You Are What You Eat, Unless What You Eat Kills You!
CATALySES Action Proposal

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Abstract

Many pathogens carry similarities in their presented symptoms, making it extremely difficult for physicians to diagnose which pathogen within a timely manner. This is where the field of Infectious Diseases and Epidemiologists comes into play, they will be the ones that go about deciphering what pathogen is afflicting the patient through the use of multiple types of labs. The most frequently used labs include the Rapid-Test Kit, and the Microarray DNA Test of which both give you instant results in order to finalize a diagnosis and proceed to identifying the treatment. However, in the case of antibiotic resistant strains which have arisen due to the natural selection of pathogens in the presence of antibiotics, thus leaving the most resistant pathogens to continue cellular division, but now including the resistant gene. The most dramatic concern of our times is to treat this, but how? Graphs and charts prove helpful in the deciphering of which symptoms apply to the patient, as well as which particular antibiotics would still be useful in the particular patients pathogenic strain (bacterial and/or viral).

Rationale

One of the most drastic concerns of our current times is the rise in antibiotic resistant bacteria and viral pathogens (PBS, 2008). Unfortunately, without the proper antibiotics to treat outbreaks, the pathogen can quickly sky-rocket from an epidemic to a pandemic. Granted there are far more factors associated with the distribution and transmission of pathogens. However, the global economic expansion has allowed for the growth of international and domestic travel. With such travel comes the added risks of transmission that include both vectors/hosts of the pathogen being transmitted by unsuspecting and at times intentional (bioterrorism being an example) hosts.

In order to do justice to this module, I am targeting my Biology students that are comprised of 9th - 10th grade students. They will be acting as a Clinician in the Infectious Disease Clinic that will ultimately diagnose Patient A with the pathogen, and then undergo two activities to relay this information to the Patient, since it is a 9 year-old girl. The activities comprise of educating the girl on what antibiotic resistance is made-up of, and how it can inadvertently affect genetic sequencing leading to variation of resistant strains of the pathogen. The students are expected to learn to extrapolate data from graphs, charts, and diagrams. This is a HUGE component of their End-of-Course Exam (EOC). The inferences that have to be drawn from these multiple resources of information allow students to be able to think critically, analytically, and relate these difficult concepts to real-life scenarios.

Students will spend a great deal of time deciphering the information they need to extrapolate from each of the sources of data, and then will be given the opportunity to move on and perform a Rapid-Kit Test, a DNA Microarray Test, an Antibiotic Resistance Lab, and a Genetic Sequencing Lab Activity. The activities were chosen in a particular order, so that they could learn new information from the passages and directly apply it to the activity and questions as they walk through the module. If an instructor were to choose to do a portion of the activity but not the other, it would be okay, because they all can be essentially done individually and still relay viable results that can be analyzed.

Description of Teaching Unit & Expected Outcomes

Of the eight Florida Sunshine State Standards for the End-of-Course Exam covered throughout this lab module, five of the annually assessed standards are touched upon the completion of this module. It is in the best interest of the student to pay very close attention to the intricacy of the underlying genetic sequencing variation that occurs in bacterial cellular division that leads to the survival or antibiotic resistant strains. One of the most pressing issues in current treatment of pathogenic diseases is the rise of antibiotic resistance in the past half-a-century, this is one of the most dramatic and compelling sources of evidence for the evolutionary theory and natural selection at the microscopic level (PBS, 2008). The module is meant to follow the diagnostic pathway of a 9 year-old girl who is the carrier of a new antibiotic resistant strain of *Salmonella*. Within the diagnosis of this patient, she is examined by a primary care physician and then is referred to an Infectious Disease Clinic which launches a full investigation on the child's blood samples, fecal sample, and genetic sequencing of the bacteria to identify which antibiotic would work for this multi-drug resistant (MDR) bacteria. The ability for this bacteria to reproduce quickly is to exchange bits of DNA in order to enable bacteria to have their own degree of adaptability to the environment (PBS).

The outcome is to decipher which pathogen is affecting the girl, and then to identify which medication is most relevant in treating her. Furthermore, students examine the genetic sequence patterns and mutations that cause genetic variation amongst the different strains as a means to educate the young girl on what she is currently carrying as a pathogen, along with modeling how antibiotic resistance works for her with a simple Marshmallow & M&M Lab Activity.

Student Learning Objectives with Standards

All students will be able to… **(Annually Assessed Strands)** *(NOT Annually Assessed)*
- **SC.912.N.1.1.** - Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space, and do the following:
    1. Pose questions about the natural world
    2. Conduct systematic observations
    3. Examine books and other sources of information to see what is already known
    4. Review what is known in light of empirical evidence
    5. Plan investigations
    6. Use tools to gather, analyze, and interpret data
    7. Pose answers, explanations, or descriptions of events

8. Generate explanations that explicate or describe the natural phenomena (inferences)
9. Use appropriate evidence and reasoning to justify these explanations to others
10. Communicate results of scientific investigations
11. Evaluate the merits of the explanations produced by others

