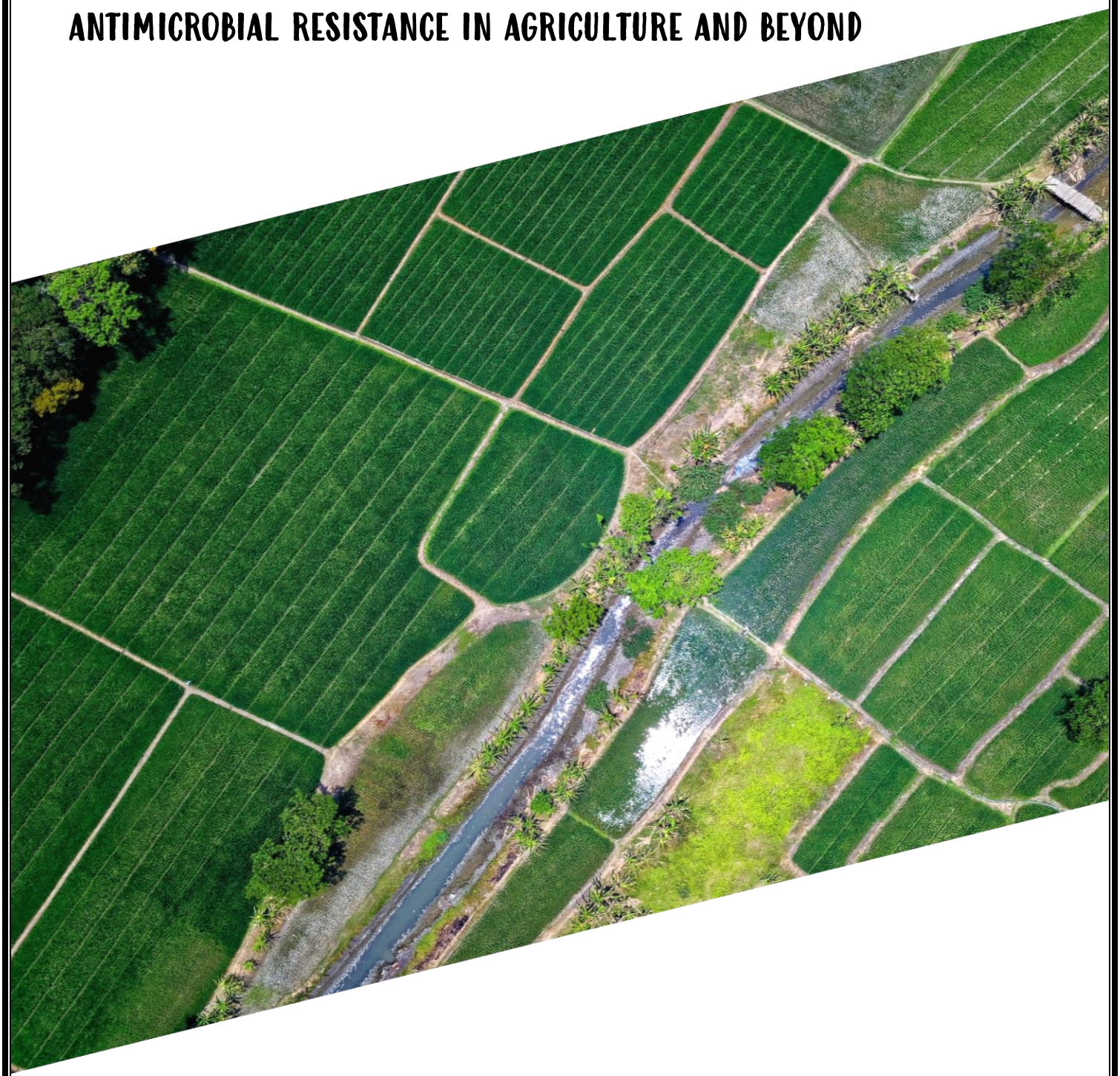
WHAT CAN WE DO?

ANTIMICROBIAL RESISTANCE IN AGRICULTURE AND BEYOND



Instructional Materials for Grades 9-12 highlighting the research of Adina Howe and Michelle Soupir

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References:

[1] https://www.news.iastate.edu/news/2016/05/04/antimicrobial
[2] https://news.engineering.iastate.edu/2013/09/02/research-looks-to-prevent-human-exposure-to-water-borne-pathogens/
[3] Natural Selection and the Development of Antibiotic Resistance - Middle School Sample Classroom Assessment
[4] https://www.nextgenscience.org/sites/default/files/MS-LS_%20Antibiotic%20Resistance-Nov%202014.pdf
[5] https://www.cdc.gov/campylobacter/outbreaks/puppies-12-19/index.html

Additional resources are noted in text.

Updated 08/2022

The activities and investigations within these curriculum modules are designed to use low-cost and locally-available supplies, where appropriate. Teachers should, however, feel comfortable substituting materials and supplies when necessary and/or when specified items are not available.

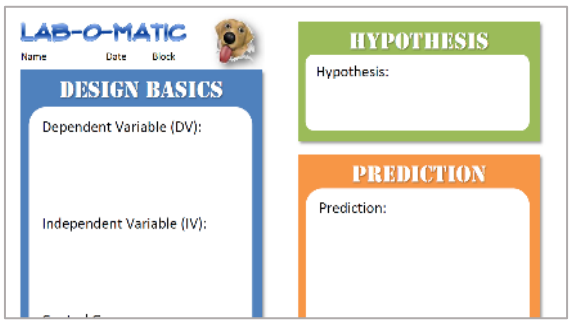
All activities and investigations should be done with safety as a priority. Teachers are responsible for ensuring that students are aware of all relevant safety concerns including, but not limited to, those mentioned in this document.

Contents

Additional Support

Lab-O-Matic

The Lab-O-Matic is a collection of student-facing tools that can support the development of students' ideas about planning and carrying out investigations. There are quite a few aspects of the Lab-O-Matic which are all available for free download here: https://hallscience.us/lab-o-matic. The Lab-O-Matic could be used as part of Module 3 in this curriculum, if "planning and carrying out investigations" is a targeted science and engineering practice (SEP).



Equitable Learning

Our promise…
This innovative collection of curriculum modules has been developed with a focus on promoting students' access to learning. While teachers will need to deploy context-specific instructional strategies to ensure skillful implementation of any science experience, we have considered these key points during curriculum development:

Students build understanding through carefully sequenced learning
The storyline approach to sequencing learning experiences allows for a broad range of pedagogical nuances that are often absent from "textbook" curriculum materials. By introducing a relevant, meaningful problem (phenomenon) before students have learned core ideas can improve the chances that students will learn transferrable knowledge and skills (National Research Council, 1999). The curriculum modules contained in this booklet build on one another and help students find meaning in each investigation that leads them closer to making sense of the anchor phenomenon.

Students use scientific practices to make sense of a phenomenon
Through the purposeful use of hands-on investigations, students will engage with many of the NGSS Science and Engineering Practices. This engagement will help them to connect what they are learning to problems that impact their own family and community. By doing so, students may be driven to use those discoveries to help solve issues of global significance. This leads them to better understand scientific ways of thinking and to value science in greater ways (National Research Council, 2012).

Students' own questions and wonderings drive learning
Activities presented in this curriculum unit are designed to encourage student-student discourse. These academic conversations provide teachers valuable insight into student thinking and provide evidence that can be used to guide the next instructional steps. Further, by eliciting students' questions and helping them use their own funds of knowledge to make sense of relevant phenomena, we are supporting student motivation and agency (Harris, Phillips, & Penuel, 2011).

Introduction

A Focus on Research

A Decade of Research

Drs. Michelle Soupir and Adina Howe, researchers at Iowa State University, are concerned about the health of our environment. Since beginning their work at ISU, they and those working in their labs have studied a variety of aspects of local watersheds and water quality. The Howe and Soupir labs have a passion for learning about what threats to our water bodies exist, and what we can do to mitigate potentially harmful situations.


