

## Action Research Proposal

Title: “Eternally Yours”: Using Antibiotic Resistance Experiments to Enhance Inquiry-Based Learning in an A.P. Biology Curriculum.

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**Abstract:** In the re-design of the A.P. Biology curriculum beginning in 2012, much emphasis has been placed on inquiry-based learning. The “dirty-dozen” laboratory activities that were part of the pre-2012 curriculum have been replaced with inquiry-based labs that are incorporated into the 4 Big Ideas, which roughly focus on concepts of ecology, evolution, energy transfer and genetics. One aspect of inquiry-based learning that would be addressed in this research proposal is to investigate the effect of antibiotic resistance in microorganisms. This is an emerging problem in society, due to the fact that in the very near future, individuals may not respond to treatment with today’s antibiotics due to the ability of such pathogens to acquire mutations that enable them to evade the action of antibiotics. Students need to understand the scope of this problem from a genetics, evolutionary, and societal point of view, so they can make informed decisions about treating potential emerging pathogens that pose a threat to the survival of the human population.

**Rationale:** The reason why I chose to do project-based learning, with a focus on antibiotic-resistance pathogens, is very simple: when students are provided with relevant projects in which they have to make decisions which have a future impact on society, the learning process becomes more authentic. The advent of emerging pathogens stemming from the antibiotic resistance of such microorganisms, is, simply put, a scary proposition for our society. “The emergence of multi-drug-resistant bacteria, or ‘superbugs’, poses an imminent threat to our society and has been accelerated by a number of factors, such as the overuse and misuse of existing antibiotics as well as diminishing antibiotic pipelines” (Yosida, M. et.al. *Nature Communications* 8, Article number 15589, 2017). In today’s fast-paced world, our students need to come up with solutions to problems such as this, by doing experiments that focus on how microorganisms become resistant to antibiotics by analyzing the problem from a genetic, evolutionary and medical points of view. One such case of antibiotic resistance is MRSA. The website: [biologycorner.com](http://biologycorner.com) offers a case study on such a problem. I plan to use such activity to introduce the topic. There are other labs which I will use which will be included in the lesson plan section of this proposal. They include one on the evolution of antibiotic resistance found on [www.faculty.virginia.edu/evolutionlabs/Antibiotic\\_Lab\\_Web\\_Page.html](http://www.faculty.virginia.edu/evolutionlabs/Antibiotic_Lab_Web_Page.html), Student Activities Measuring Antibiotic Resistance found at [www.colorado.edu/outreach/BSI/pdfs/resistance.activity.pdf](http://www.colorado.edu/outreach/BSI/pdfs/resistance.activity.pdf), as well as DNA sequencing sections of the lab “A Mouthful of Microbes” presented at the 2017 CATALySES Emerging Pathogens seminar sponsored by CPET at the University of Florida in Gainesville. Such labs and investigations are the “area of focus” of my proposal. To make sure my students are exposed to cutting-edge technology that can enable them to perform inquiry-based learning to understand the real threat of antibiotic resistant microorganisms.

**Intervention:** In the past, I have been more of the “sage on the stage” type of teacher. The opportunity of attending the “Emerging Pathogens” seminar at UF this summer has allowed me to step out of my comfort zone and apply novel methods to my teaching. The lesson on microorganisms using genetics in their context was taught using power point presentations and virtual labs on gel electrophoresis, as opposed to the actual hands-on approach. I did try a bacterial transformation lab this past year, in which the students were finally exposed to real-life procedures of genetic manipulation. Unfortunately, the transformation efficiency was zero for all the lab groups. Many factors contributed to such “failure”, which are not pertinent to discuss at this point. I am ready to try a novel approach by introducing the study of antibiotic resistant microorganisms to my A.P. Biology curriculum. Such intervention would serve my students by enhancing their research and critical thinking skills, as well as making them aware of the problem that such microorganisms pose to society and letting them experience a real-world application of genetics and microbiology.

**Data Collection and Analysis:** Not very clear yet on how I will approach this. However, a simple pre-test and post-test on their knowledge on antibiotic resistance can be implemented. Also, opinion surveys about the effectiveness of the laboratory experience will be administered.