- **SC.912.L.14.52**
    - Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics
        - *SC.912.L.14.6.*
            - Explain the significance of genetic factors, environmental factors, and, and pathogenic agents to health from the perspectives of both individual and public health.
- **SC.912.L.15.13**
    - Describe the conditions required for natural selection, including: overproduction of offspring, inherited variation, and the struggle to survive, which result in differential reproductive success
        - *SC.912.L.15.15*
            - Describe how mutation and genetic recombination increases genetic variation
- **SC.912.L.16.3 (Annually Assessed- but L.16.4 is focused on here)**
    - **SC.912.L.16.4**
        - Explain how mutations in the DNA sequence may or many not result in phenotypic change. Explain how mutations in gametes may result in phenotypic changes in offspring.
- **SC.912.L.16.10**
    - Evaluate the impact of biotechnology on the individual, society, and the environment, including medical and ethical issues
- **H.E.912.C.1.8.**
    - Analyze strategies for prevention, detection, and treatment of communicable and chronic diseases

Data Collection & Assessments
- **Diagram, Graphs, & Chart Analysis throughout activity is required**
- **Science-TakeOut Kit: A Medical Mystery of Epidemic Proportions Activity Lab**
  (1 Kit per Group of Students)
  Resource: https://www.sciencetakeout.com/product/a-medical-mystery-of-epidemic-proportions/
    - Rapid Test Kit
        - PCR Tubes (one of sample diarrhea / one of sample positive for virus)
        - 2 Dipsticks
    - DNA Microarray Test
        - 1 DNA Microarray Test Paper
        - 1 PCR Tubes of DNA
        - 1 Pipettes
- **Antibiotic Resistance Simulation Lab**
    - 2 -3 bags of small marshmallows

- - 2-3 bags of M&M's (or any other candy that is similar)
  - 1 Large Box of Toothpicks
- **Genetic Sequences Activity**
  (Pre-Cut: 15 Reference Sequences per Group of Students)
  15 Reference Sequences on Pg. 13 of the following Resource:
  https://drive.google.com/file/d/0B9ccH8PAEIuobDlxVTNiWVJmUmV6dGtXXzM1SXk3djJsTWdr/view?usp=drive_open

CATALySES Summer Institute Included Elements
- *The Medical Mystery of Epidemic Proportions (Rapid Test-kit, DNA Microarray Test, and Antigen / Antibody Diagram analysis)*
- *Disease Detectives -Broad Institute (Genetic Sequence Reference Sheet Pg. 13)*

Differences Between Past Lessons and This Lesson
In the past I have taught this unit of information on biotechnology, pathogens, genetic variation, natural selection, evolutionary theory, antibiotic resistance, etc. individually, through individualized labs and activities targeting only one of the strands at a time. I have not created a module that touched briefly on various topics and put it together in a real-world simulation. My preference is to create these sort of real-world cases, so that the student gets emersed in the storyline and learns the skills through the process of assimilation, and self-gratification of having solved the "mystery". I would probably use this as a class introduction into the concepts of bacteria and viruses, and focus heavily on the factor of antibiotic resistance being due to natural selection affecting genetic sequences of the pathogen as it replicates. This will allow the students to better understand the premise of the study is focused on the overall view of this concept, granted I will go in greater detail within their subsequent lessons, but this works as a talking piece, and comparison unit when I am teaching, so I can refer back to the module on multiple occassions.

Budget and Budget Justification
- 15 Bags of "A Mystery of Academic Proportions"
  - Students will be arranged in groups of 2 -3 students per Kit
    - Will be utilizing the Rapid-Test Kit, DNA Microarray Test, and Antigen/Antibody Diagram
  - Cost each individual Bag $14.67
  - ***TOTAL COST:*** *$ 220.05*

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Lesson Plan for CATALySES Action Proposal

Title: You Are What You Eat, Unless What You Eat Kills You!

Science Subject: Science, Biology, Bioscience, Biotechnology, Microbiology, Anatomy & Physiology

Grade and Ability Level: 9th-12th Grade (Advanced Placement, Honors & Regular)

Topics Covered:
Pathogens, Pathogen Transmission, Epidemics, Pandemics, Antibiotic Resistance, Genetic Sequencing & Evolutionary Genetic Variation

Learning Styles: Visual, Auditory, Kinesthetic, Logical, Social

Key Questions:
- What is a pathogen?
- What is the difference between a virus and a bacteria?
- What is the correlation between genetic variation and the evolutionary theory?
- What is natural selection in relation to bacterial growth?
- How is bioinformatics and genetic sequencing correlated?
- How is genetic sequencing correlated to the new emerging field of phyloanatomy?

Overall Time Estimate: 1 - 2 Class Day

Vocabulary: (20 Vocabulary Words)
- Antibiotic
- Antibiotic Resistance
- Antibodies
- Immune Response
- Adaptive Immune System
- Innate Immune System
- Genetic Engineering
- Genetic Sequencing
- Virus
- Bacteria
- Bioinformatics
- Epidemic
- Pandemic
- Pathogen
- Codons
- Nucleotides (nucleic acids)
- Natural Selection
- Genetic Variation
- Phylogeny
- Phyloanatomy

Lesson Summary:

A New York resident has just arrived to the physician's office with a pathogen, and is going to be diagnosed by the attending physician and referred to the Infectious Diseases Clinic for further testing. Students are the clinicians at the Infectious Disease Clinic and will run a Rapid-Kit Test, a DNA MicroArray Test, and perform an Antibacterial Resistance Lab and a Genetic Sequencing activity to explain the diagnosis to the individual 9 year-old patient.

