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The cost of money: an inquiry-based laboratory activity identifying bacterial populations on foreign currencies

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Abstract

The pedagogical landscape in the scientific domain has transformed, with the integration of personalized and experiential learning. This paper explores the development of innovative pedagogical approaches, which fuse together personalized and experiential learning, targeting individual learner needs while providing engaging and learner-centric experiences. A semester-long group activity is presented, which focuses on the development of microbiology lab preparatory skills, such as making growth medium, and performing phenotypic and genotypic testing on unknown organisms. More specifically, this activity was completed in groups of 2-3, requires ten 2-hour labs and incorporates biochemical tests and barcoding to uncover the identity of bacterial populations on foreign currency. Each student group must formulate a unique hypothesis that they test using the laboratory techniques available to them. Students use the data they have collected to formulate conclusions based on their hypothesis. This paper presents a novel framework that intertwines personalized and experiential learning, elaborating on its implementation, potential benefits, and challenges.

Keywords: Personalized learning, Experiential learning, semester, hypothesis formulation

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INTRODUCTION

The pedagogy landscape, specifically in the scientific domain, has undergone significant transformation in recent years due to the integration of personalized and experiential learning methodologies (Makhambetova et al., 2021). This integration holds the potential to create a profound and learner-centric educational experience within biology laboratories, where direct hands-on encounters play a fundamental role. In this article, we will introduce concepts

regarding personalized learning and experiential learning methodologies in the scientific domain, and what led to the development of innovative pedagogical approaches.

At the core of this transformation lies personalized learning, a concept that has garnered considerable attention and has undergone notable evolution. While there is an increasing emphasis on this strategy, the combination of personalized learning with advanced technologies remains an underexplored area, offering promising opportunities for continued innovation and refinement of personalized learning paradigms (Makhambetova et al., 2021). Learners can explore topics and phenomena that resonate with their curiosity and align with their career aspirations. Personalized learning approaches leverage technology, adaptive learning platforms, and data analytics to provide customized learning experiences. Learners can engage with interactive simulations, virtual laboratories, and multimedia resources that cater to their individual learning styles and preferences. This customization enables learners to delve deeper into scientific concepts that captivate their interest and progress at their own pace.

Concurrently, experiential learning presents a powerful methodology that complements personalized learning exceptionally well. The fusion of these strategies holds great promise for the development of a revolutionary model for biology laboratory education, one that not only caters to the unique needs of individual learners but also supplies an engaging and hands-on experience (Shemshack & Spector, 2020). Experiential learning emphasizes hands-on experiences and real-world applications. In the scientific domain, this translates into engaging learners in practical experiments, fieldwork, and problem-solving activities that reflect the work of scientists in various disciplines. Experiential learning allows learners to actively participate in the scientific process, developing critical thinking skills, collaboration abilities, and a deeper understanding of scientific principles. Through experiential learning, learners gain practical skills and knowledge that can be applied in real-life contexts.

The laboratory activity

The laboratory activity is over the span of an entire semester and requires student in groups of 2 to 3 to develop a hypothesis using the laboratory activities outlined at the beginning of the semester. Students were given a selection of foreign currency and the ability to build a hypothesis which examined: 1) microbial communities on multiple foreign currencies and their resistance to one antibiotic dosage, 2) microbial communities on one foreign currency and its resistance to multiple antibiotic dosages, or 3) microbial communities on one foreign currency and its resistance to multiple different antibiotics. Each week, students followed a series of experimental protocols that involved creating their own growth media and performing numerous physiological and metabolic tests on their samples. The results from their findings were used to help test their hypothesis. At the end of the term, students created a scientific poster, and we held a poster session similar to a poster session at a typical scientific conference.

STUDENT OUTLINE

The cost of money: an inquiry-based laboratory activity

Objectives

- Be able to list and explain the specific steps required to uncover the identity of the bacteria
- To perform a variety of biochemical analysis on bacteria
- To use technology to sequence and submit bacterial sequences
- To arrange and assemble findings into oral presentations
- To develop critical thinking and problem-solving skills, specifically in laboratory inquiry and investigation
- To cultivate an attitude of curiosity, confidence, perseverance, and responsibility for laboratory science

Introduction

In this activity, students work in groups of 2-3 and participate in ten 2-hour labs where they perform biochemical tests. Each student group is responsible for creating a distinct testable question, which they will investigate using the available laboratory techniques. Initially, the activity was designed with the expectation that students would choose two different currencies and test them with one specific antibiotic dosage. However, some groups opted to deviate from this plan by selecting only one currency and conducting tests with multiple antibiotics or dosages.