(Pre and post-test will be sent next week)

**Connections to CATALySES Summer Institute:** They are already mentioned in the “Rationale” and “Interventions” sections of the action proposal.

**Literature Cited:**

Yosida, M. et.al. *Nature Communications* 8, Article number 15589, 2017).

Case Study: How Do Bacteria Become Resistant?

[www.biologycorner.com/worksheets/case\\_study\\_bacteria\\_resistance.html](http://www.biologycorner.com/worksheets/case_study_bacteria_resistance.html)

[www.faculty.virginia.edu/evolutionlabs/Antibiotic\\_Lab\\_Web\\_Page.html](http://www.faculty.virginia.edu/evolutionlabs/Antibiotic_Lab_Web_Page.html)

[www.colorado.edu/outreach/BSI/pdfs/resistance.activity.pdf](http://www.colorado.edu/outreach/BSI/pdfs/resistance.activity.pdf)

## THE LESSON PLAN

**Title:** “Eternally Yours”: Using Antibiotic Resistance Experiments to Enhance Inquiry-Based Learning in an A.P. Biology Curriculum.

**Key Questions:** This lesson will ask students to identify antibiotic resistance in microorganisms. They will explore how bacteria become resistant to specific antibiotics by analyzing a case study of MRSA. They will also perform a lab in which they actually measure how different bacteria become resistant to different types of antibiotics. As a concluding activity, they will perform a PCR analysis on such antibiotic resistance pathogens and analyze what specific genetic mutations they have that confer antibiotic resistance. For this part of the activity, students will attend the facilities at the University of Florida and conduct their experiments there.

**Science Subject:** A.P. Biology

**Grade Level:** 11<sup>th</sup> and 12<sup>th</sup> grade.

### Science Concepts for A.P. Biology:

Essential Knowledge 4.C.1: Variation in molecular units provides cells with a wider range of functions.

Essential Knowledge 3.C.1: Changes in genotype can result in changes in phenotype.

Essential Knowledge 3.B.1: Gene regulation results in differential gene expression, leading to cell specialization.

Essential Knowledge 3.B.2: A variety of intercellular and intracellular signal transmissions mediate gene expression.

Essential Knowledge 4.C.2: Environmental factors influence the expression of the genotype in an organism.

Essential Knowledge 3.C.2: Biological systems have multiple processes that increase genetic variation.

Essential Knowledge 4.C.3: The level of variation in a population affects population dynamics.

Essential Knowledge 1.A.3: Evolutionary change is also driven by random processes.

Essential Knowledge 1.C.3: Populations of organisms continue to evolve.

Essential Knowledge 2.D.3: Biological systems are affected by disruptions to their dynamic homeostasis.

Essential Knowledge 3.E.1: Individuals can act on information and communicate it to others.

**Overall Time Estimate:**

For the Case Study on MRSA: one 90 minute class period

For the Lab: Student Activities Measuring Antibiotic Resistance: two 90-minute class periods

For the Culture of the Antibiotic Resistance Bacteria: two days.

For the Analysis of DNA from the Resistance Bacteria at UF: two days.

**Learning Styles:** Mostly visual and kinesthetic. Auditory as well, since they need to carefully listen to specific instructions.

**Vocabulary:**

**Pathogen:** a bacterium, virus, or other microorganism that can cause disease.

**Antibiotic:** is a chemical compound that **kills bacteria** or slows their **growth**. They are used as **medicine** to treat and **cure diseases** caused by bacteria.

**Antibiotic Resistance:** is the ability of a microbe to resist the effects of medication previously used to treat them.

**Antibiotic Sensitivity:** is the **susceptibility** of **bacteria** to **antibiotics**.

**Zone of Inhibition:** The area around an antibiotic disc that contains no bacterial growth.

**Bacterial Culture:**

The growing of microorganisms on a culture medium, such as agar, in an incubator kept at body temperature, for purposes of identification.

**Nosocomial Infection:** Infection acquired or occurring in a hospital.

**Gram positive bacteria:** in a gram staining reaction, will react in a **crystal violet** dye and will stain dark purple or violet. Its **cell** wall is unique because it is composed of several peptidoglycan layers (multilayered) making it thicker.