Student Learning Objectives with Standards
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    2. Conduct systematic observations
    3. Examine books and other sources of information to see what is already known
    4. Review what is known in light of empirical evidence

5.Plan investigations

6. Use tools to gather, analyze, and interpret data

7. Pose answers, explanations, or descriptions of events

8. Generate explanations that explicate or describe the natural phenomena (inferences)

9. Use appropriate evidence and reasoning to justify these explanations to others

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  - Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics
- SC.912.L.14.6.
  - Explain the significance of genetic factors, environmental factors, and, and pathogenic agents to health from the perspectives of both individual and public health.
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- SC.912.L.15.15
  - Describe how mutation and genetic recombination increases genetic variation
- SC.912.L.16.4
  - Explain how mutations in the DNA sequence may or many not result in phenotypic change. Explain how mutations in gametes may result in phenotypic changes in offspring.
- SC.912.L.16.10
  - Evaluate the impact of biotechnology on the individual, society, and the environment, including medical and ethical issues
- H.E.912.C.1.8.
  - Analyze strategies for prevention, detection, and treatment of communicable and chronic diseases

Materials
- **Science-TakeOut Kit: A Medical Mystery of Epidemic Proportions Activity Lab**
  (1 Kit per Group of Students)
  Resource: https://www.sciencetakeout.com/product/a-medical-mystery-of-epidemic-proportions/
  - Rapid Test Kit
    - PCR Tubes (one of sample diarrhea / one of sample positive for virus)
    - 2 Dipsticks
  - DNA Microarray Test

- ■ 1 DNA Microarray Test Paper
- ■ 1 PCR Tubes of DNA
- ■ 1 Pipettes
- ● **Antibiotic Resistance Lab**
  - ○ 2 -3 bags of small marshmallows
  - ○ 2-3 bags of M&M's (or any other candy that is similar)
  - ○ 1 Large Box of Toothpicks
- ● **Genetic Sequences Activity**

  (Pre-Cut: 15 Reference Sequences per Group of Students)

  15 Reference Sequences on Pg. 13 of the following Resource:

  https://drive.google.com/file/d/0B9ccH8PAEIuobDlxVTNiWVJmUmV6dGtXXzM1SXk3djJsTWdr/view?usp=drive_open

You Are What You Eat, Unless What You Eat Kills You
Case Study
STUDENT HANDOUT

Background Information

We have a patient (Patient A) who has reported to our Infectious Diseases Clinic, and has exhibited symptoms that are in correlation with a pathogenic disease. However, with the recent series of multiple differing outbreaks in the nation (such as ***Zika, Chikungunya, Cholera, Salmonella, etc.***) we intend to narrow down the cause of this patients illness through a quick

rapid-test kit to confirm the presence of the bacteria in the biological sample, as well as collection of blood samples, first hand observations, and case study diagnostic analysis of prior recent medical history. Any of the above **pathogens**, *bacterial or viral diseases that are infectious,* have different manners of transmission and **vectors** *(the host organism that stores the pathogen asymptomatically while the pathogen is not in an outbreak).* **Bacteria** are still *considered living things, because they are unicellular and contains a nucleus with genetic information and organelles,* while a **virus** is considered *a non-living pathogen because it requires a host to replicate*, it must successfully enter the cell and the nucleus of its host to replicate.

Patient A, is a 9 year-old girl who came back to Indiana, USA from visiting family in China, and exhibited an onset of symptoms within 24 hours of arriving. At first her family thought she just had the common flu but after once she began to vomit in large quantities they sought assistance from the Emergency Room (ER). Considering most diagnoses of pathogens happen within the first 72 hours it was vital to acquire blood samples.The local hospital reached out the Infectious Disease Clinic to consult with the patient and identify the culprit bacterium or viral pathogen.

Doctor Observations

- *Patient recently visited China and returned, and within the first 24 hours of return had a sudden onset of : <u>vomiting, diarrhea, high fever, and gastroenteritis</u> (inflammation of the stomach and intestines).*

- *Suspect the contamination of one of our current outbreak pathogens, referring patients samples to the local Infectious Disease Clinic for further testing.*

- *Child has been hospitalized and treated with IV fluids during her stay to avoid complications of dehydration*

- *Blood samples, bacterial cultures, urinary samples, saliva-test sample, fecal sample were all sent to the Infectious Disease Clinic for analysis and further culturing*
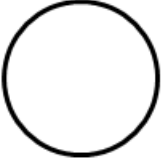
1. Use the **"Clinician Analysis of Symptoms Associated with most Common Pathogens"** to decipher a plausible diagnosis based on symptoms, which pathogen(s) would you narrow it down to?
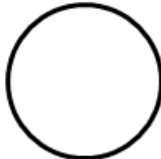
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Table 1: Clinician Analysis of Symptoms Associated with most Common Pathogens (PBS, 2008; UF ICBR CPET, n.d.)
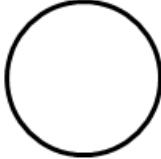
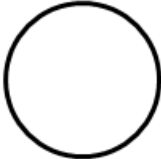| Pathogen Name	Zika	Chikungunya	Salmonella	E. Coli
Symptoms	● Fever ● Rash ● Headache ● Joint Pain ● Red Eyes ● Muscle Pain	● Fever ● Rash ● Headache ● Severe Joint Pain ● Joint Swelling ● Muscle Pain	● Fever ● Diarrhea ● Bloody Stool (poop) ● Headache ● Abdominal	● Fever ● Rash ● Headache ● Joint Pain ● Red Eyes ● Muscle Pain