Dr. Michelle Soupir


Dr. Adina Howe

Some of their studies focus on the use of innovative strategies to remove nitrates from field runoff water. These investigations often employ the use of 'woodchip bioreactors' to house nitrogen-removing bacteria that lower nitrate levels prior to the runoff joining other bodies of water. While these experiments make use of 'helpful' bacteria, the labs are also studying the presence of potentially harmful bacteria in our local watersheds.

The concern is around the presence of antimicrobial-resistant bacteria in these areas, including livestock systems. Antimicrobial-resistant bacteria are those with genetic variations which render them "resistant" to the use of some antibiotics.

Why does it matter?

Antimicrobials are used widely in animal production. They improve animal health and animal welfare, and also enhance animal growth rates and raise animal productivity. The use of antimicrobials, however, can lead to the emergence of resistance and the transmission of resistant genes and resistant bacteria between species.

Access to effective and cost-efficient antimicrobials is critical for human and animal health, animal welfare and food security. The potential consequences of antimicrobial resistance include reduced food production, reduced food security, greater food safety concerns, higher economic losses to farm households, and contamination of the environment.

The condition of antimicrobial resistance (AMR) develops when potentially harmful organisms such as bacteria, viruses and fungi no longer respond to medications generally used against them. AMR continues to pose a growing threat to the health of both humans and animals, since infections will linger and spread if the treatments we have for them are no longer effective. [1]

In this curriculum, students will learn about resistance, how it spreads, the dangers of exposure and the ultimate impacts resistance has on agriculture, the economy, and our health.

The Water Quality Research Lab's studies look at understanding the occurrence and movement of antibiotics, antibiotic-resistant bacteria and genes responsible for antibiotic resistance in manure and manure's effect on soil and water quality from repeated application.

Research teams are conducting studies at research farms around Iowa, which have a long-term history of managed agricultural systems and at least 30 years of data looking at the impacts of water quality from repeated swine manure application. In the past, confined animal feeding operations fed low doses of antibiotics to animals to promote growth, but now focus is on keeping the herds healthy and treating disease, when necessary.

Experts say previous studies have found that up to 75 percent of the antibiotics administered to animals can pass through their systems. Questions have arisen about whether the antibiotics are able to move from the soil to the water, eventually making their way to lakes and streams, ultimately affecting humans.

Even though the bacteria are exposed to antibiotics meant for livestock, they could develop resistance to human drugs if they belong in the same class of antimicrobials.

~based on an ISU College of Engineering News article [2]

What to Expect

The information, activities and assessments included in these curriculum modules aim to tell a story. This storyline will help students learn the basics of how populations of bacteria become resistant to antibiotics, and how that knowledge can help make sense of the phenomenon presented. Students will learn that local conditions and actions can have a significant impact on global issues. The activities with which students will engage constitute a meaningful pathway to understanding and are not intended to be used in isolation. As you make plans for how these modules will be used, carefully consider the connections and interdependence of the activities, which make it difficult to separate the activities and is not advised.

Each module consists of two or three activities. Each activity provides opportunities to develop and use specific elements of the Next Generation Science Standards (NGSS) science and engineering skills and practice(s) to make sense of phenomena and/or to design solutions to problems. They also provide students with the chance to use conceptual understanding that spans scientific disciplines and develop deep understanding of core ideas and content.

Finally, please maintain your own sense of curiosity as you use these materials. Resources and ideas for classroom implementation are included at the end of this guide. Consider your own professional growth an integral part of implementation - always value your own learning, as well as that of your students.

Why focus on antimicrobial resistance?

"Adaptation by natural selection acts over generations to change the characteristics of a population, particularly in response to new environmental conditions. Traits that support successful survival and reproduction in the new environment become more common; those that do not, become less common. As a result, the distribution of traits in a population changes." [3]

"Over time...the wide spread use of antibiotics has led to the development of resistant strains of bacteria. Infectious diseases such as staphylococcal infection are becoming increasingly difficult to treat because the bacteria that cause them are becoming resistant, through mutations and natural selection, to the antibiotics used to treat them. New types of antibiotics are being developed, but bacteria continue to develop resistance to these new medicines. This antibiotic resistance makes it difficult to eliminate infections because existing medicines are becoming less effective. Thus, diseases that were once highly treatable are now becoming a problem once again." [4]

Suggested Learning Plan

Teacher Planning and Suggested Pacing Guide

Each 🕒 represents approximately 45 minutes of class time. This includes time for the entire activity, including pre– and post-activity discussions or work.

Although the activities listed below should be done in the order listed, they need not be done on consecutive days. Please use the schedule below as a guide for determining how each activity will support the learning your students will do throughout time you have.

Module 1
Activity #1: Getting to Know the Puppy Problem 🕒

Module 2
Activity #2: What are microbes? 🕒
Activity #3: How does AMR happen? 🕒
Activity #4: What role does DNA play in the life of a bacterium? 🕒🕒

Module 3
Activity #5: Determining resistance in bacterial populations 🕒🕒🕒
Activity #6: Transforming bacterial DNA: ampicillin resistance 🕒🕒🕒🕒
Activity #7: One Health in Action 🕒🕒

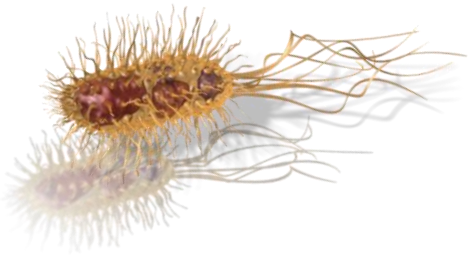
Module 4
Activity #8: Jyll & Jakk Dilemma– Assessment Project 🕒

Connection to Standards

The connections seen here should be considered POSSIBLE connections to the Next Generation Science Standards (NGSS). Depending on how each module and activity is implemented, teachers may choose to emphasize additional/different science & engineering practices, disciplinary core ideas, and cross cutting concepts beyond those indicated below.

	SCIENCE & ENGINEERING PRACTICES								DISCIPLINARY CORE IDEAS				CROSS CUTTING CONCEPTS						
	Asking Questions & Defining Problems	Planning & Carrying out Investigations	Analyzing & Interpreting Data	Developing & Using Models	Constructing Explanations & Designing Solutions	Engaging in Argument from Evidence	Using Mathematics & Computational Thinking	Obtaining, Evaluating & Communication Info	DCI: Physical Science	DCI: Life Science	DCI: Earth & Space Science	DCI: Engineering, Technology & Applications	Patterns	Cause & Effect	Scale, Proportion & Quantity	Systems & System Models	Energy & Matter: Flows, Cycles, & Conservations	Structure & Function	Stability & Change
MODULE 1 ACTIVITY #1	X							X											
MODULE 2 ACTIVITY #2				X				X		LS1.A					X			X	
MODULE 2 ACTIVITY #3				X						LS3.B								X	
MODULE 2 ACTIVITY #4				X				X		LS3.A				X				X	
MODULE 3 ACTIVITY #5	X	X	X			X				LS1.D			X	X					
MODULE 3 ACTIVITY #6		X	X		X					LS1.D		ETS2.B	X			X			
MODULE 3 ACTIVITY #7					X	X		X					X						
MODULE 4 ACTIVITY #8	the summative task provides opportunities for students to demonstrate the SEPs, DCIs and CCCs indicated above																		

Storyline



A Plan for Student Learning

Driving Question

How can something so small cause so many problems?