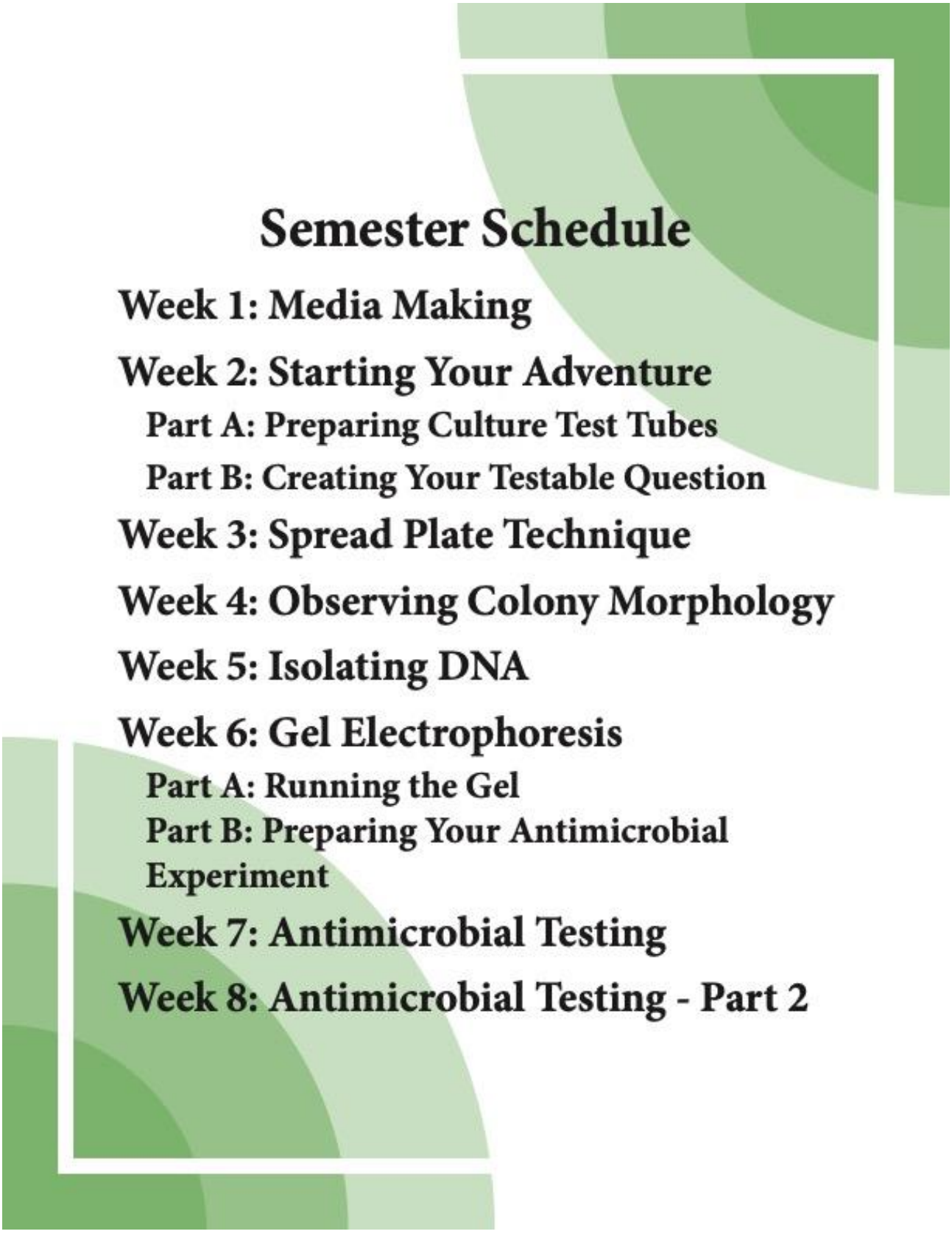

Figure 1. The weekly laboratory schedule for undergraduate students to follow.

Week 1. Media Making

This week, you will be making a typical growth medium, Trypticase Soy Agar. We will use this throughout the semester to grow various types of cultures.

Instructions

Use the scale to measure out each ingredient needed for this recipe. Make sure to zero the scale with the weight boat present before scooping the ingredient onto the scale. For the purposes of maintaining accuracy, it is important to weigh out each ingredient separately. Do not add all ingredients to the weight boat at the same time.

TSA Recipe

Ingredient	**Quantity**
Casein Peptone	8.5g
Soybean Peptone	1.5g
NaCl	2.5g
Dipotassium Phosphate	1.25g
Glucose	1.25g
Distilled Water	500mL

Fill your autoclavable media container with ~250ml of water and a magnetic stir bar. Place the container on the stirring hot plate and begin to slowly warm and stir your liquid. I would recommend keeping the rotation speed at 1 or 2. Then slowly add each of the above ingredients, following the order they are presented in the table above.

Gradually heat your mixture until you start to see the formation of small bubbles at the base on the container. Once these bubbles begin to rise, we can turn off the heat and, WHILE WEARING OVEN GLOVES, remove your container from the hot plate.

**After the laboratory period, your instructor will autoclave your media in preparation for next lab.

Week 2. Starting your adventure!

Part A

This week, you will be preparing your own culturing test tubes. The nutrient medium will be used from the previous week. Pipetting media into culture test tubes is the final step in preparing for growing cultures.

Procedure

1. Prepare your workbench for aseptic technique
2. Collect your autoclaved growth medium and ~25 sterile test tubes. You will also need a 10ml pipette and bulb.
3. Pipette 10ml of TSA into each test, while being careful to follow aseptic technique.

Part B

Today, you will form your own experimental question using the techniques in the following weeks. Additionally, you will receive a control organism. For today, you will not do anything with your control group. However, in future weeks all experimental methods will be applied to your control organism and your experimental group. (Remember that your experimental group is the microbial cultures you are collecting from the different currency.)