			Cramping Chills Nausea Vomiting Muscle Pain	
Host Vector and Transmission	• Mosquito's • Pregnant Women to her fetus • Through sex	• Mosquito's	• Raw Fish (especially Tuna, Salmon, Sushi, Shellfish, etc.) • Raw Poultry • Raw Beef	• Uncooked Beef • Uncooked Pork • Contaminated Water • Contaminated vegetation (spinach)
Virus or Bacteria	Virus	Virus	Bacteria	Bacteria
Sites of Current Outbreaks	• China • Africa	• USA -Florida • Carribean • Africa • Europe • Southeast Asia • Pacific Islands • Indian Islands	• USA -Connecticut - Iowa -Illinois -Minnesota -New York -Indiana -North Dakota -Washington • Japan • China • South America • Southeast Asia • Africa • Europe • Oceania	• USA • Haiti • China • Japan
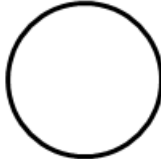
DNA Microarray Laboratory Observations
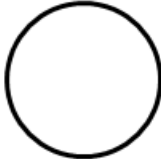
(1) (2) (3)

(4) (5) (6)

Testing Patient A Sample Against Most

Gene 1- Zika viral gene
Gene 2- Chikingungya viral gene
Gene 3- Salmonella gene

Conclusions:

_____

_____

Instructions: Follow the directions on the test-kit for **DNA MicroArray Lab Test** in order to fill out your observations above.

2. What is your conclusions based on the DNA MicroArray Lab Test?

_____

Rapid-Test Kit : Clinician Testing Results

Based on the sample collected we have identified the patient to have *Salmonella enterica serovar*, so the DNA MicroArray Test tested for their saliva and blood plasma samples. We are testing the diarrhea for the presence of *Salmonella* with *Vibrio salmonellae.*

3. Test a sample of patient diarrhea provided by local ER hospital, and test a sample of *Vibrio salmonellae* as your positive control group.

   a. Record your observations by coloring and labeling the pictures of your dipsticks

Virio Salmonellae Sample

Patient Diarrhea Sample

   b. Is your test positive or negative for *Salmonella*?

_____

c. What is your conclusions based on this Rapid-Test?

_____

Aftermath of Diagnosis

During 2015, Patient A, was considered Patient Zero in Indiana, since she was the first patient within that state, however there had been an outbreak of sporadic locations across the USA in the last few months. Her being the first patient in Indiana, meant an eminent outbreak could ensue within that state, thereby she was quarantined.

An outbreak of ***Salmonella enterica serovar*** *(Bacteria species variety)* within the USA had become an epidemic, but recently the pathogen has crossed over to other countries, thus now considered a full blown pandemic that included China, Japan, and sporadic outbreaks in parts of Europe, South America, Africa and Oceania.

However, while being quarantined the **epidemiologists** *(doctor or researcher that studies epidemics and pandemics)* and **pathologists** *(doctor or researcher that studies diseases)* identified that the bacterial strain of *Salmonella* was different than the ones they saw in the other states. The **genetic sequencing** *(the order of nucleotides in DNA)* in her strain showed mutations within the bacterial plasmid DNA, and thereby had shown adaptation from its original exposure. This **natural selection** of *the fittest bacterium surviving to pass on its new strain of genetic material through cell division*, gave rise to the emergence of **antibiotic resistance** amongst many of these bacterial and viral pathogens which is making it extremely difficult to treat the pathogen effectively.

"**Bacteria** are single-celled **prokaryotic organisms**, which means that they *lack a nucleus.* Compared to **eukaryotic organisms**—*multicellular organisms such as yeast and humans*—which corral their DNA inside the cell nucleus, bacterial DNA floats freely in the cytoplasm. Most often, this is in the form of a single, *circular chromosome of DNA* called **plasmids**. These structures carry genes in addition to the genes on the bacterial chromosome and can replicate independently of the chromosome. Plasmids often contain genes for antibiotic resistance" (PBS, 2008).

Unfortunately, with the over exposure patients get to general broad spectrum antibiotics, the naturally genetically fittest bacterium will survive (due to natural selection as described in the evolutionary theory) and replicate through **unicellular** *(one-celled organism)* **binary fission** *(mitosis of a bacteria),* thereby passing on their antibiotic resistant genes to each subsequent generation. This is of utmost concern for physicians and patients, because most antibiotics that are used to treat the resistant strain, now requires Carbapenem (which is considered the last resort medication for this particular resistant strain), now referred to as ***Salmonella indiana*** or ***MDR Salmonella*** *(MDR = multi-drug resistant)* . However, there is an occurrence of a MDR (multi-drug) resistant that exhibits resistance towards Carbapenem as well, thus putting us in the unfortunate situation of not having a viable method of treating the patient if they contract this newly mutated version of *MDR Salmonella*.

Instructions:

4. Read the following quote from the Head of the Center of Disease Control (CDC) and describe why the process of natural selection and evolutionary theory affect the development of new *MDR Salmonella* in the space below. Include the following words in your description: **bacteria, antibiotic resistance, genetic sequence, evolution, natural selection, genetic variation.**

> *" The rise of antibiotic resistance over the past half-century is one of the most dramatic and compelling examples of evolution in action. Bacteria have adapted to nearly every antibiotic we've developed. Their ability to reproduce quickly and exchange bits of DNA enable bacteria to have this degree of adaptability." (PBS, 2008)*

_____
_____
_____
_____
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5. Below you will find the "**Antibiotic resistance phenotype of the tested S. Indiana strains**", *Table 2*, on the top you will find the antibiotics abbreviations and on the left the strain number and year it was isolated. Decipher which antibiotics our patient S1501 (Patient A) is capable of being treated with, and describe using evidence from the chart as to why you feel this would be the best course of treatment (you are allowed to use the abbreviations of antibiotics in your discussion) . Your analysis is what the attending physician will use to treat Patient A, so make sure to not make any mistakes in deciphering which are the antibiotics this patient can still benefit from.