Anchoring Phenomenon

Disease outbreak in pet store puppies

Phenomenon-driven Lessons	Making Sense (Learning)	Connection with Next Investigation
What are microbes?	Microbes come in a variety of types, each having a distinct structure and way of living.	The structure of a microbe may influence its ability to cause disease
How does AMR happen?	Bacteria gain resistance to antibiotics through a known process that involves multiple systems and mechanisms.	Genetic mutations play a significant role in AMR in bacterial populations.
What role does DNA play in the life of a bacterium?	Most bacteria have circular chromosomes consisting of DNA which codes for the production of RNA and proteins.	Bacteria that produce proteins which enable them to resist antibiotics will survive while the rest of the population will not (Module 3)
How can we know if bacteria are resistant to antimicrobials?	Because antimicrobials inhibit bacterial growth, we can use lab processes to find resistant colonies.	With the right techniques, we can confer resistance directly to bacteria.
How can we make bacteria resistant to antibiotics?	By using cutting edge techniques, we can impart the genetic information needed to create antibiotic resistant bacteria.	Knowing how and why bacteria become resistant to antibiotics may help us make better choices when it comes to antibiotic use and management.

Engagement with photos → Module 1: Explore the anchor phenomenon → Module 2: Get to know the microbes → Module 3: AR Investigations → Module 4: Assessing our learning

Getting to Know the Puppy Problem

Module 1, Activity #1

Overview
This activity will allow students to begin developing questions related to the anchor phenomenon – the disease outbreak among "pet store puppies". It's organized in such a way that students are presented with a variety of sources of information related to the phenomenon and after each new artifact is presented, students generate additional questions. These student-generated questions should remain visible in the classroom for the duration of the unit and be referenced when class time is spent investigation a question or collection of similar questions.

Students will…

- Obtain and evaluate information from a variety of sources.
- Write, categorize and prioritize questions generated by themselves and other class members.
- Ensure questions developed relate directly to the phenomenon.

Time Required: Approximately 60 minutes

Materials Needed

- ✓ 4 large pieces of poster paper (24x36"). Title the posters: SCIENCE, SOCIAL/POLITICAL, SOLUTIONS, and OTHER. Hang them around the classroom.
- ✓ 3x3" sticky notes, approximately 5-10 per student
- ✓ 10-15 color prints of photos related to antimicrobial resistance (could include diagrams, short articles, photographs…anything that might spark conversation about the phenomenon)

Teacher Tips

- Get students excited by having the photos out when they come into the classroom. It helps if photos are laminated, but they don't have to be. It's okay (and even encouraged) if students start talking about the phenomenon before class "starts".
- Monitor students closely when creating questions on the sticky notes. Each student should write at least a couple questions, that way they're invested in subsequent conversations which reference the questions. If they don't have a question posted, they're less likely to be interested in the discussion.
- Leave the posters and sticky notes UP for the duration of the unit/project. It helps you remember to reference them at various times during learning.

Activity Procedure

1) Place photos on students' desks before class begins. (not all students need one, as they can share during discussion)
2) As students enter the classroom, encourage them to discuss the photos.
3) To begin the activity, as students to quietly generate questions about the photos they've seen. Write one question per sticky note.
4) As a class, watch this video on YouTube: https://youtu.be/DQGi6AyqUDQ
5) After watching the video, again ask students to quietly generate more questions and write them on sticky notes.
6) Lastly, read this article (or one similar, depending on the reading level of your students): https://www.cidrap.umn.edu/news-perspective/2021/09/highly-resistant-bacteria-pet-store-puppies-continue-cause-illness
7) One more time, allow students to generate and write down questions on sticky notes.
8) Now, students should categorize their questions according to the titles on the poster papers hanging around the room. Students should quietly move from one poster to another and leave their questions on the appropriate poster. The OTHER poster is for questions that don't fit into the other 3 categories.
9) Using a 'gallery walk' or similar strategy, ask students to look over the questions one last time to ensure that all questions directly relate to the phenomenon.
10) Then, ask students to visit each poster and prioritize the questions by considering the question: "which questions do we think are more important when considering how to deal with the drug-resistant disease outbreak among puppies?". Highest priority questions should be moved to the top of the poster, and so on.
11) Let students know that these questions will guide their learning over the coming days, and that they will eventually be able answer many of the questions they've posed.

Don't Forget to Loop Back!

As students complete each activity, don't forget to help them connect with the anchor phenomenon. Doing activities in isolation is not what science is about. Continue building the storyline with students by asking questions like:

- How does what we just learned help us understand the pet store puppies problem?
- Does this activity help you answer any of the questions on your sticky notes from Activity #1?
- What questions did this activity bring up for you?
- How should we try to answer these questions?
- At this point, what solutions to the pet store puppy problem do you have?

It's A Puppy's Life

Our Anchor Phenomenon

In December of 2019, the Centers for Disease Control (CDC) issued a reminder for dog owners. The announcement came after an outbreak of disease caused by a type of bacteria that had become resistant to several antimicrobial drugs. The bacteria were being carried by "pet store puppies" and had infected more than two dozen people in the U.S.

The advice from the CDC [5] included:

- People should wash their hands thoroughly with soap and water after handling their pets
- Pet owners must use care when cleaning up pets' poop, pee and vomit
- Owners should try to prevent dogs from licking around peoples' mouth and face

The people identified had been infected with a strain of Campylobacter jejuni. This strain of bacteria causes bloody diarrhea, cramps, fever and pain in humans and other animals, including these pet store puppies.

The CDC investigated the outbreak and discovered that, of the twenty-four people interviewed:

- 88% reported recent contact with a puppy
- 71% reported recent contact with a puppy from a pet store
- 80% were linked to Petland, a national pet store chain
- 42% were Petland employees

Laboratory evidence suggested that bacteria from ill people in this outbreak are closely related genetically to bacteria from ill people in an earlier outbreak (2016-2018) of drug resistant Campylobacter, which was also linked to pet store puppies. [5]

Most importantly, scientists discovered that the Campylobacter jejuni from people in this outbreak are resistant to commonly recommended, "first-line" antibiotic medications.

Scientists continue to monitor these outbreaks. So far, states affected include Nevada, Utah, Wyoming, Minnesota, Illinois, Ohio, Kentucky, Tennessee, Georgia, South Carolina, Florida, Maryland and Connecticut. States with the highest rates of infection are the Midwestern states of Minnesota and Ohio. [5]

How can the Howe and Soupir labs help us to better understand how antibiotic-resistant bacteria develop, how they are transmitted and what we can do to protect ourselves and animals from them?

What are Microbes?

Module 2, Activity #2

Overview: This activity is designed to introduce students to some of the basic information about bacterial types, with the purpose of helping them understand that a bacteria's structure may influence its ability to cause disease.

Students will…

- Access a variety of resources to develop claims about microbe structure
- Develop an initial model/explanation of their current understandings

Time Required: 30 - 45 minutes

Materials Needed

- Technology access for all students
- Activity 2 handout (paper copy or e-copy)

Teacher Tips

- Make time to reconnect with the anchor phenomenon before beginning the activity. This could include a simple conversation, review of the article or use of another relevant resource that you've found.
- Ensure students have adequate opportunity to explore resources provided.
- If technology access is an issue, students may complete this activity in pairs.
- Allow adequate time for sense-making discussion after the students have completed the handout, using the strategy provided or another one that serves the same purpose.

Activity Procedure

1) Make activity 2 handout available to all students. Ask them to read through the handout, providing time to answer any questions they may have or provide necessary clarification.
2) Allow adequate time for students to access websites and complete the table.
3) Students may use a "jigsaw-type" strategy to share findings and ensure collective understanding of the information that was gathered from the internet.
4) Allow time for students to revisit their questions/sticky notes from Activity #1. They may want to add or modify questions, depending on their new learning.

Activity 2 Handout for Students

Name _____ Date _____

LEARNING ABOUT MICROBES

Using the resources provided by your teacher and others you find on your own, attempt to complete the table below. You need not create exhaustive lists of examples and characteristics! Focus on just a few for reach microbe type. You may or may not use all of the spaces provided in the table below.