Work with your group to form a testable question. Begin by reviewing the PowerPoint slides from the pre-lab talk “What is a testable question?”, the laboratory activities in future weeks, and the country bios for China, Laos, and Vietnam. Below are some additional questions to consider when forming your ideas.

1. Which currency will you test? Read the attached background information on each country.
2. Future labs will examine colony morphology and identify gram-positive and gram-negative microorganisms. How can you use this information to answer your question?
3. Future labs will use molecular techniques to determine whether the microbial communities present on your currency are resistant to the antibiotic tetracycline. Within the lab you will use spectrophotometry to further examine the antibiotic resistance of the microbial community on the currency. Refer to the whiteboard for the full list of antibiotics available for testing. You must decide which of these you wish to work with in future weeks.

State your testable question below:

Once you have developed your testable question, please complete the procedure below.

Procedure

1. Now, you will select currency from two different countries.
2. Take one bill from each country to your bench and use sterile tweezers to remove the bill from the ziplock bag.
3. You will place the bill within one of the test tubes filled with liquid medium.
4. Place the cap on the test tube and gently vortex with the bill inside the test tube for 30 seconds.
5. Remove the bill from the test tube and place the wet bill in the sink.
6. Parafilm your test tube, label with your information, and place in the incubator.
7. Important note: You must place your control test tube in the same test-tube rack as your experimental cultures in the growth chamber.

**After the laboratory period, your cultures will incubate in the growth chamber and your instructor will move them to cooler temperatures in preparation for the next class.

Week 3. Spread plate technique

You will be creating a spread plate for each of your three samples (two currency cultures and one control) on three different growth mediums, TSA, MSA, and MacConkey Agar. At this end of this activity, you will have created a total of 9 plates. Below is a description of each medium type you'll be working with today.

Trypticase Soy Agar

Trypticase soy agar (TSA) or tryptone soya broth (TSB) with agar are both growth media utilized in the cultivation of bacteria. These media serve as general-purpose and nonselective environments, offering an ample supply of nutrients that facilitate the growth of a diverse array of microorganisms. Their applications span a broad spectrum, encompassing culture storage, cell enumeration (counting), isolation of pure cultures, and general-purpose cultivation.

MacConkey Agar Test

MacConkey Agar functions as a specialized medium with the purpose of distinguishing Gram-negative bacteria based on their capacity for carbohydrate fermentation. The mechanism involves restraining the proliferation of Gram-positive bacteria by incorporating crystal violet and bile salts into their cell walls. Certain Gram-negative bacterial types, like E. coli, Salmonella, or Shigella, are closely associated with the gastrointestinal flora.

A pH-sensitive indicator integrated within the agar undergoes a color transformation, typically to pink or red, as a response to the generation of acidic by-products. Notably, E. coli is recognized for its ability to ferment the lactose present in the medium, leading to the release of acid. Conversely, Salmonella and Shigella are identified as non-lactose fermenters, which results in the appearance of their colonies in white or tan hues. The majority of Gram-negative bacteria can tolerate bile salts due to the protective nature of their outer membrane.

Mannitol Salt Agar Test

Mannitol Salt Agar (MSA) is a widely employed growth medium in the field of microbiology, serving as both a selective and differential medium. Its purpose is to promote the growth of specific bacteria while hindering the growth of others. This medium boasts a high concentration of salt (NaCl), typically ranging from 7.5% to 10%, which acts as an inhibitor against most bacteria. Consequently, MSA selectively favors Gram-positive bacteria such as Staphylococcus, Enterococcus, and Micrococcaceae that can tolerate elevated salt levels, while discouraging the growth of Gram-negative bacteria.

Additionally, MSA functions as a differential medium for staphylococci that ferment mannitol. It contains mannitol, a sugar alcohol, and phenol red, a pH indicator. The purpose of phenol red is to detect the acidic byproduct produced by mannitol-fermenting staphylococci. Staphylococcus aureus, for example, generates yellow colonies with surrounding yellow zones, while other coagulase-negative staphylococci produce small pink or red colonies without altering the medium's color. In the presence of mannitol fermentation, the acidic byproduct causes the phenol red within the agar to turn yellow.

Procedure

1. Collect 3 plates of each media type (TSA, MSA and MacConkey Agar) from the front of the room. Label each one using numbers 1-9. Do not repeat your numbers. Include the plate details in the table below.

Plate Label	What is the media type and sample information?
1	
2	
3	
4	
5	
6	
7	
8	
9	

2. Pipette 0.1ml of the bacterial culture onto the middle of an agar plate.
3. Obtain a L shaped glass rod. To sterilize the glass rod, fill a beaker with ethanol and allow the rod to soak for one minute.
4. Wave the glass rod through the Bunsen burner flame briefly to burn off any excess alcohol. Allow the rod to cool by placing on the lid of the petri plate. Do not place the rod on the bench.
5. Obtain your bacterial samples and use the rod to evenly spread the bacteria on the agar plate. Ensure the whole surface is covered.
6. Using the same rod repeat the procedure (steps 3 to 5) for the other agar plates and bacterium.
7. Incubate the plates for 24 to 48 hours at room temperature. Note: make sure the plates are upside down to prevent condensation from forming, disturbing the bacteria on the agar.