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_____
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Table 2: Antibiotic resistance phenotype of the tested S. Indiana strains
(Gong, Zeng, Zhang, Zhang, Wang, & Lin, 2019)   (R- resistant)

Strain	Grou	Antibiotics

(Isolation year)	p	AMP	AMC	CTX	CRO	CAZ	ATM	IPM	STR	GEN	KAN	AMK	TET	NAL	CIP	SUL	TMP	SXT	CHL	NIT
ATCC51959	1																			
S0802 (2008)	2	R							R		R		R	R	R	R			R	
S1104 (2011)									R		R			R	R	R	R	R	R	
S1105 (2011)													R	R	R					
S1106 (2011)			R						R				R		R	R	R	R		
S1064 (2010)	3	R							R	R	R		R	R	R	R	R	R	R	R
S1319 (2013)			R						R	R	R	R	R	R	R	R	R	R	R	R
S1402 (2014)			R						R		R		R	R	R	R	R	R	R	
S1430 (2014)			R						R	R	R	R	R		R	R		R		R
S1204 (2012)	4	R		R	R		R		R	R	R	R	R	R	R	R	R	R	R	R
S1318 (2013)			R	R	R	R	R		R	R	R			R	R	R	R	R	R	R
S1407 (2014)			R	R	R				R	R	R		R	R	R	R	R	R	R	
S1443 (2014)			R	R	R	R	R		R	R	R		R	R	R	R	R	R	R	R
S1445 (2014)			R	R	R	R	R		R	R	R		R	R	R	R	R	R	R	R
S1447 (2014)			R	R	R	R	R		R	R	R		R	R	R	R	R	R	R	R
S1454 (2014)			R	R	R		R		R	R	R	R	R	R	R	R	R	R	R	R
S1459 (2014)			R	R	R	R	R		R	R	R		R	R	R	R	R	R	R	R
S1467 (2014)			R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	
S1501 (2015)			R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	
S1515 (2015)			R	R	R		R		R	R	R		R	R	R	R	R	R	R	

Medication Abbreviations:

- AMP: ampicillin
- AMC: amoxicillin/clavulanic acid
- CTX: cefotaxime
- CRO: ceftriaxone
- CAZ: ceftazidime
- ATM: aztreonam
- IPM: imipenem
- STR: streptomycin
- GEN: gentamicin
- KAN: kanamycin

- AMK: amikacin
- TET: tetracycline
- NAL: nalidixic acid
- CIP: ciprofloxacin
- SUL: sulfafurazole

- TMP: trimethoprim
- SXT: trimethoprim
- CHL: chloramphenicol
- NIT: nitrofurantoin

Transmission

"Emergence of multidrug-resistant (MDR) *Salmonella enterica* serovar Indiana (*S.* Indiana), a dominant *Salmonella* serovar in China, has raised global awareness because the MDR *S.* Indiana also was rapidly emerged in other countries recently. To improve our understanding of underlying MDR mechanism and evolution of this emerging zoonotic pathogen" (Gong, Zeng, Zhang, Zhang, Wang, & Lin, 2019), we must begin to examine its **zoonotic** (*diseases transferred from one organism to another)* method of transmission.

The most common method of transmission is the exposure to contaminated food, water, raw and uncooked meats such as beef and pork, poultry, and fish. Raw shellfish is a particular strong vector, as is tuna and salmon when left uncooked, raw, or in sushi. The most recent outbreak has been in 2019, and has been caused by an MDR strain that is now prevalent in Tuna that came from Japan, and has been used in sushi recipes and/or sold to be cooked later and/or frozen. The main contamination is caused by exposure to these raw and uncooked foods, however, the secondary method of exposure is transmission from bodily fluids to other bodily fluids and open cuts (like when animals are butchered and prepared for cooking).

ANTIBIOTIC RESISTANCE LAB EXPERIMENT

Instructions: Perform the following **Antibiotic Resistance Lab** (KBS, 2017) for the 9 year-old patient so that she can better understand what is happening to her, and why she is ill.

Materials: Toothpick, mini-marshmallows, M&Ms, stop watch or phone, paper.

Reasoning: The mini-marshmallows represent the bacteria that are causing you to feel sick. Start by putting 25 marshmallows on the paper.

6. The toothpick represents the antibiotic your doctor prescribed. Name your toothpick after an antibiotic _____.

Step 1: You now have 5 seconds to pick up as many marshmallows as possible using the toothpick (i.e. kill as many bacteria as possible).

7. But before you begin list two ways that antibiotics kill bacteria?

_____and _____

Dose 1: Now start the clock for 5 seconds (one person at a time, and one person use the toothpick) **GO!**

8. How many marshmallows were you able to grab in 5 seconds? _____

 Those that remain are is representative of how many harmful bacteria are still alive in your body. Record your results in the table.

Step 2

- ❖ **(Info) Certain bacteria may not have been killed by the antibiotic because the dose was not strong enough or because they are resistant to the antibiotic.**
- ❖ **(Info) Many bacteria are naturally resistant to bacteria and others develop resistance through beneficial mutations that prevent the antibiotic from working.**

Directions for Setup of Dose 2:

> ➢ *To represent mutated bacteria, <u>take one marshmallow away and replace it with an M&M.</u>*
> ➢ **Then to represent binary fission (asexual reproduction), <u>double the number of marshmallows and M&Ms!</u>**

Dose 2: Now it is time for the second dose of antibiotics. This time the antibiotic is stronger and you will have 10 seconds to pick up as many marshmallows and M&Ms as possible with the toothpick.