**Gram negative bacteria:** This group shows a different gram staining reaction as it normally does not react to a **crystal violet** dye. However, it usually stains pinkish red and can be decolorized in accepting safranin (a counterstain). It has a thinner peptidoglycan cell wall layer because it is just made of one single layer as opposed to gram positive's multi-layered wall.

**Polymerase Chain Reaction (PCR):** a technique used to replicate a fragment of DNA and produce a large amount of that sequence.

**Lesson Summary:** The lesson will consist of three lab activities which follow. Procedures and steps are outlined. This covers student learning objectives, materials, background information, advance preparation, and procedure and discussion questions.

Activity #1.

## **Case Study: How Do Bacteria Become Resistant?**



### **Part 1: What is MRSA?**

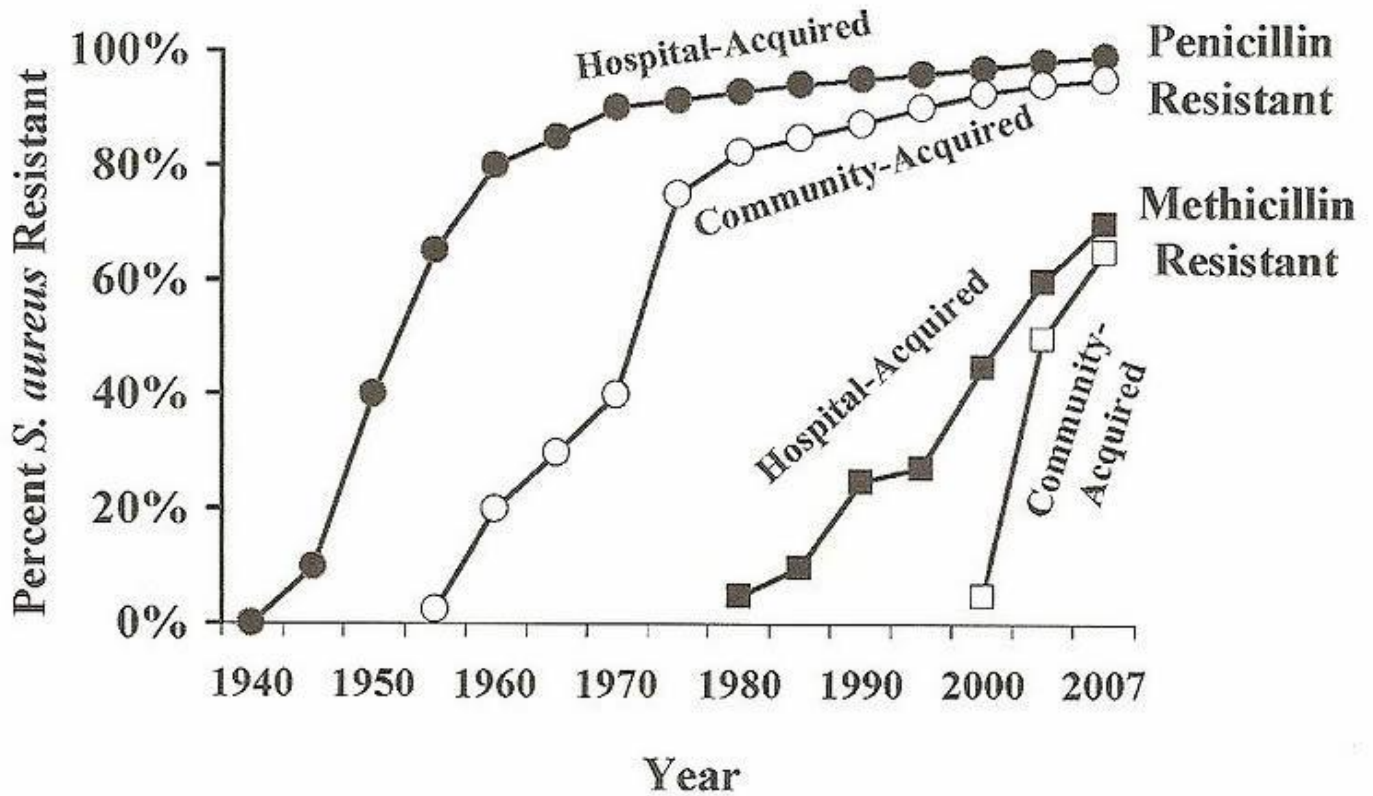
No matter what doctors did, the baby's oxygen levels were dropping as a drug resistant bacteria were eating holes in the lungs of the 7 week old. Even the most powerful antibiotics could not stop the infection. Just two days ago, Madeline had started coughing, a symptom the doctor dismissed as a viral infection. Then Madeline's mother found her limp and blue in her crib and she was rushed to the hospital. She was diagnosed with sepsis and a virulent pneumonia that was destroying her lungs. It was MRSA.