Week 4. Observing colony morphology

Colonies can display different patterns when grown in solid media. Colony morphology is one of the first things one can notice on a macroscale. The main descriptors are color, surface texture, shape, and elevation.

Procedure

Perform the following steps for all three media types.

1. Observe and describe physical characteristics. Start by colony color and colony pigments secreted into the agar.
2. After color observations, estimate and record the size, shape, and texture. Also, note if your colony has edges or elevations.
3. If possible, take pictures of your colony.

Plate #	Color/Pigments	Shape	Surface	Edges and Elevations	# of colonies with similar appearance
1					
2					
3					

4					
5					
6					
7					

8					
9					

Week 5. Isolating DNA

This week, we will be extracting DNA from your bacterial samples, so that in future weeks we can detect whether your cultures contain the antibiotic resistance genes for tetracycline. Before beginning today's lab, please read the 'Background information' handout.

Extracting DNA from bacterial cells can be quite difficult, relative to extractions from plant or animal tissue. Bacterial cells have tough cell walls that can be very difficult to break open or lyse. A significant amount of time must be spent on lysing these cells, thus your instructor has completed some of the preliminary lysing steps.

Prior to today's lab…

Your instructor completed the following initial cell lysis steps:

1. One of the culture test tubes you created during week 2 was centrifuged and the biomass was transferred to a 1.5ml microcentrifuge tube.
2. Step 1 was repeated using the other currency culture test tube.
3. Step 1 was repeated using your control.
4. Lysis solution and microbeads were added to each microcentrifuge tube.
5. Each sample was vortexed for 40 mins.
6. The microcentrifuge tubes were centrifuged, and the biomass was transferred to a new 1.5ml microcentrifuge tube.
7. Samples were frozen until the start of today's lab.

Procedure

1. Collect the following items from the front of the class: distilled water (250 µL), lysis solution (700 µL), silica resin (10 µL), wash buffer on ice (2.5 mL), 3 microcentrifuge tubes (1.5 mL), and 2 plastic pestles.
2. Obtain your 3 frozen samples (2 currency samples and 1 control).
3. Add 300 µL of lysis solution into tube and grind sample using pestle for approximately 2-5 minutes.
4. Incubate tube in a water bath at 65˚C for 10 minutes.
5. After incubation, obtain tube and place in microcentrifuge for 1 minute at maximum speed. Note: Make sure the microcentrifuge is balanced properly.
6. Label a new tube, Tube 2, and transfer 150 µL of the supernatant into the tube. Note: Be careful to not disturb the white pellet at the bottom of the tube when removing supernatant. Throw out the tube with the pellet.
7. Add 3 µL of silica resin into the second tube. Mix well and incubate the tube for 5 minutes in a water bath at 57˚C.
8. Again, centrifuge after incubation for 30 seconds at maximum speed.
9. Remove and discard the supernatant.
10. Add 500 µL of ice-cold wash buffer to the pellet in Tube 2. Mix well using a pulse vortex or pipetting up and down to resuspend the resin.

11. Once mixed properly, centrifuge for 30 seconds at maximum speed.
12. Remove and discard the supernatant.
13. Add 500 µL of ice-cold wash buffer to the pellet in Tube 2. Mix well using a pulse vortex or pipetting up and down to resuspend the resin.
14. Once mixed properly, centrifuge for 30 seconds at maximum speed.
15. Remove and discard the supernatant.
16. Add 100 µL distilled water to Tube 2 and mix well. Incubate for 5 minutes at 57˚C.
17. Place your tube in the centrifuge with the hinge placed outwards. Centrifuge for 30 seconds at maximum speed.
18. Label a clean tube, Tube 3. Pipet 90 µL of the supernatant from tube Tube 2 into Tube 3. Discard tube 2. Store Tube 3 sample on ice or at -20˚C.

** After this lab, your instructor will use PCR techniques to amplify the genetic information extracted from your samples. Primers for 16S, as well as primers for tetracycline resistance genes tetB and tetM were used so that we can amplify and detect the presence of these two antibiotic resistance genes.

Week 6. Gel electrophoresis

Part A

Gel electrophoresis is an effective way to visualize your samples to determine if you successfully extracted genetic material. For this experiment, the presence of bands at 492 base pairs would indicate if any DNA is present in your sample. The presence of bands at 406 or 659 base pairs would indicate the presence of tetM or tetB genes, respectively.

Prior to today's lab…

Your instructor completed the following initial steps:

1. A 2% agarose solution was poured into the gel electrophoresis tray to a depth that covers one-third the height of the comb teeth.
2. Wait ~20 minutes for the agarose to solidify.
3. The gel was placed into the electrophoresis chamber and 1xTBE buffer was added to just cover the surface of the gel.
4. The come was carefully removed from the gel.

Procedure:

1. Review the class signup sheet which indicates which groups have loaded their samples into each lane.
2. Write your group name on the sheet in the empty lane where you will be loading your sample. Additionally, for your own note keeping, please indicate which lane you loaded your sample in.

   Gel Lane #: _____ Remember, count from left to right on the gel.