Switch off who was timing. Set the clock for 10 seconds. **GO!**

9. Record below how many marshmallows and M&Ms are still in the population. If there are no M&Ms at the end of a dose, a new mutation will arise and you should add one M&M. ***Now double the number of marshmallows and M&Ms.***

Dose 3: Apply a third dose of antibiotics that is the strongest yet. This time you have 15 seconds to pick up marshmallows and M&Ms with the toothpick.

10. Record how many marshmallows and M&M's are left in the population after the third dose of antibiotics? You can keep going for 3 more doses, but do not extend the time limit any longer as you are already taking the strongest legal dose of antibiotics.

M&M and Marshmallow Lab

	Marshmallows Start	Marshmallows Finish	M&M's Start	M&M's Finish
Dose 1	25		--	--
Dose 2			2	
Dose 3				
Dose 4				
Dose 5				
Dose 6				

11. Graph the number of marshmallows and M&Ms *at the end of each dose (before doubling)*. Before you begin determine what to plot on the X and Y axes.

X- axis: _____Y- axis: _____

12. Label the line in your plot that represents non-resistant bacteria and resistant bacteria. Use this information to answer the questions below. For the graph, focus only on **how many bacteria** are left at the finish of each round.
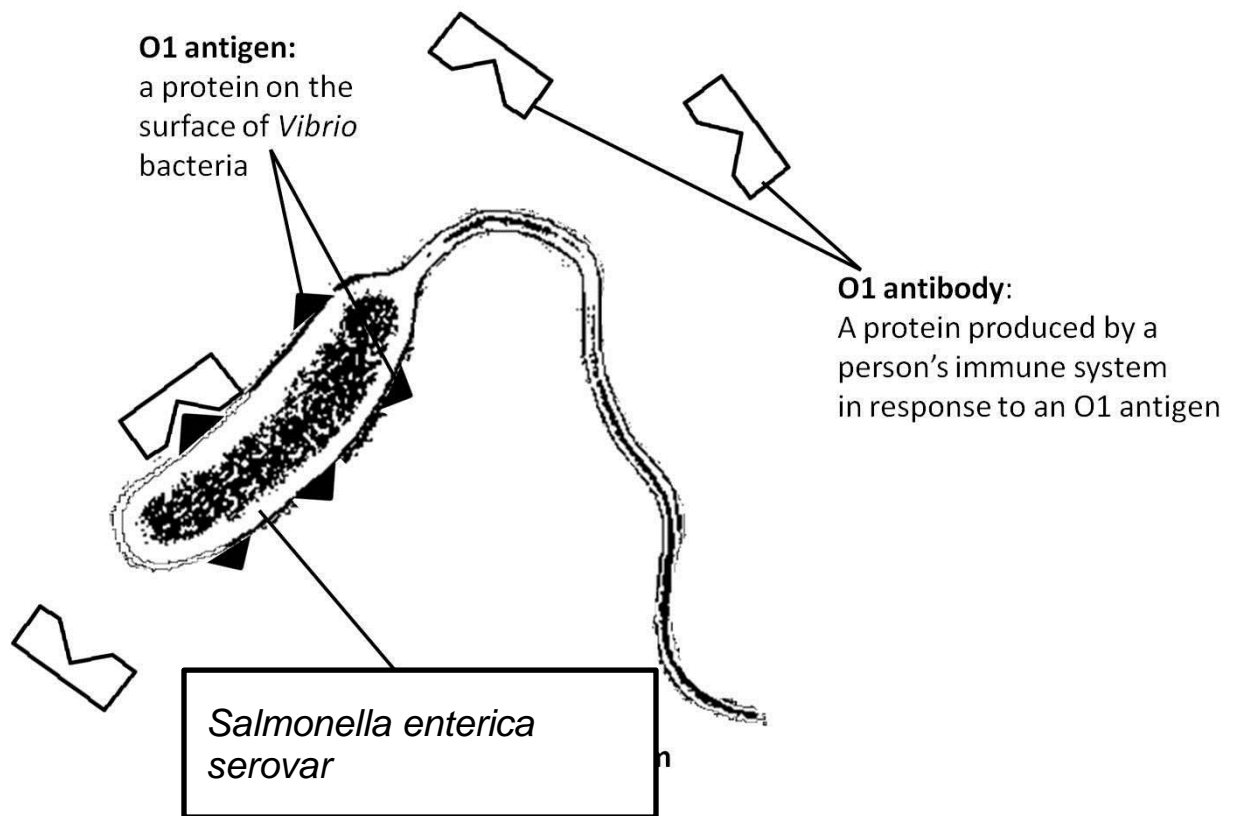
13.What can you conclude about the influence of the antibiotic on the population of bacteria?
_____

14. How effective will this same antibiotic be when prescribed to this patient again?

_____

15. What is one human practice that increases the prevalence of antibiotic resistance?

_____

Instructions:

Use the diagram below with the *Salmonella* bacterium and describe how antigen and antibodies work.

