thousands and thousands of lives? The short answer is yes, antibiotics are life-saving medications that when used correctly can speed us along to a rapid recovery. But here's the situation, some bacteria are now becoming resistant to antibiotics. What does that mean? Well, that's what this WebQuest is about.
Grade Level: 9-12
Curriculum: Science
Keywords: Antimicrobial Resistance and immunology
Author(s): Lori Herrington

Introduction:

In April 2011, the World Health Organization adopted “Combating Drug Resistance and its Global Spread” for its World Health Day theme. With this in mind and after working at an immunology research laboratory through the American Association of Immunologists, I wanted high school science students to understand how antimicrobial resistance (AMR) develops and how new therapies might help stem the problems associated with AMR. You, the student, will perform internet-based research on public health issues of antimicrobial resistance. The goal is to deepen your understanding of the human immune response and how AMR presents a global public health issue.

Task:

YOUR task, if, you accept it, is to discover how antimicrobial resistance (AMR) develops and how AMR currently affects our world. You will need to read current research, analyze current facts about the topic and learn how an amazingly, life-saving medicine like antibiotics can become ineffective in fighting certain bacterial infections. Learn how and why AMR happens and what may happen if scientists can't find a way to stop bacteria.

Process:

Phase 1 - Individual Work

BEFORE YOU BEGIN, TAKE THE PRE-TEST TO FIND OUT WHAT YOU KNOW. Print out your result.

1. Go to the website: *Get Smart: Antibiotic Resistance Questions and Answers* (see link)

Write out and answer the following questions:

1. What are bacteria and viruses?
2. What kinds of infections are caused by viruses and should not be treated with antibiotics?
3. What is an antibiotic?
4. What is antibiotic resistance?
5. Why should I be concerned about antibiotic resistance?
6. Why are bacteria becoming resistant to antibiotics?
7. How do bacteria become resistant to antibiotics?
8. How can I prevent antibiotic-resistant infections?
9. How can healthcare providers help prevent the spread of antibiotic resistance?
10. Are antibacterial-containing products better for preventing the spread of infection? Does their use add to the problem of resistance?
11. Can antibiotic resistance develop from acne medication?
12. Do probiotics have a role in preventing or treating drug resistance or drug-resistant infections?

2. Go to the Website: *World Health Organization (WHO) AMR Fact Sheet*. (See link.)

Read the fact sheet. Write out and answer the following questions:

1. List 6 reasons *why* AMR (antimicrobial resistance) is a global concern.
2. List 6 factors that drive AMR.
3. List 5 ways how the WHO is engaged in guiding the response to AMR.

Watch the Video - Antimicrobial Resistance (see link below)

Phase 2 - Group Work

1. Go to http://www.cdc.gov/drugresistance/index.html (link is below)
2. In the bottom, left-hand corner find the hyperlink to the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) report.

3. Open the report.
4 Go to the Executive Summary page. Read this page. What is the purpose of TATFAR?
5. Go to the Introduction/History section (page 5) Answer the following:

a. What was the first antibiotic introduced in 1940?

b. What does selective pressure exerted by antimicrobial drugs favor? In other words, how have organisms changed in response to being in contact with antimicrobial drugs? What adaptation have bacteria developed to survive?

c. Name two things that could happen in the future because of antimicrobial resistance.

d. How many deaths are there per year in the European Union (EU) from a multi-drug resistant bacterial infection? United States?
e. True or False: There has been an increase in the number of new antibacterial drugs entering the market place over the past few decades.
f. Why won't development of new antimicrobial drug alone suffice to stop the growing resistance problem?
5. Go to the CDC website http://wwwn.cdc.gov/dls/master/ams/intro/open.swf (use this link or the link below). Click on SKIP INTRO. Go through the modules.
6. Use the NIH website as a review page: http://www.niaid.nih.gov/topics/antimicrobialresistance/understanding/pages/default.aspx
7. Take the Post-test. Print out your results.