Microbe type (common identification)	Specific examples (scientific names) and the diseases caused	General characteristics including shape (morphology), nutrition, reproduction and growth, metabolism, genetic properties, etc.

How Does AMR Happen?

Module 2, Activity #3

Overview: This activity asks students to sort out the various processes by which bacteria become resistant to antibiotics/antimicrobials by completing an inductive card sort so that they begin to understand the complex relationships involved.

Students will…

- Create a visual model of the process by which bacteria become resistant to antibiotics.
- Begin to understand the role humans play in the development of antibiotic resistant microbes.

Time Required: 30 - 45 minutes

Materials Needed

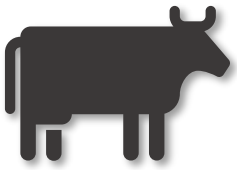
- ✓ One set of cards for each student or group of students OR use a Jamboard (https://jamboard.google.com) or similar

Teacher Tips

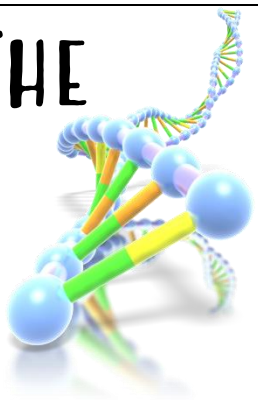
- Inductive card sorts can be a source of struggle for students. Do your best to allow this struggle, as long as it's productive. Ask students to work for 5, 10 or maybe even 15 minutes before they're permitted to ask you questions.
- Encourage student discourse. Conversations within and across groups is a good thing, especially if it's not just seeking the "correct" answer. I particularly like using a "fish bowl" strategy, where students will gather around another student who is allowed to ask the "audience" questions to help them make progress.
- Do your best to NOT provide answers or to do the work for your students.

Activity Procedure

1) Each student or group should be provided a full set of cards.
2) Let students know that the goal of the card sort is to create a visual model of process of becoming antibiotic resistant that shows the correct relationships among the various parts and processes represented on the cards. Students SHOULD INCLUDE arrows and any connecting words that help make sense of their model.
3) Allow time for students to work through the card sort. Monitor work closely, listen to discussions and ask probing questions when appropriate.
4) Once students can show you a model that the believe correctly represents the relationships involved, ask students to copy the model into a notebook or onto a separate sheet of paper for future reference and refinement.

Animals get antibiotics 	**Bacteria in animal systems develop drug resistance over time** 
Fertilizer or water containing animal feces and drug-resistant bacteria is used on food crops 	**Drug resistant bacteria spreads to humans and can remain in the human digestive system** 
Drug resistant bacteria can remain on meat if not handled or cooked properly 	**Human patient receives antibiotics and develops resistant bacteria in their system** 
Human patient spreads drug-resistant bacteria to family and others in the community 	**Human patient receives care at the hospital, if needed** 
Drug-resistant bacteria spread to other patients in the health care facility via unclean hands or surfaces 	**Human patients go home** 

What Role Does DNA Play In The Life Of A Bacterium??



Module 2, Activity #4

Overview: This activity asks students to engage with videos which introduce the idea of bacterial genomes and how bacteria become resistant to antibiotics.

Students will…

- Watch a brief video about the DNA structure, size and shape of bacterial DNA (https://www.youtube.com/watch?v=yQETaLlqub0)
- Watch another brief video about how "mega-plate" Petri dishes can be used to visualize bacteria becoming resistant to antibiotics (https://vimeo.com/180908160)
- Incorporate their learning into a "final" model of their understanding of the anchor phenomenon

Time Required: 45 minutes

Materials Needed:

- Technology access for all students

Teaching Tips

- The majority of this activity is focused on making some additional revisions to students' ongoing consideration of the anchor phenomenon.
- At this point, students still may not know how antimicrobial resistance "fits into" their ideas about the anchor phenomenon, but they should have a good understanding of bacterial structure and the role DNA plays in a bacteria's life.

Activity Procedure

1) Reconnect students with the anchor phenomenon and facilitate a brief discussion (small groups or whole-class) about the questions generated in the initial Activity #1. Be sure to include conversation about relevant questions that students have developed and how each activity so far has provided additional information that may have improved answers.
2) Make sure you've completed step 1 ^^^^ Really…it's that important!
3) Allow students to view the TWO videos linked above.
4) Provide time for students to re-visit their sticky note questions from Activity #1. They may decide to re-write questions, remove questions from the posters that have already been answers, or add additional questions that have come up as a result of viewing the videos.

Module 2 Debrief

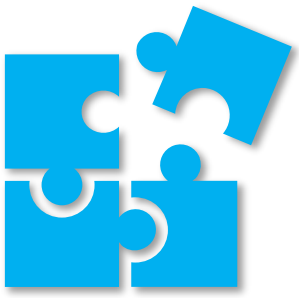
Helping Students Make Sense

What did we learn from Activity #2?
Students were introduced to some basic information about bacterial types. The resources they accessed online should have provided an opportunity to learn how bacterial structure impacts function – specifically, how many bacteria have the ability to cause disease in humans and animals. Students may have been asked to discern which sources were of high enough quality to use as a source, depending on how the teacher structured the activity. After doing their own research, students should have shared their findings with classmates and developed an initial explanation of the anchor phenomenon.

What did we learn from Activity #3?
This activity asked students to consider the various processes that lead to development of antimicrobial resistance in bacterial populations. This inductive card sort provides a chance for students to visualize how these processes, people, and places interact. While there are several "correct" ways these cards can be organized, it is important that students understand the role that humans play in the development of antimicrobial-resistant microbes.

What did we learn from Activity #4?
Students should learn about the role that DNA plays in the life of bacteria. They should know that bacteria contain circular chromosomes consisting of DNA which codes for the production of RNA and proteins. These proteins may enable them to resist antibiotics through a variety of mechanisms, allowing resistant bacteria to better survive in the presence of antimicrobial substances and drugs.

Putting the Pieces Together
At this point, students show be able to see how bacteria are able to resist antimicrobials on both a micro- and macro-scale. Students should be asking questions like "how did the bacteria that made the pet store puppies ill become resistant to drugs?" or "if bacterial DNA can lead to protection from antimicrobials, how might humans change behavior to manipulate bacteria to mitigate the effects of drug resistance?". It's crucial that teachers help connect each activity back to the anchor phenomenon in concrete ways, and allow students to reflect on their initial sticky note questions so they understand how their work has helped them to answer some of their questions.



Teacher Lab Prep

Module 3, Activities #5 and #6

The following table outlines the preparation that needs to be done prior to students working on Activities #5 and #6. Quantities provided in the first column below are PER GROUP OF 3 STUDENTS. You should write in the number of groups you'll have across all classes, then multiply to determine the total number of each item required. You may want to have a few extras of each item available to students, just in case!