O1 antigen: a protein on the surface of *Vibrio* bacteria

O1 antibody: A protein produced by a person's immune system in response to an O1 antigen

Salmonella enterica serovar

Antigen: a protein on the surface of a bacteria or other pathogen; different bacteria have different antigens

Antibody: a protein produced by the immune system to recognize a specific antigen and then to initiate an immune response. In the presence of the matching antigen, antibodies will attach to the antigen and cause **agglutination** *(experimental result that results in clumping of cells with antigen on surface being matched to corresponding antibodies)*

16. What are the main differences between the antigen and the antibodies involved with Salmonella?

_____

17. Explain why people diagnosed with Salmonella enterica serovar will not develop symptoms of Salmonella if they were exposed to subsequent similar strains of the bacterial pathogen?

_____

18. What experimental results will you observe

_____

Bioinformatics Application

     A combination of **bioinformatics**, *the integration of mathematical principles and data analysis using technology and computer sciences to analyze science data.* In bacterial growth and mutations that lead to beneficial gene sequencing traits can be further analyzed using bioinformatics as a means of deciphering data. Databases like PubMed (www.pubmed.gov) who qualify as bioinformatic databases store the genetic sequences of entire organism genomes for a multitude of living organisms (animals, plants, bacteria, etc.). The genetic sequences provided in the activity below used PubMed to acquire the data. *A new emerging field that focuses on the differences in genetic variation of a pathogen as it evolves in different portions of the organism is referred to as*, **phyloanatomy**.

19. What sort of bioinformatic question would you ask as a means to analyze your M&M/ Marshmallow Lab Results that describes the genetic variation and phyloanatomy of the pathogen?

_____

Genetic Sequencing

Reference	C	T	A	T	G	C	A	A	G	C	A	G	T	T
Sequence 1	C	C	A	T	G	T	A	A	G	T	G	G	T	T
Sequence 2	T	C	A	T	G	T	C	A	G	C	A	A	C	T
Sequence 3	C	C	G	T	G	T	A	A	G	C	A	G	T	T
Sequence 4	C	C	A	T	G	T	A	A	G	C	A	G	T	T
Sequence 5	T	C	A	T	G	T	C	A	G	C	A	A	C	T
Sequence 6	C	C	A	T	G	T	A	A	G	C	A	G	T	T
Sequence 7	T	C	A	T	G	T	C	A	G	C	A	A	C	T
Sequence 8	T	C	A	T	G	T	C	A	G	C	A	A	C	C
Sequence 9	C	C	A	T	G	T	A	G	G	C	A	G	T	T
Sequence 10	T	C	A	G	G	T	C	A	A	C	A	A	C	T
Sequence 11	C	C	A	T	G	T	A	A	G	C	A	G	T	T
Sequence 12	T	C	A	T	G	T	C	A	G	C	A	A	C	C
Sequence 13	C	C	A	T	G	T	A	A	G	C	A	G	T	T
Sequence 14	C	C	A	T	A	T	A	A	G	C	A	G	T	T
Sequence 15	T	C	A	T	G	T	C	A	G	C	A	A	C	T

Directions:
- Move the *Salmonella* genetic sequences around to compare them to each other and find the patterns in the sequences
- Group similar sequences together using the patterns you identify

The **reference sequence** is the *one you use to compare all other sequence changes* to. Remember the colored base-pairs show the genetic mutation that has occurred to that sequence

20. How many genetic sequence groups did you make when arranging them based off of patterns (multiple arrangements are possible, no answer is the ONLY right answer)?

_____

21. Why did you use that number of groups?

_____

22. Would it be useful to just have a group with all the 15 sequences (why or why not?)?

_____

23. Would it be useful to have 15 groups of 1 sequence each?

_____

24. Why do these genetic sequences with mutations enhance the survival of the strain of bacteria?

_____

25. Why is it important to analyze the genetic variation of the sequences pertaining to the current bacterial strain?

_____

VOCABULARY DEFINITION REFERENCE SHEET

Note: Not ALL bolded vocab words from reading included, please refer to the italicized portion of the reading when reading passages for those definitions not included here

Vocabulary Word	Definition
Antibiotic	a type of medicine (such as penicillin or its derivatives) that inhibits the growth of or causes destruction of microorganisms
Antibiotic Resistance	antimicrobial resistance is the ability of a microbe (bacteria or virus) to resist the effects of a medication that once could successfully treat the microbe, due to multiple generations of microbes being treated with the same medicine and the natural selection process of evolution taking effect where the fittest microbe survived and got to reproduce (these being the resistant microbes)
Antibodies	a free floating blood protein that is produced when exposed to a specific

	antigen (**antigen= a receptor on the blood cell or any cell**) of which it will attach to if exposed to it as a foreign substance the body recognizes as not their own, such as bacteria, viruses, etc.
Immune Response	the reaction of the cells and fluids of the body in protecting itself from the presence of a substance which is not recognized as a part of the body itself
Adaptive Immune System	adaptive immunity is an immunity that occurs after exposure to an antigen either from a pathogen or a vaccination there are two types of adaptive responses: the cell-mediated immune response, which is carried out by T cells, and the humoral immune response, which is controlled by activated B cells and antibodies Both types attempt to prevent the foreign microbe from ever attacking again by targeting the microbe for destruction by creating antibodies that will target it for destruction if ever exposed again
Innate Immune System	innate immunity refers to nonspecific defense mechanisms that come into play immediately or within hours of an antigen's appearance in the body. These mechanisms include physical barriers such as skin, chemicals in the blood, and immune system cells that attack foreign cells in the body generically not targeted like in adaptive immune response
Genetic Engineering	the deliberate modification of the characteristics of an organism by manipulating its genetic material
Genetic Sequencing	genome sequencing is figuring out the order of DNA nucleotides, or bases, in a genome—the order of As, Cs, Gs, and Ts that make up an organism's DNA. The human genome is made up of over 3 billion of these genetic letters.
Virus	A virus is a small infectious agent that can only replicates inside its host's cells within an organism. Viruses can infect all types of organisms, from animals and plants to microorganisms, such as bacteria
Bacteria	a member of a large group of unicellular microorganisms which have cell walls but lack organelles and lack an organized nucleus some bacteria are bad and some are good, the bad ones can cause infections/diseases
Bioinformatics	the collection, classification, storage, and analysis of biological and chemical information using computers as applied research to decipher complex issues in biological sciences, especially in genetic sequencing and engineering
Epidemic	a widespread infectious pathogen within a community or regional location at a particular time
Pandemic	A widespread infectious pathogen within multiple countries across the globe at a particular time
Pathogen	a bacterium, virus, prion (prion = defective and contagious proteins), or