Check List:
1. Pre-Test completed, printed and turned in.
2. All Phase 1 and Phase 2 questions have been written out, answered and turned in.
3. Post-test completed, printed and turned in.

Evaluation Rubric

	not so hot	getting better	almost perfect	perfection	Score
Completing tasks in Phase 1	Questions were not written out. Answers were not in complete sentences, very few correct answers.	Questions were written out, but answers weren't in complete sentences and mostly correct.	Questions were written out, answers were in complete sentences and mostly correct.	Questions were written out, answers were in complete sentences and all answers were correct.	25
Completed tasks in Phase 2	Questions were not written out. Answers were not in complete sentences, very few correct answers.	Questions were written out, but answers weren't in complete sentences and mostly correct.	Questions were written out, answers were in complete sentences and mostly correct.	Questions were written out, answers were in complete sentences and all answers were correct.	25

Total Score: 50

So you've read about AMR and seen how it is affecting the entire world. This exercise was not meant to scare you, but hopefully, make you want to do something to help effect change. So, think about what you could do to help. I know that you can't go out in your school and create a new antibiotic, but you can spread what you know. Remember to take all your medications as directed, find out about regulations that effect how antibiotic use is monitored in agriculture--you are a citizen and do have a voice. Better yet, go to college and become a scientist ... there is always more to learn about, discover and create.

Teacher Page:

This WebQuest should take 2, 55-minute periods. You should be able to complete phase 1 on day one, and phase 2 on day 2.

Standards

After this WebQuest, your students should be able to:

1. Know how antimicrobial resistance (AMR) develops.
2. List the causes of AMR.
3. Identify multinational task forces and other agencies committed to stopping AMR.
4. Work cooperatively with other classmates to create an oral presentation highlighting key points of AMR.
5. List several ways to prevent or lessen AMR.

California Content Standards for Science (9-12) covered in the WebQuest are as follows:
Cell Biology
1. The fundamental life processes of plants and animals depend on a variety of chemical reactions that occur in specialized areas of the organism's cells. As a basis for understanding this concept:
c. Students know how prokaryotic cells, eukaryotic cells (including those from plants and animals), and viruses differ in complexity and general structure.
Genetics
2. Mutation and sexual reproduction lead to genetic variation in a population.
4. Genes are a set of instructions encoded in the DNA sequence of each organism that specify the sequence of amino acids in proteins characteristic of that organism. As a basis for understanding this concept:
c. Students know how mutations in the DNA sequence of a gene may or may not affect the expression of the gene or the sequence of amino acids in an encoded protein.
Evolution
7. The frequency of an allele in a gene pool of a population depends on many factors and may be stable or unstable over time. As a basis for understanding this concept:
c. Students know new mutations are constantly being generated in a gene pool.
8. Evolution is the result of genetic changes that occur in constantly changing environments. As a basis for understanding this concept:
a. Students know how natural selection determines the differential survival of groups of organisms

Physiology
10. Organisms have a variety of mechanisms to combat disease. As a basis for under-standing the human immune response:
b. Students know the role of antibodies in the body's response to infection.
d. Students know there are important differences between bacteria and viruses with respect to their requirements for growth and replication, the body's primary defenses against bacterial and viral infections, and effective treatments of these infections.

Credits

A special thanks to the members of the American Association of Immunologists who helped me with my quest for knowledge.

This WebQuest was created using information provided by the CDC, World Health Organization and the NIH.

Other

This is a lot of fact finding for students. If you want, you can break the class into groups, divide up the tasks and do a jigsaw where a member from each group shares what they learned with a different group. You could also have the students create a "web collage," where they find pictures and graphics that show what antimicrobial resistance means to them.

Pre-Test

Description: Pre-test knowledge assessment

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1. Antimicrobial resistance is a new, emerging problem in the world

- [x] a) False
- [ ] b) True

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2. What is antimicrobial resistance?