	Quantity (per group of 3)	# of groups	Total needed	Description	Notes (quantities indicated will produce enough for ONE group of 3 students)	Used in Activity #
STERILIZATION OF SUPPLIES	200mL ×		=	Sterile water	Begin by sterilizing enough water for the items listed below. Do this by placing distilled water in a container (loosen cap, if present) in a boiling water bath for 30 minutes.	#5 and #6
	4			Wooden toothpicks	Sterilize toothpicks by wrapping in foil and baking at 350°F for 15 minutes	#6
	4			Paper clip "spreaders"	Sterilize paper clips by wrapping in foil and baking at 350°F for 15 minutes	#6
	3			1.5mL microcentrifuge tubes	Sterilize tubes by wrapping in foil and baking at 250°F for 30 minutes. Use to store the $CaCl_2$ (2 tubes) and plasmid (1 tube)	#6
MEDIA PLATES	5			Nutrient agar plates	Add 3.2g LB premix + 1.9g agar 125mL sterile water. Carefully heat to boiling. Allow to cool briefly, then pour plates (~25mL per plate). Store lid-down in refrigerator.	#5 (2) and #6 (3)
	2			Nutrient agar plates with ampicillin	1.25g LB premix + 0.75g agar to 50mL sterile water. Carefully heat to boiling. Allow to cool, then add 1 drop of ampicillin solution, swirl, and pour plates (25mL per plate). Store lid-down in refrigerator.	#6
	1			Master E. Coli MM294 culture plate	Use one of the no-Amp agar plates to make one master plate from the slant culture provided by the BOEC.	
OTHER SUPPLIES	1			Nutrient broth solution	Add 0.125g LB premix to 5mL sterile water. Further sterilize by boiling the container for 30 minutes (loosen cap if present). Allow to cool completely. Pour into a glass tube with cap and store in refrigerator.	#6
	1			Ampicillin solution	Add 1.0mL sterile water to the tube labeled "Ampicillin salt". Refrigerate.	#6
	2*			$CaCl_2$ solution	Dissolve 0.75g $CaCl_2$ into 50mL of sterile water. Further sterilize by boiling the container for 30 minutes (loosen cap if present). Allow to cool completely. Transfer 2 drops into each of two microcentrifuge tubes. Store in refrigerator.	#6

*while each group of 3 students needs two (2) tubes of $CaCl_2$, 50mL should be plenty for an entire class

Determining Resistance in Bacterial Populations

Module 3, Activity #5

Safety protocols must be observed throughout the following investigations. Specific techniques and protocols may be required in your school/district, and it is important to remember that many bacteria can cause disease, especially when present in larger-than-usual quantities, such as on a petri dish. Additionally, should any colonies of antibiotic resistant bacteria be present, special care should be taken to contain and properly dispose of these cultures. We suggest the use of appropriate personal protective equipment throughout the investigation, proper sterilization and transfer techniques, as well as proper disposal of waste. Please consult your science specialist or other documentation to ensure all safety precautions are taken.

Overview: Students will prepare bacterial cultures, which will be added to a petri dish with ampicillin-infused agar. They will monitor growth and determine if there are any antibiotic resistant bacteria present.

Students will…

- Engage in a hands-on investigation to determine whether or not antibiotic resistance is present in a population of bacteria
- Use the information from the investigation to continue to refine their thinking around the anchor phenomenon

Time Required: Two consecutive days of 30-45 minutes each

Materials Needed:

- Lab supplies – read through the procedure below for details

Teacher Tips

- The procedure below is merely a suggestion. There are MANY versions of this lab that can be found online, so look for one that will best suit your circumstances. Some of this work will need to be done by the teacher ahead of time, and some can be done by students depending on age and ability.
- Re-read the safety precautions above. Keep your students safe!
- You may need to help students make the connection between what they learned about DNA in bacteria in the previous activities, and the learning that comes from this investigation. Eventually, students should incorporate into their ideas the concept that antibiotic (antimicrobial) resistance is a result of a **genetic change in a single bacterium**. This change enables the bacteria to survive in the presence of an antibiotic, reproduce and create entire populations that are resistant.

Activity Preparation and Procedure

Note: The procedure below is merely a suggestion. There are MANY versions of this lab that can be found online, so look for one that will best suit your circumstances, students, supply stores and level of expertise.

Part I – Students Inoculate "Clean" Petri Dishes

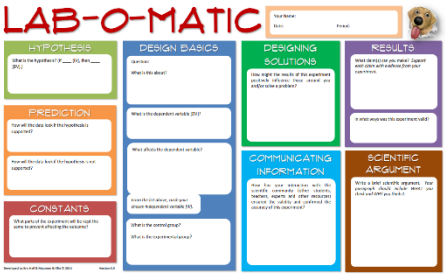
1. Each group of students should have access to two Petri dishes containing simple nutrient agar – one for a control and one for an experimental group.
2. Using all safety procedures and sterile techniques, students should collect bacteria from various surfaces around the classroom using a cotton swab or similar and transfer to a Petri dish. Repeat for the other Petri dish.
3. In the middle of <u>one</u> plate (experimental group), which students should label as "ANTIMICROBIAL", students should place a 0.5cm diameter circular disc of filter paper. The filter paper disc should be saturated with an antimicrobial of the student's choice (cleaning products, antibiotics (purchased by teacher), salt water solution, etc.).
4. The other plate will serve as the control and does not need any additional preparation.

Part II – Culture Growth

5. Students should seal the two Petri dishes using tape or Parafilm and label each with their name, class and date of inoculation.
6. Invert and place the two plates in an incubator at 37°C for 24 hours.
7. Students should wash their hands with soap and water and clean all lab surfaces well before moving on.
8. When viewing the plates, students should NOT open them. Wash hands frequently and dispose of the cultures using your district's hazardous waste disposal guidance.
9. Students should view the plates after 24 hours, looking for a zone of inhibition around the antimicrobial disc. If there is a "clean" zone (no colonies growing) there are no bacteria that are resistant to that particular antimicrobial.

Part III – Student Reflection & Experiment Wrap-Up

10. Once students have had a chance to look at their plates and they (or you) have properly disposed of the plates per your district's disposal guidelines, they should spend some time reflecting on the investigation.
11. One way to engage students in the process of planning and carrying out an investigation is to use a scaffolding tool like the Lab-O-Matic. This series of student-facing documents can help your students identify key aspects of their experiment's design, as well as create arguments and consider how collaboration and communication aided their lab efforts.
12. The Lab-O-Matic can be found at https://hallscience.us/lab-o-matic, and is free to download.



Transforming Bacterial DNA: Ampicillin Resistance

Module 3, Activity #6

Safety protocols must be observed throughout the following investigations. Specific techniques and protocols may be required in your school/district, and it is important to remember that many bacteria can cause disease, especially when present in larger-than-usual quantities, such as on a petri dish. Additionally, should any colonies of antibiotic resistant bacteria be present, special care should be taken to contain and properly dispose of these cultures. We suggest the use of appropriate personal protective equipment throughout the investigation, proper sterilization and transfer techniques, as well as proper disposal of waste. Please consult your science specialist or other documentation to ensure all safety precautions are taken.

Overview: The process of bacterial transformation refers to the addition of exogenous DNA in to a host cell. Transformation generally means that the DNA is incorporated into bacterial, yeast or plant cells in an attempt to transfer a particular functionality. In this lab, students will work to transfer resistance to ampicillin to E. Coli MM294 cells. Transformed cells will then be selected for using nutrient agar plates to which ampicillin has been added.

Students will…

- Engage in a complex investigation to better understand the methods used by researchers to learn more about antibiotic resistant populations.
- Discuss how bacterial DNA transformation in a lab might lead to solutions for problems arising from antibiotic resistance in agriculture.

Time Required: 180 minutes (four consecutive days of 45 minutes each, but not the entire class period)

Materials Needed:

- Lab supplies – read through the procedure below for details

Teacher Tips

- As mentioned in Module 2 Activity #5, it is extremely important that proper safety protocols are observed when working with transformed bacteria. Follow all local/district disposal guidelines. Consult a science education specialist if you have questions about the safe handling of the bacterial colonies in this activity.