	other microorganism that can cause disease
Codons	a sequence of 3 nucleotides which together make an unit of genetic code (a gene) within a DNA or RNA molecule
Nucleotides (one nucleic acid)	nucleotides form the basic structural unit of nucleic acids such as DNA. Examples include Adenine, Thymine, Guanine and Cytosine
Natural Selection (part of evolutionary theory)	the process whereby organisms better adapted to their environment (the fittest organisms) tend to survive and produce more offspring. The theory of its action was first fully expounded by Charles Darwin and is now believed to be the main process that brings about the concept of evolutionary theory
Genetic Variation	genetic variation is the difference within DNA sequences between individuals within a population. Variation occurs in gamete (sex) cells i.e. sperm and egg, and also in somatic (all other) cells
Phylogeny	phylogenetic tree or evolutionary tree is a branching diagram or "tree" showing the evolutionary relationships and ancestry amongst various biological species or other entities—their phylogeny —is based upon similarities and differences in their physical or genetic sequences
Phyloanatomy	tracking the genetic sequence changes of pathogenic disease spread within the one host organism Ex: the e.coli changed genetically the more it replicated because it adapted to include genetic sequences from the host organism and now carry it in their genetic code

You Are What You Eat, Unless What You Eat Kills You!

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SALMONELLA

The Challenge

- The Module simulates the process of diagnosing a Patient that has become afflicted with a pathogen
    - **Role:** as the Infectious Disease Clinician you will be using various strategies to diagnose the pathogen
    - **Diagnostic Pathogen Tests used**
        - *Vibrio Salmonellae* Rapid-Kit Test
        - DNA Microarray Test
        - Genetic Sequencing Analysis for Antibiotic Resistant Strain
   - Antibiotic Resistance and Medication Compatibility Analysis

Focus Rationale

-targets the process of genetic variation that affects the evolution of pathogens and has led to antibiotic resistant strains that require compatibility assessments

-to expose our students to the variability of treating pathogens depending on type and antibiotic resistance of the pathogen

Experiment data

Record the information you get from your experiment

Include a table or graph to display what you see
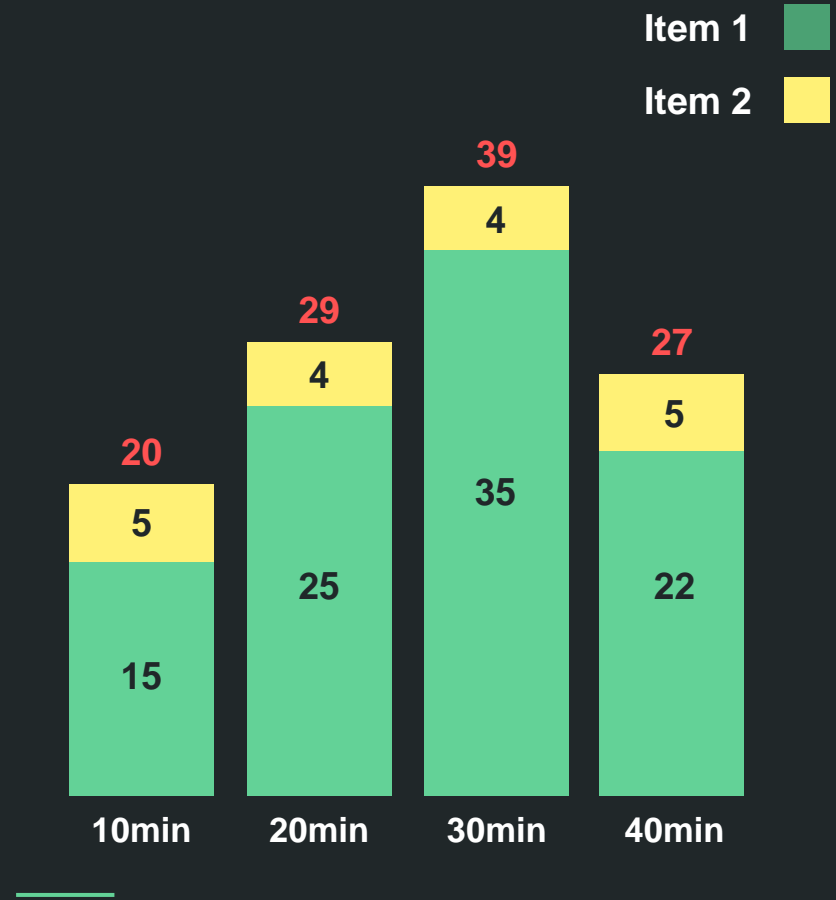
This is the most important takeaway that everyone has to remember.

References