- [ ] a) bacteria that cannot replicate
- [ ] b) bacteria that possess genes that enable the bacteria to grow when antibiotics are present
- [ ] c) bacteria that do not exist
- [ ] d) bacteria that possess genes that cause them to glow
- [ ] e) bacteria that are not harmful and easily treated

---

3. What is the percentage of all hospital-related infections resistant to at least one common drug?

- [ ] a) 10%
- [ ] b) 30%
- [ ] c) 50%
- [ ] d) 70%
- [ ] e) 90%

---

4. AMR (antimicrobial resistance) emerges as a result of all of the following EXCEPT

- [ ] a) natural adaptations
- [ ] b) spontaneous changes in DNA
- [ ] c) when different bacteria exchange genes with each other
- [ ] d) heat induced stress
- [ ] e) mutations

---

5. Factors that play a role in the development of antimicrobial resistance include all of the following EXCEPT

- [ ] a) overuse of antibiotics
- [ ] b) not taking the antibiotics as prescribed (example: stop taking medicine when you feel better and there are pills leftover)
- [ ] c) the widespread use of antibiotics in farm animals
- [ ] d) taking antibiotics when they aren't needed
- [ ] e) keeping hospitals clean

---

6. Antibiotics are used to treat which illness?

- a) colds
- b) flu
- c) bacterial infections
- d) coughs caused by non-bacterial illness
- e) viral infections

---

7. MRSA is a/an _____.

- a) special antibiotic
- b) methicillin-resistant Staphylococcus aureus
- c) a strain of bacteria that is resistant to antibiotics
- d) both B and C
- e) both A and C

---

8. What was the first antibiotic introduced in 1940?

- a) aspirin
- b) penicillin
- c) tetracycline
- d) amoxicillin
- e) azithromycin

---

9. Microbes will always find a way to escape the harmful actions of new drugs.

a) true b) false

---

10. People infected with antimicrobial-resistant organisms are more likely to have longer, more expensive hospital stays, and may be more likely to die as a result of the infection.

a) true b) false

Post-Test

Description: Post-test Description goes here.

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1. People infected with antimicrobial-resistant organisms are more likely to have longer, more expensive hospital stays, and may be more likely to die as a result of the infection

- a) true
- b) false

---

2. Microbes will always find a way to escape the harmful actions of new drugs.

- a) true
- b) false

---

3. What was the first antibiotic introduced in 1940?

- a) penicillin
- b) azithromycin
- c) tetracycline
- d) aspirin
- e) amoxicillin

---

4. MRSA is a/an _____.

- a) methicillin-resistant Staphylococcus aureus
- b) special antibiotic
- c) a strain of bacteria resistant to antibiotics
- d) both A and C
- e) both B and C

---

5. Antibiotics effectively treat

- a) flu
- b) bacterial infections
- c) colds
- d) viral infections
- e) coughs caused by non-bacterial illness

---

6. Factors that play a role in the development of antimicrobial resistance include all of the following EXCEPT

- a) overuse of antibiotics
- b) not taking the antibiotics as prescribed
- c) the widespread use of antibiotics in farm animals

- [ ] d) taking antibiotics when they aren't necessary
- [ ] e) keeping hospitals clean

---

7. Antibiotic resistance emerges as a result of all of the following EXCEPT

- [ ] a) natural adaptations
- [ ] b) spontaneous changes in DNA
- [ ] c) when different bacteria exchange genes with each other
- [ ] d) heat induced stress
- [ ] e) mutations

---

8. What is the percent of all hospital-related infections resistant to at least one common drug?

- [ ] a) 10%
- [ ] b) 30%
- [ ] c) 50%
- [ ] d) 70%
- [ ] e) 90%

---

9. What is antimicrobial resistance?

- [ ] a) bacteria that are not harmful and easily controlled
- [ ] b) bacteria that possess genes that make them glow
- [ ] c) bacteria that do not exist
- [ ] d) bacteria that possess genes that enable the bacteria to grow when antibiotics are present
- [ ] e) bacteria that cannot replicate

---

10. Antimicrobial resistance is not a new, emerging problem in the world

- [ ] a) false
- [ ] b) true