3. Load your sample onto the gel.
4. Run the gel for approximately 30 minutes at 130V.
5. View the gel using a UV, blue light, or white light transilluminator depending on the stain used.

Part B

While you wait for your gel to run you can prepare cultures for your antimicrobial experiment next week. You will be using the test tubes filled with liquid media that you created in week 2. You should have ~21 tubes.

1. Using aseptic technique, pipette 0.5ml of your currency culture, that you created in week 2, into 9 of your culture test tubes.
2. Repeat step 1 with your other currency culture, resulting in the inoculation of the other 9 test tubes.
3. Repeat step 1 with your control culture, but (instead of inoculating 9 test tubes) you will only inoculate 3 test tubes.
4. Place these into the growth chamber.

Week 7. Antimicrobial testing

Spectrophotometry is a technique used to measure microbial growth by analyzing the optical density (OD) of a microbial culture. It involves the use of a spectrophotometer, an instrument that measures the absorbance or transmission of light through a sample at different wavelengths.
Today, you will be setting up your antimicrobial tests. You will be using the test tubes filled with liquid media and culture from last week. The spectrophotometer has already been set to measure OD at 600 nm (OD_{600}). Note, your instructor has already created a calibration curve, so that you can convert your photometry values into viable counts.
You must decide which antibiotics you wish to test. The list of available antibiotics is listed on the board, please write below which ones you have selected. (Note, you could also pick multiple doses of the same antibiotic.)
Scenario A
Antibiotic and dose: _____
Scenario B
Antibiotic and dose: _____

Procedure

1. Each currency has 9 culture tests, which must be organized as follows: 3 test tubes are negative controls (no antibiotic is added), 3 test tubes are scenario A and 3 test tubes are scenario B.
2. The 3 remaining test tubes containing the control culture will act as a positive control using a 0.5 mg/L dose of tetracycline.
3. Double check your dose calculations with your instructor before completing the next step.
4. Using aseptic technique, pipette the appropriate dose of antibiotic into the correct test tubes. Record this information in the table below.
5. Follow the instructions on the spectrophotometer to measure the initial microbial density of each culture test tube. Record your findings in the table below.

Test tube label	Antibiotic	Antibiotic concentration (mg/L)	Initial Photometry values
A			
B			
C			
D			
E			
F			
G			
H			
I			
J			
K			
L			
M			
N			

O			
P			
Q			
R			

Week 8. Antimicrobial testing (Part II)

Spectrophotometry allows for rapid and non-destructive measurement of microbial growth, making it a widely used technique in microbiology research and industrial applications. It provides valuable information about the growth rate, biomass production, and growth kinetics of microbial cultures.

Procedure

1. Follow the instructions on the spectrophotometer to measure the final microbial density of each culture test tube. Record your findings in the table below.

Test tube label	Final Photometry values	Difference of Initial and Final Photometry values	Calculated viable counts
A			
B			
C			
D			
E			
F			
G			
H			
I			
J			
K			
L			
M			
N			
O			
P			
Q			
R			

MATERIALS

Weeks	The materials/methods utilized by the students in collaboration with the entire class	Sources of materials	The materials/methods employed by the students exclusively within their respective groups
Week1. Media making: Students will make a typical growth medium, Trypticase Soy Agar. Students will use this throughout the semester to grow several types of cultures.	Scale	- https://www.fishersci.com/	Students from each group gathered the same materials as the corresponding groups and therefore performed the experiment individually within their groups while using the same materials but different currencies.
	Weight boat	- -https://www.fishersci.com/	
	8.5g of Casein Peptone	- -https://www.fishersci.com/	
	1.5g of Soybean Peptone	-https://www.fishersci.com/	
	2.5g of NaCl	- https://www.sigmaaldrich.com/	
	1.25g of Dipotassium Phosphate	- https://www.thermofisher.com/	
	1.25g of Glucose	- https://us.vwr.com/	
	500mL of Distilled Water.	- https://www.fishersci.com/	
	Autoclavable media container	- https://www.thermofisher.com/	
	Magnetic stir bar	- https://www.fishersci.com/	
	Hot plate	- https://www.fishersci.com/	
Week 2. Starting your Adventure: This week students prepared will prepare their own culturing test tubes. The nutrient medium was used from the previous week. Pipetting	Collect autoclaved growth medium and ~25 sterile test tubes	- https://www.thermofisher.com/	Control organism
	10ml (about 0.34 oz) pipette and bulb	- https://us.vwr.com/	microbial cultures by collecting from the different currencies
	Selecting currency from two different countries	- https://www.coleparmer.com/	Preparing your own culturing test tubes

media into culture test tubes is the final step in preparation for growing cultures.	Tweezers	- https://www.sigmaaldrich.com/	Forming a testable question
	Test tubes	- https://www.fishersci.com/	Using spectrophotometry to further examine the antibiotic resistance of the microbial community on the currency
	Liquid medium	- https://www.bd.com/	
Week 3. Spread plate technique: This week students will create a spread plate for each of the three samples (two currency cultures and one control) on three different growth mediums, TSA, MSA, and MacConkey Agar. At the end of this activity, students will have created 9 plates.	Spread plate	- https://us.vwr.com/	Bacterial samples
	Collecting 3 plates of each media type	- https://www.thermofisher.com/	
	Pipette	- https://www.eppendorf.com	
	Glass rod	- https://www.fishersci.com	
	Bunsen burner	- https://www.coleparmer.com	
	Petri plate	- https://www.sigmaaldrich.com	