The methicillin-resistant form of the bacterium commonly known as staph was first identified in the 1970's in hospitals, but it has since spread across the world, showing up in day cares, schools and other public spaces. Today, 1.2 million MRSA infections occur in hospitals in the U.S. and invasive MRSA kills

over 19,000 per year. The bacterium can sometimes "colonize" a person and not cause illness. The person can carry it on their bodies for years and pass them to other people or leave them on surfaces. Hospitals have mounted aggressive campaigns to eliminate MRSA from their facilities.

Madeline's parents wondered how she had contracted this dangerous bacterium. Madeline's family agreed to tests to determine if any of them were carrying the deadly bacteria or if the child contracted the bacteria from the hospital. The hospital protested, claiming that their facility is not the source of MRSA.

In the past, penicillin was used to treat *Staphylococcus aureus* infections. Shortly after, *S. aureus* became resistant to penicillin. During the 1950s, derivatives of penicillin was discovered by pharmaceutical companies that could treat *Staphylococcus aureus*. The graph below depicts the Spread of Antibiotic-Resistant *S. aureus* Infections in the United States. Separate curves are shown for bacteria that caused infections in the hospital ("Hospital-Acquired") and in healthy people in the community ("Community-Acquired").



1. Based on the graph, make an inference about where the "community acquired" penicillin resistant *S. aureus* originated from.

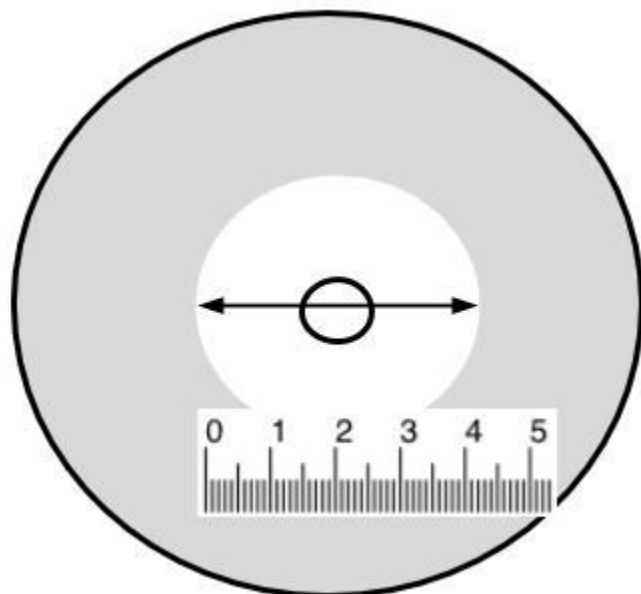
2. Why did methicillin resistance lag behind penicillin resistance? Based on the trend seen with penicillin, what would you expect to see happen with methicillin?

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## Part 2: MRSA Screening

A methicillin resistant *Staphylococcus aureus* (MRSA) screen is a test that looks for the presence of MRSA and no other pathogens. It is primarily used to identify the presence of MRSA in a colonized person. On a community level, screening may be used to help determine the source of an outbreak. On a national level, additional testing may inform clinicians and researchers about the unique genetic characteristics of the strains of MRSA circulating in the community or health care setting.

A nasal swab is collected from the nares (nostrils) of an asymptomatic person and cultured (put onto a special nutrient medium, incubated, and then examined for the growth of characteristic MRSA colonies). A swab may be collected from a wound site or skin lesion of a person who has been previously treated for a MRSA infection and cultured similarly. A screening culture identifies the absence or presence of MRSA and usually takes 1 to 2 days for a result.