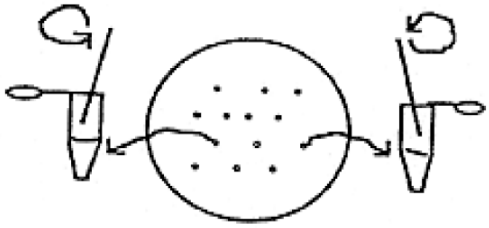
STUDENT INSTRUCTIONS

Genes control the traits that living organisms possess. Bacteria, such as E. coli, have genes on their chromosome and on a small circular piece of DNA called a plasmid. Genes can be transferred from one bacteria to another on the plasmid by a process known as transformation. In this experiment, a plasmid with a gene (DNA) for resistance to the antibiotic ampicillin will be used to transfer the resistance gene into a susceptible strain of the bacteria. The same technique is used to transfer genes (DNA) for production of insulin, growth hormones, and other proteins into bacteria. The transformed bacteria are used in fermentation to produce commercial quantities of the protein for treating diabetes and other conditions.

You will work with two other people while conducting this investigation.

DAY 1 (pre-lab)

☐ Step 1. Use a separate sterile toothpick to transfer a colony of E. coli about the size of this 0 into each of two tubes of calcium chloride. Use the toothpick to stir the cells vigorously and thoroughly into the solution. The solution should appear milky. Close the caps of both tubes and discard the toothpicks into the container provided for that purpose. One person in the pair should label one of the tubes "B1". The other person should label the other tube "B2".

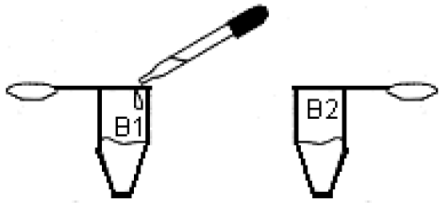


☐ Step 2. Place the tubes back in the ice and place the container of ice with tubes back in the refrigerator. (DO NOT FREEZE) (*The cold calcium chloride, in the tubes, conditions the surface of the bacteria for DNA uptake the following day.)*

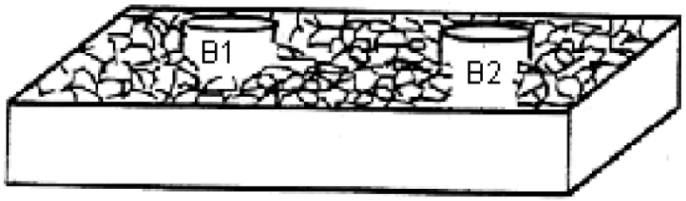
DAY 2

☐ Step 1. Finger flick tube to resuspend cells.

☐ Step 2. Open the tube labeled "B1" and with a sterile pipette add one drop of solution from the "P" tube. Close the tube. Do not add anything to the tube labeled "B2". (*The plasmid DNA, from the "P" tube, added to the tube has a gene for resistance to ampicillin.)*



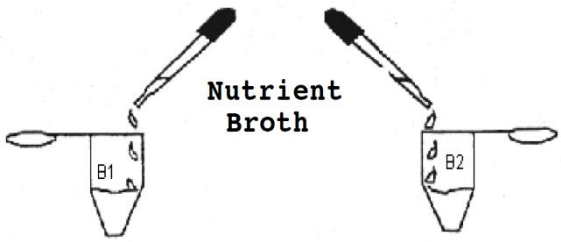
☐ Step 3. Place the tubes on ice for 15 minutes. (*The cells are kept cold to prevent them from growing while the plasmids are being absorbed.*)



☐ Step 4. Remove the tubes from the ice and immediately hold them in a 42°C water bath for 90 seconds. (*The marked temperature change causes the cells to readily absorb the plasmid DNA*).

Steps 3 and 4 can be repeated for up to a total of 3 times. This may improve plasmid incorporation into the competent cells.

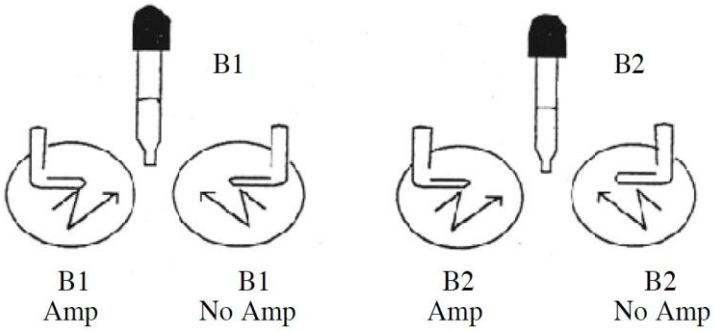
☐ Step 5. Use a sterile pipette to add 5 drops of sterile nutrient broth to each of the tubes. Close the tubes. Mix by tipping the tube and inverting it gently (*The bacteria are provided nutrients to help them recover from the calcium chloride and heat shock treatments*).



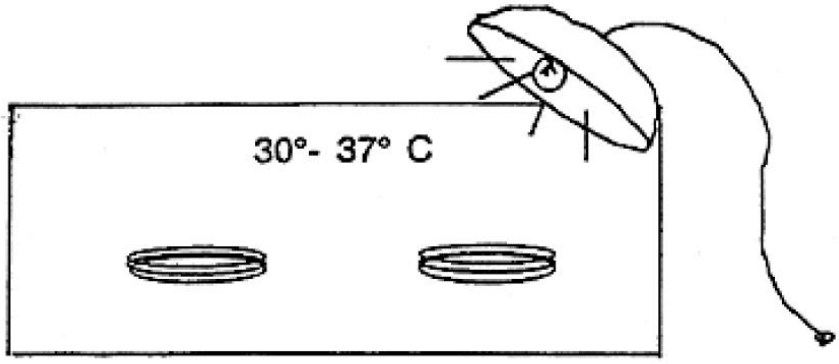
Note: For better results allow cell recovery at 37°C for any amount of extra time, 20 minutes preferred.

☐ Step 6. Label the underside of the four petri dishes with your name. On one "Amp" plate, print "B1" and on the other "Amp" plate print "B2". On one "No Amp" plate print "B1" and on the other "No Amp" plate print "B2".

☐ Step 7. Use a fresh sterile pipette to place 3 drops of cell suspension from the tube labeled "B1" onto the center of a petri dish labeled "Amp"/"B1" and 3 drops to the center of a dish labeled "No Amp"/"DNA". Use another fresh sterile pipette to place 3 drops of cell suspension from the tube labeled "B2" onto the center of the dish labeled "Amp"/"B2" and 3 drops to the center of the dish labeled "No Amp"/"B2". Use a fresh sterile paper clip to spread the liquid evenly across the surface of each plate. Do not touch the part of the paper clip that comes in contact with the agar.

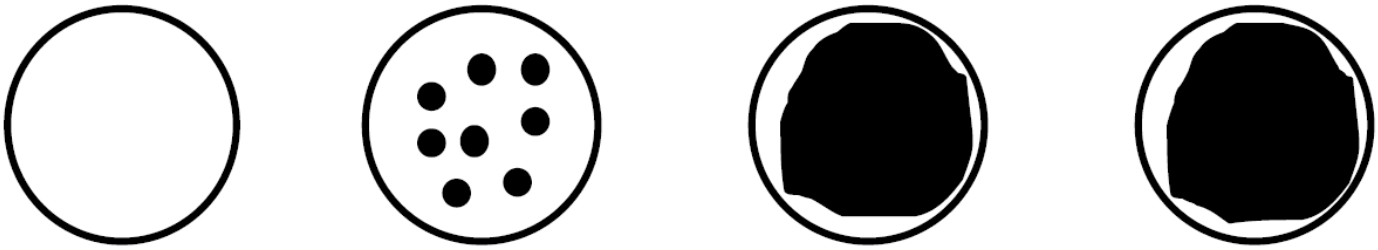


☐ Step 8. Incubate the plates upside down for 24 hours at 37°C.