Week 4. Observing colony morphology: Students will observe colony morphology. When cultivated on solid media, colonies can exhibit various patterns. On a macroscale, colony morphology is among the first noticeable characteristics. The primary descriptors for colony morphology include color, surface texture, shape, and elevation.	Describe physical characteristics		Students from each group gathered the same materials as the corresponding groups and therefore performed the experiment individually within their groups while using the same materials but different currencies.
	Estimate and record the size, shape, and texture		
	Picture taking		
Week 5. Isolating DNA: Students will extract DNA from bacterial samples to detect whether cultures contain the antibiotic resistance genes for tetracycline.	Distilled water	- https://www.sigmaaldrich.com/	Students from each group gathered the same materials as the corresponding groups and therefore performed the experiment individually within their groups while using the same materials but different currencies.
	Lysis solution	- https://www.thermofisher.com/	
	Silica resin	- https://www.qiagen.com/	
	Wash buffer on ice	- https://www.bio-rad.com/	
	3 microcentrifuge tubes	- https://www.eppendorf.com/	
	2 plastic pestles	- https://www.fishersci.com/	
	3 frozen samples (2 currency samples and 1 control)		
	Water bath	- https://www.labdepotinc.com/	
	Pipette	- https://www.gilson.com/	
	Centrifuge	- https://www.beckmancoulter.com/	

Week 6. Gel electrophoresis: Students will indicate the presence of DNA in their samples using bands at 492 base pairs.	Gel	- https://www.bio-rad.com/	Students from each group gathered the same materials as the corresponding groups and therefore performed the experiment individually within their groups while using the same materials but different currencies.
	UV, blue light,	- https://www.analytik-jena.com/	
	white light transilluminator	- https://www.analytik-jena.com/	
	Tubes	- https://us.vwr.com/	
	Pipette	- https://www.gilson.com/	
Week 7. Antimicrobial testing: Students will set up their antimicrobial tests. Students will use the test tubes filled with liquid media and culture from last week.	Test tubes	- https://www.fishersci.com/	Each group will choose antibiotics they wish to test
	Pipette	- https://www.eppendorf.com/	
	~~Which antibiotics? Concentration?~~Penicillin (5ug and 10ug)	- https://www.sigmaaldrich.com/	
	Gentamicin (5ug and 10ug)	- https://www.sigmaaldrich.com/	
	Kanamycin (5ug)	- https://www.sigmaaldrich.com/	
	Streptomycin (5ug)	- https://www.sigmaaldrich.com/	
	Tetracycline (15ug and 30ug)	- https://www.sigmaaldrich.com/	
	Chloramphenicol (5ug and 10ug)	- https://www.sigmaaldrich.com/	
	Novobiocin (5ug)	- https://www.sigmaaldrich.com/	
Week 8. Antimicrobial testing (Part II): Students will continue working on part 2 of antimicrobial testing.	Collecting photometry values	- https://www.thermofisher.com/	This week was the final procedure that students conducted with each of their groups.
	Collecting Initial and Final Photometry values		
	Calculating viable counts		

NOTES FOR THE INSTRUCTOR

I have provided additional resources that will help with implementation of this semester-long activity. In Appendix A, I have provided an example of a completed poster from previous students that have completed this project. In Appendix B, I have included rubrics for all the graded components.

CITED REFERENCES

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APPENDIX A

Microbes Around the World: A Study of Antibacterial Resistance in Southeast Asia

[redacted]

[redacted]

Abstract

Our primary objective is to determine if developed countries have more bacteria that are resistant to antibiotics compared to lesser developed countries. By using currency from a Thailand restaurant and various techniques, we determined which bacteria grow and survive in our specific setting. We measured the phenotypic characteristics of our bacteria by performing a MacConkey agar test, catalase test, gram staining, observing colony morphology, and sulfide and indole production and motility experiment. Our TSA bacteria is coccus and our blood bacteria is coccus with few rods present. Each bacteria had catalase present and both bacteria tested positive for fermentation. Once the tests were completed each bacteria was streaked onto a plate and divided into sections ranging from four to eight with a different antibacterial disk in the center. The results are important because they will help us determine if more developed countries have bacteria that are resistant to antibiotics compared to less developed countries. Eventually, the results of this experiment could be used as an example of how antibiotics are impacting developed nations and how to combat antibacterial resistance.