When studying how bacteria respond to antibiotics, the Kirby-Bauer disk diffusion method is used. In this technique, discs containing antibiotics are placed on agar where bacteria are growing, and the antibiotics diffuse out into



the agar. If an antibiotic stops the bacteria from growing, we can see circular areas around the wafers where bacteria have not grown. This area is called the "zone of inhibition." The diameter of these zones is measured as shown below.

3. What methods would hospitals employ to eliminate MRSA from their facilities?

4. What is a "strain" of bacteria? How is it possible that some strains of *Staphylococcus aureus* can be harmless, but others can be deadly? (*You may need to google this.*)

5. A young scientist suggests that a chemical found on the skin of frogs can be used as an antibiotic. Explain how the Kirby-Bauer disk technique could be used to support this hypothesis.

6. Consider the data gathered from the frog-skin experiment. What conclusion would you draw from the data?

Site	Zone of Inhibition
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Frog Skin	1.2 cm
Penicillin	3.9 cm
Amoxicillin	3.6 cm
Control	0.1 cm

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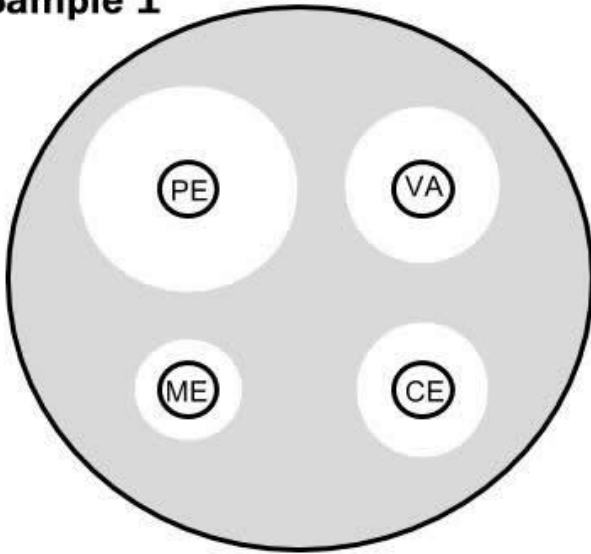
### Part 3: Analyzing the Plates

Each plate below represents a sample taken in the investigation. Nasal swabs were taken from individual family members and two samples were taken from the hospital delivery room. The samples were grown on agar with antibiotic disks added.

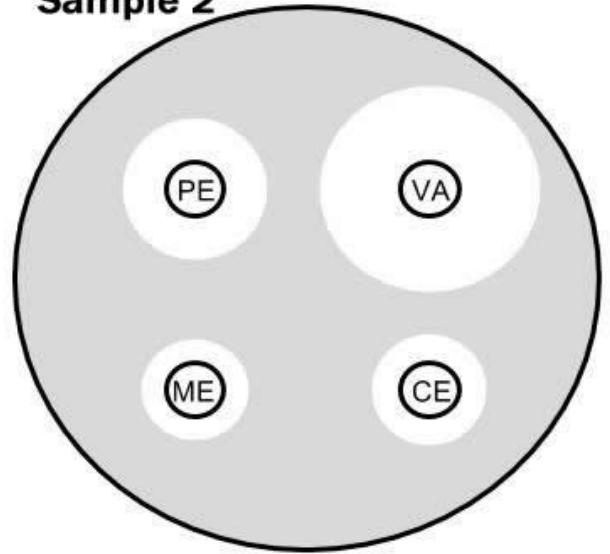
PE = penicillin | ME = methicillin | CE = cephalothin | VA = vancomycin

Measure the zones of inhibition on the plates and record the data in the table.