DAY 3

☐ Step 9. Analyze the results of the transformation by placing the two plates labeled "Amp" and the two plates labeled "No Amp" together. (*The plate labeled "Amp"/"B2" should not have bacterial growth because the bacteria are killed because they did not have resistance to the antibiotic ampicillin. Bacterial growth on the "Amp"/"B1" plate is from cells that took up plasmids added in step 2 and that became resistant to ampicillin. There is extensive bacterial growth on both of the "No Amp" plates because the antibiotic was not present and both resistant and nonresistant bacteria could grow.*)



Note: the above images from Step 9 are removed from the instructions located in the Student Investigation Notebook. The notebook is available for download on the BOEC website: https://boec.biotech.iastate.edu/curriculum-materials/

On Health in Action

Module 3, Activity #7

Students will…

- Thoughtfully discuss the major tenets of the One Health initiative and how it can be used to mitigate the effects of antimicrobials in agriculture and beyond
- Explore the antibiotic use pathways in each of the One Health domains and connections among them
- Negotiate peer-to-peer conversations in order to develop consensus understanding of the One Health initiative

Time Required: 60-90 minutes

Materials Needed:

- Technology access for all students
- Student notebooks or similar method to record findings
- Optional: poster paper, dry erase board

Teacher Tips:

- This activity is a structured discussion around the One Health initiative. Students will use a format similar to that found in the STEM Teaching Tools student talk protocol as outlined below.
- It's important that you, the teacher, become familiar with the One Health initiative. We have provided a collection of links to relevant resources at the end of this module. These resources may also be provided to students when researching, as indicated below.
- The One Health approach to whole-Earth care includes professionals in each of 3 domains: HUMAN health, ANIMAL health and ENVIRONMENTAL stewardship. Other areas of expertise may need to be part of the conversations, when necessary.

Activity Procedure:

1. Pose an open ended-question to the class. It should be a question that requires students to grapple with a complex issue or system (One Health, in our case) and does not have a yes/no answer that's "Google-able". Possibilities include:
    a. Who/what are the relevant players in the One Health approach to protecting our health?
    b. How are the major domains of the One Health approach (Humans, Animals, and the Environment) connected? What connects them, and how should those connections be monitored?
    c. What pathways exist between humans, animals and the environment which could allow antimicrobials to pass from one to another?
    d. How prevalent are antimicrobials in each domain? What are they used for? Where do they come from?
2. Students should be provided time to think, research, write, discuss and negotiate with each other in pairs or small groups. At this time, the teacher can provide resources for research (such as the links included at the end of this module) or not, depending on the students' comfort with online researching.

3. Once time has been provided for research and small-group discussion, students should turn their desks toward the center of the room for the 'science talk' about One Health. As you monitor the discussion, look for direct reference to the questions you posed, the main tenets of One Health, and the domains and players which are part of the One Health approach.
4. While facilitating the discussion, you should avoid giving answers or asking closed-ended questions. Your task is to ensure the conversation stays on track and addresses the key issues mentioned previously.
5. At the end of the science talk, students should record their answers in a notebook or similar. Provide time for individual thought and reflection, as well as large-group share out. You may use other strategies to record large group thoughts, as necessary (dry erase boards, poster paper, etc.).
6. Encourage some meta-cognition after the science talk. As students to document the evolution of their thinking or talk about what may have made them change their mind at any point during the process.
7. Important equity note from STEM Teaching Tools: "Try to take an open-minded stance during all discourse activities, but especially whole-class ones. Research shows that teachers often subtly privilege students who "talk like scientists" by using mainstream or standard English. This should be a space for all students to be free to share, not just ideas of students you agree with."

Supporting Resources provided by the Soupir Lab

One Health
https://www.cdc.gov/onehealth/index.html

https://blogs.cdc.gov/global/2016/11/01/one-health/

https://onehealthinitiative.com/

https://www.who.int/news-room/q-a-detail/one-health

https://www.cdc.gov/onehealth/images/multimedia/one-health-definition-graphic-with-bats.jpg

One Health - AMR
https://www.health.state.mn.us/onehealthabx/

https://www.ecdc.europa.eu/en/antimicrobial-consumption/facts/infographics

https://www.cdc.gov/globalhealth/infographics/pdf/World_Antibiotic_Awarness_Infographic-1st.pdf

https://www.ecdc.europa.eu/en/publications-data/antibiotic-resistance-how-does-antibiotic-resistance-spread

https://www.amr.gov.au/resources/infographic-how-antibiotic-resistance-can-spread

Module 3 Debrief

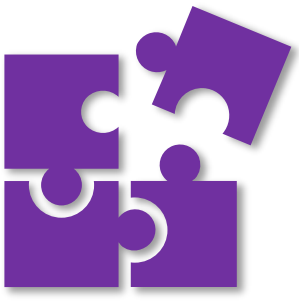
Helping Students Make Sense

What did we learn from Activity #5?
This activity serves as an introduction to hands-on learning about antibiotic/antimicrobial/drug resistance in bacterial populations. Students will collect ubiquitous bacteria samples from around the classroom and determine if there are any antimicrobial-resistance cell present. Students can be creative when it comes to the antimicrobial used. Of course, safety should be a priority when performing this and subsequent investigations involving bacteria. Students should recognize that there are practical ways we can see whether or not bacteria are resistant using fairly simple lab techniques.

What did we learn from Activity #6?
Students engage in a complex investigation to better understand the methods used by researchers to learn more about antibiotic resistant microbe populations. Over the course of several days, students prepare competent E. Coli cells, introduce a plasmid which confers ampicillin resistance, and grow up potentially resistant cells on selective media. Most importantly, students should discuss how bacterial DNA transformation in a lab might allow researchers to 1) better understand the mechanisms of resistance, and 2) develop potential solutions to the problem.

What did we learn from Activity #7?
Researchers at Iowa State University emphasize the importance of thinking about the health of the Earth system in holistic ways. It's important to understand how people, animals, and the environment interact and coexist to support one another. The One Health initiative provides a structure in which to discuss these impacts and allows students to better see the "50,000 foot" perspective after having been "in the weeds" throughout Activities #5 and #6. Students should learn that systems are complex and interconnected, but work is being done to promote healthy ecosystems and processes.

Putting the Pieces Together
These activities push students to consider scale when it comes to the problem of antimicrobial resistance in agriculture. While the lab-based activities provide concrete evidence of resistance and how it can be conferred to and within bacterial populations, students are asked to "zoom out" during the final activity to consider how previous learning about antimicrobial resistance is crucial to discussing the larger picture…and eventually developing our next steps toward solutions.



Jyll & Jakk Dilemma

Module 4, Activity #8

Assessment Summary

Using thought experiments can be a powerful means to assess student thinking. The unique format engages students through the use of a provocative pairing of similar, yet different thoughts. As students wrestle with the nuanced differences between the two positions presented, they begin to formulate their own position…which usually falls somewhere outside of the two presented!

This Jyll & Jakk thought experiment asks students to navigate two different perspectives around antimicrobial resistant bacteria. The assessment should be used to evaluate and support student learning related to these questions:

- How do antibiotic-resistant bacteria develop?
- How are they transmitted?
- What can we do to protect ourselves and animals from them?

While the means of communicating their position may vary, each student will develop an argument for one of the positions presented – or another position, should you allow them to develop their own. These arguments will be presented to peers allowing assessment of each students' progress toward the identified learning goals, their understanding of the questions presented above, and their use of practices and skills associated with researching and communicating their findings.

Time for Sense-Making

Introduction of the Jyll & Jakk thought experiment must be accompanied by individual student reflection time, followed by in-depth class discussion. The NGSS require students to make sense of phenomena and authentic problems using the three dimensions. Specifically, students should use reasoning to sense make about problems, demonstrate grade-appropriate use of the three dimensions (SEP elements, CCC elements and DCI elements), integrate multiple dimensions in service of sense-making and make their thinking visible. (NGSS Science Task Screener, 2018)

Structured Discussions

Discussions that allow for students to share ideas in pairs or small groups is preferred over whole-class discussion. When students talk to each other to share their thinking, they are more likely to: (1) connect to their personal and cultural sources of knowledge, (2) take risks with new language, and (3) use community-based linguistic practices to support their science learning. (STEMTeachingTools.org, Brief #35)

Teachers must consider the purpose of a discussion, however. While many discussion structures exist, each will best serve a particular purpose toward student sense-making. Do you want students to explain their thinking? Consider putting them in pairs and having one student explain while the other jots down key ideas and questions. Should students revise their thinking during conversation? Consider a "four corners"-type discussion where each student aligns with an idea and the groups attempt to convince others of their position. You get the idea! It's important to consider what purpose a discussion will serve before opting into a format or strategy.