Introduction

The overall goal of this experiment is to determine if developed countries have more bacteria that are resistant to antibiotics than lesser developed countries. The results we collected are important because they will help validate our hypothesis; that more developed countries have bacteria that are more resistant to antibiotics compared to lesser developed countries. For our experiment we treated China as the most developed. This is because, China is the most technologically advanced and has a medical system that allows for a large percentage of the population to have access to healthcare. Our second country of study, Laos, is what we considered to be a lesser developed country in terms of this study. We came to this conclusion because Laos still relies heavily on its neighboring countries for medical needs and only about 20% of its population has basic medical care [6]. Laos also has a presence of preventable infectious diseases like Oriental, Rickettsia, and Leptospira, which is likely a direct result of their developing healthcare system [4]. Thailand had similar preventable diseases but also had outbreaks of Tuberculosis, Cholera, and the Plague, diseases that more developed nations like China have not seen for decades [5]. Thailand was considered the second most developed country in our experiment. Thailand is not as underdeveloped as Laos but also not as developed as China. The vast majority of Thailands' citizens have access to healthcare systems, but hospitals and private specialists do not have the technological advances like hospitals in China. We chose those three countries to test because they provided us with a scale of developed and developing nations. Easily transferable diseases in highly populated countries like Thailand, China, and Laos The results of our study could be used as an example or model in the public health field for how antibiotics are impacting developed nations.

Materials and Methods

Bacteria were collected and cultured off of the currency. Colony morphology tests were then preformed which included gram staining, MacConkey agar test, catalase test, and sulfide and indole test. Each bacteria was then identified using Bergeys manual. Each bacteria was streaked onto a plate of either blood or TSA agar that was then divided into either four or eight sections with an antibacterial disc placed in the middle of each section. Each plate was incubated for two days at 21°C. Zones of Inhibition were then observed. DNA was extracted from each of our bacteria following the instructions of the Carolina DNA Barcode Amplification and Electrophoresis Kit with GelGreen. PCR cycles were run according to the Carolina DNA Barcode Amplification and Electrophoresis Kit with GelGreen specifications. Once PCR was finished we ran Gel Electrophoresis. After each step was completed and PCR was finished our bacteria was sent to Genewiz to be identified. Once we received the bacteria back from Genewiz the sequences were run through Basic Local Alignment Tool (BLAST).

Results

Figure 5: Percentages of Zones of Inhibitions observed on blood agar containing bacteria from restaurants

Figure 4: Percentages of Zones of Inhibitions observed on TSA agar containing bacteria from restaurants

Discussion

The purpose of this experiment was to evaluate if developed countries, like China, have bacteria with greater antimicrobial resistance in comparison to developing countries like Laos and Thailand. According to Figures 4 and 5 Laos had the smallest percentage of reactions. This means that the bacteria from Laos showed the most resistance and had the least amount of zones of inhibition present. According to the Human Development Index, Laos had the lowest level of development in comparison with Thailand and China [1]. The primary source of revenue for developing countries comes from the service industry, while developed countries generally profit from industrialization and technological innovation [2]. This means that money will change hands more frequently in developing nations due to customers buying and selling goods and services. This higher frequency of exchange could lead to increased rates of bacterial infections amongst a population. According to our original hypothesis Laos should have had the highest percentage of zones of inhibition recorded since it is the country with the lowest level of development; this result leads our hypothesis to be untrue. Thailand had the highest percentage of reactions on both Blood and TSA Agar, which means that the bacteria collected from Thailand reacted the most to the antibacterial discs; again, this disproves our hypothesis because Thailand should have had a lesser amount of reactions than Laos. According to Figures 4 and 5 China had lesser percentage of reactions than Thailand but more reactions than Laos did. This result could depend on what part of China the currency was collected from; meaning that the bill may have come into contact with someone who may not be under health coverage. Another factor that could account for the behavior of the Chinese bacteria could be if the bacteria on the currency had not been previously exposed to the types of antibiotics we tested it against. Again, this goes against our hypothesis because China does have nationwide health care and is the most developed nation out of the three countries we tested [2]. Although our hypothesis was proven to be wrong by the reactions of the bacteria, there are some interesting observations. In Figure 3, all total hotel bacteria that were found on Blood agar had only three reactions out of the ten antibiotics tested against it. A possible theory for this phenomenon could be that, hotels house many different types of people from many different parts of the world, which could have exposed the various bacteria to different antibiotics throughout time, allowing it to become resistant to seven out of the ten antibiotics. Restaurants were proven to have more reactions than hotels which could be due to the constant sanitation restaurants have to do. This constant disruption in bacterial evolution and growth could inhibit the formation of antibiotic resistance. Another interesting facet of this experiment was the fact that Penicillin exhibited no reaction to any of the bacteria used. This is more than likely due to the fact that Penicillin was the first antibiotic discovered and its use was widespread in the 20th century. This circumstance clearly demonstrates antibiotic resistance.

References

A full list of references used can be located at the following link:

https://docs.google.com/document/d/1zxopbxHRZ7gBJKzPbnaHw-WhyAS4bIWFwEDm_8mxCUvE/edit?usp=sharing

Figure 2. An example of a student generated poster.