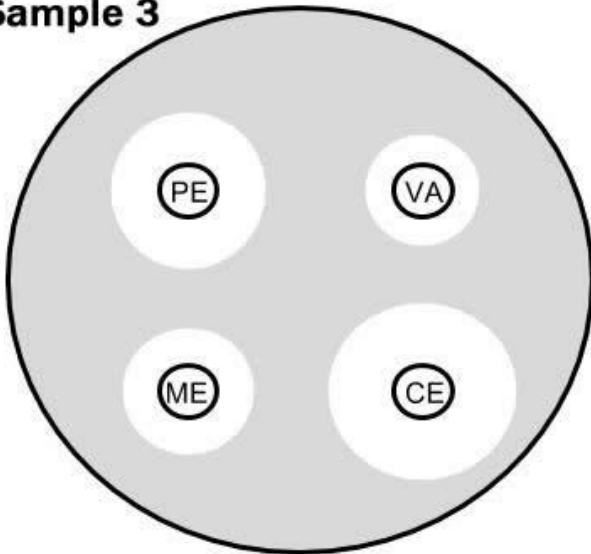
**Sample 1**



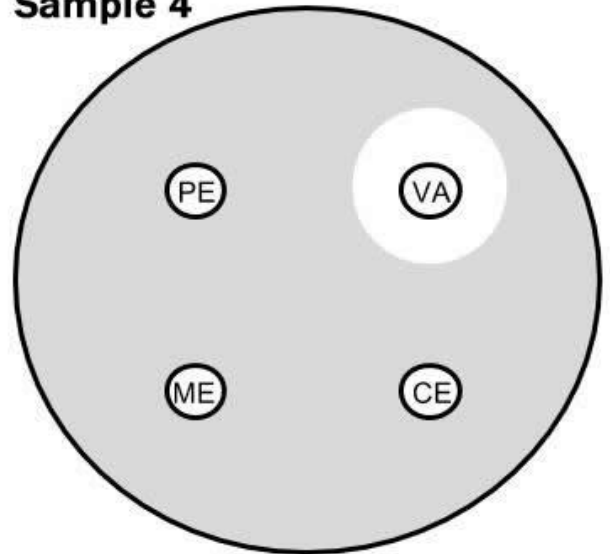
**Sample 2**



**Sample 3**



**Sample 4**



Sample	Disk	Zone Size	Sample	Disk
1	PE		3	PE
	ME			ME
	CE			CE
	VA			VA

2	PE		4	PE
	ME			ME
	CE			CE
	VA			VA

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### Part 4: Conclusions

6. The following table identifies the sample sources. Which sample contains MRSA? How do you know?

Sample 1	Madeline's Mother
Sample 2	Madeline's Sister
Sample 3	Madeline's Father
Sample 4	Delivery Room Surface

7. Sample 2 was taken from a nasal swab of a family member who has been having sinus infections. What course of antibiotics would you recommend?

8. What recommendations would you make to Madeline's family and the hospital where Madeline was delivered. Your recommendations should include evidence-based reasoning and details from the case to support your position.

Activity #2:

Refer to the following website: [www.colorado.edu/outreach/BSI/pdfs/resistance.activity.pdf](http://www.colorado.edu/outreach/BSI/pdfs/resistance.activity.pdf)

Activity #3:

Once antibiotic resistant bacteria have been identified they will be cultured using appropriate protocols. Students will travel to the University of Florida to do polymerase chain reaction to detect which target genes confer antibiotic resistance to specific strain of bacteria to be analyzed. PCR protocol to follow.

Updated protocol:

Naturally antibiotic resistant bacteria will be inoculated in the classroom and DNA will be extracted from them at the UF labs. Such bacterial will be grown in the UF labs and my students will travel to UF then, extract DNA from the bacteria, run PCR and check the results on a gel. If we get a band, the bacteria should be able to survive with the antibiotic present. Then, the bacteria will be plated on the antibiotic and see if it grows on the next day. A “parallel lab” will be run with other bacteria without the resistance gene to see that those do not have the ability to survive. A unit on SnapGene will be run as well which allows for nucleic acid analysis/primer design (extracted from an email sent by Ryan Chastain-Gross, Ph.D., Program Coordinator UF-CPET, on Aug 3, 2017)

### **Resources/References:**

[www.biologycorner.com/worksheets/case\\_study\\_bacteria\\_resistance.html](http://www.biologycorner.com/worksheets/case_study_bacteria_resistance.html)

[www.colorado.edu/outreach/BSI/pdfs/resistance.activity.pdf](http://www.colorado.edu/outreach/BSI/pdfs/resistance.activity.pdf)

Chastain-Gross, Ryan, Ph.D., email sent on Aug 3, 2017. Antibiotic-resistant bacteria DNA extraction and PCR analysis protocol.