APPENDIX B

Table 1. Rubric: Week 2 - Creating Your Testable Question

Criteria	3 Points	2 Points	1 Point
Clarity of the Testable Question	The testable question is clear, concise, and focused, demonstrating a deep understanding of the techniques and concepts covered in the course.	The testable question is somewhat clear and focused, but may lack conciseness or demonstrate a partial understanding of the techniques and concepts covered in the course.	The testable question is unclear, lacks focus, or shows a limited understanding of the techniques and concepts covered in the course.
Alignment with Course Content	The testable question demonstrates a strong alignment with the course content, incorporating knowledge gained from the pre-lab talk, laboratory activities, and country bios.	The testable question somewhat aligns with the course content, but may not fully incorporate knowledge gained from the pre-lab talk, laboratory activities, and country bios.	The testable question does not align with the course content, failing to incorporate knowledge gained from the pre-lab talk, laboratory activities, and country bios.
Creativity and Originality	The testable question is highly creative and original, showcasing a unique approach to uncovering the identity of the bacteria.	The testable question is somewhat creative and original, but may lack uniqueness or fail to showcase a distinct approach to uncovering the identity of the bacteria.	The testable question lacks creativity and originality, relying on generic or unremarkable approaches to uncovering the identity of the bacteria.

Table 2. Rubric: Week 7 - Antimicrobial Testing

Criteria	5	4	3	2	1
Selection of Antibiotics	Student selected a variety of appropriate antibiotics and provided a clear rationale for their choices.	Student selected several appropriate antibiotics but did not provide a clear rationale for their choices.	Student selected a few appropriate antibiotics but did not provide a clear rationale for their choices.	Student selected only one appropriate antibiotic or did not provide a rationale for their choice.	Student did not select any antibiotics.
Setup of Antimicrobial Tests	Student accurately set up all antimicrobial tests and followed all instructions correctly.	Student accurately set up most of the antimicrobial tests and followed most instructions correctly.	Student accurately set up some of the antimicrobial tests but made a few minor errors in following instructions.	Student inaccurately set up the antimicrobial tests and made several errors in following instructions.	Student did not set up the antimicrobial tests or did not follow any instructions.
Data Analysis	Student accurately performed biochemical analysis on bacteria and demonstrated a thorough understandi	Student performed biochemical analysis on bacteria with minor errors, but overall showed a good understandi	Student performed biochemical analysis on bacteria, but made several errors and showed some gaps in	Student attempted to perform biochemical analysis on bacteria, but made significant errors and showed limited	Student did not perform biochemical analysis on bacteria or demonstrated a lack of understanding of the process.

	ng of the process.	ng of the process.	understandi ng.	understandi ng.	
Communicat ion of Results	Student effectively communica ted the results of the antimicrobia l tests in a clear and organized manner.	Student communica ted the results of the antimicrobia l tests adequately, but with some minor issues in clarity or organization .	Student communica ted the results of the antimicrobia l tests, but with noticeable issues in clarity or organization .	Student attempted to communica te the results of the antimicrobi al tests, but with significant issues in clarity or organization .	Student did not effectively communic ate the results of the antimicrobi al tests.

Table 3. Rubric: Week 9 - Poster Presentation

Criteria	3 Points	2 Points	1 Point
Content	The poster clearly and effectively presents findings and their relevance to the hypothesis. All necessary components are included.	The poster presents findings and their relevance to the hypothesis, but some components may be missing or lack clarity.	The poster lacks clear presentation of findings and their relevance to the hypothesis, with significant components missing.
Organization	The poster is well-organized, with a logical flow of information and clear sections for different aspects of the findings.	The poster is mostly organized, but some sections may lack clarity or a clear flow of information.	The poster is disorganized, with unclear sections and a lack of flow in presenting the findings.
Visual Appeal	The poster is visually appealing, with appropriate use of color, images, and graphics to enhance understanding and engagement.	The poster is visually acceptable, but the use of color, images, and graphics could be improved to enhance understanding and engagement.	The poster is visually unappealing, with little or no use of color, images, or graphics to enhance understanding and engagement.
Language and Clarity	The language used in the poster is clear, concise, and appropriate for the target audience. Information is effectively communicated.	The language used in the poster is mostly clear and appropriate, but some areas may lack conciseness or effectiveness in communication.	The language used in the poster is unclear, verbose, or inappropriate for the target audience, making it difficult to understand the information.
Oral Presentation	The student delivers a confident and	The student delivers an acceptable oral	The student's oral presentation is

	engaging oral presentation, effectively explaining the findings and their relevance to the hypothesis.	presentation, but may lack confidence or struggle to effectively explain the findings and their relevance.	unclear, lacks organization, or fails to effectively explain the findings and their relevance.

Table 3. Rubric: Week 1-8 Overall Laboratory Technique and Safety

Criteria	3 Points	2 Points	1 Point
Scientific Procedures	Student consistently followed all appropriate scientific procedures as described in the lab manual.	Student followed most appropriate scientific procedures as described in the lab manual, with minor inconsistencies.	Student did not follow appropriate scientific procedures as described in the lab manual.
Personal Protective Equipment (PPE)	Student wore proper PPE at all times and followed safety guidelines for PPE usage.	Student wore proper PPE most of the time but may have had some instances of not following safety guidelines for PPE usage.	Student did not wear proper PPE or consistently failed to follow safety guidelines for PPE usage.
Safety Protocol and Technique	Student consistently followed appropriate safety protocol and demonstrated excellent technique throughout the experiments.	Student followed most safety protocols and demonstrated good technique, but with occasional lapses or minor safety concerns.	Student did not consistently follow safety protocols and demonstrated poor technique, resulting in significant safety concerns.

Mission, Review Process & Disclaimer

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