Make Thinking Visible

First, let's dispel the myth that "make thinking visible" means that students must publicly display visual or representations of their thoughts. When students make their thinking visible, it simply means that others are able to better understand what – or how – a student is thinking though the use of written work, visual representations, peer discussion or virtually any other means of communication that provides a window in the student's mind.

In the context of a Jyll & Jakk thought experiment, that might mean that a student's thinking is made visible during a structured conversation. The teacher can monitor the conversation and record qualitative observations of the student's understanding. Or, students may discuss the nuances of each position in pairs, and then create a poster that outlines their thinking using a concept map or t-chart. Whatever the strategy, the goal is for the student to demonstrate their current level of progress toward the identified assessment goals.

Connecting with NGSS

The connections presented here are merely suggestions. Depending on how you structure the Jyll & Jakk thought experiment, students may – and likely will – engage with additional or other aspects of 3-dimensional learning.

SCIENCE & ENGINEERING PRACTICES

Developing and Using Models

As students consider the positions presented, the development or use of models which depict the development of antimicrobial resistance (including visual models and mathematical models) will likely support their work.

Engaging in Argument from Evidence

Students will need to provide evidence for their chosen position – either that of Jyll or Jakk. Reasoning and discussing arguments based on evidence will be essential to identifying the "best" position.

CROSSCUTTING CONCEPTS

Cause and Effect

Understanding of the mechanisms of antimicrobial resistance may involve students' understanding of both simply and complex causal relationships.

Stability and Change

Antimicrobial resistance occurs in natural systems. The factors which affect the stability of these systems, as well as the factors which promote change must be a part of students' understanding to make sense of the phenomena.

DISCIPLINARY CORE IDEAS

Ecosystems: Interactions, energy & dynamics

LS2.A: Interdependent relationships in ecosystems

Biological Evolution: Unity & diversity

LS4.B: Natural selection

Earth & Human Activity

ESS3.B: Human impacts on Earth systems

Jyll's Position

Recap: Jyll thinks that the use of antibiotics in agriculture should be stopped completely. She is concerned that bacteria will continue becoming resistant unless alternate methods are found to keep livestock healthy and of high quality.

Considerations: Students who agree with Jyll's position will likely be concerned about the possibility that antimicrobial resistant bacteria will find their way into human populations in appreciable numbers, thereby causing disease and possibly death. The phenomenon – It's a Puppy's Life – may have struck a sympathetic chord with them, too.

It's important to recognize that Jyll's position seems to be an "all-or-nothing" approach to the control of antimicrobial resistance, citing the need to "invest in scientific research that gives us new ways to improve livestock health and quality without the use of any antibiotics." This position, while effective, may have students questioning its implementation. They will likely have had experience with the use of antibiotics in humans (maybe themselves or family) to treat an infection, and not be willing to get rid of them altogether.

Conceptual Understanding: Students who align themselves with Jyll's thinking are likely to rely on the power of scientific research to identify alternatives to antibiotics for livestock use. They may use models to demonstrate how other methods of bacterial control could be implemented, or use models of bacterial genomics to demonstrate an innovative way to disrupt the development of resistance.

Jakk's Position

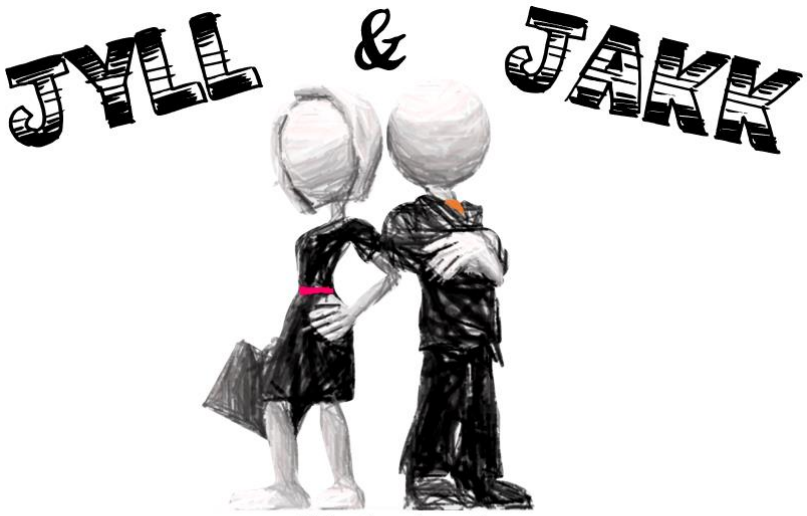
Recap: Jakk argues for taking a slightly more measured approach to the control of antimicrobial resistance. He notes that it doesn't seem realistic to completely remove antibiotic use from livestock production, and advocates for careful monitoring of use, instead.

Considerations: This position begins with the mention that "antimicrobial resistance in bacteria is a naturally-occurring phenomena." Thinking this way might resonate with some students who appreciate the notion that preventing nature from doing something is going to be difficult.

Conceptual Understanding: Students who align with Jakk's thinking will likely rely heavily on the concept of natural selection, understanding that random mutation in bacterial genomes will happen, regardless of the degree of antimicrobial use.

Students may also look to Jakk's final sentence as evidence to support his thinking. Researching the transmission of antibiotic resistant bacteria from livestock to humans will yield minimal results. There's just not a lot of evidence that it happens. While this thinking isn't necessarily a reason to not worry about antimicrobial resistance at all, it may bolster some students' arguments.

Antimicrobial Resistance Project Assessment – Student Handout



Jyll thinks:

The fact that there are an increasing number of bacteria that are resistant to common antibiotics is a problem that we must address quickly! We need to invest in scientific research that gives us new ways to improve livestock health and quality without the use of any antibiotics. If we don't do this, more and more bacteria will be resistant to antimicrobials and more and more people and animals will die of infections.

Jakk thinks:

Antimicrobial resistance in bacteria is a naturally-occurring phenomenon. We should make sure the use of antibiotics in livestock is carefully monitored by expert agriculturists, but getting rid of them altogether isn't a realistic solution. The number of cases of people being infected with resistant bacteria from an animal source is very low and doesn't really pose a problem, anyway.

Student instructions: using what you know about antimicrobial resistance and the use of antibiotics in agriculture, decide who you agree with more – Jyll or Jakk. Providing evidence of your choice is important and will help you build an argument for your decision.

Appendix A: Additional Notes for Activity #6 Teacher Prep

Preparation for the DNA transformation experiment should begin at least 24 hours in advance of the laboratory period.

The following supplies can be provided to the class in groups of three students:

- 2 microcentrifuge tubes (1.5 ml) containing 2 drops of sterile CaCl2 and labeled "CaCl2". The tubes can be put in the same ice container used to provide the DNA to the group of three students.
- 1 aluminum foil packet containing 4 sterile toothpicks
- 4 sterile plastic pipettes from the Office of Biotechnology
- 1 aluminum foil packet containing 4 sterile paper clips that are large and smooth. The clips should be opened into a 90o angle and the small end bent to close it.
- 1 Sharpie marking pen
- 1 glass test tube with a cap (provided by the Office of Biotechnology) containing 2 ml of sterile nutrient broth and labeled "Broth"
- 2 petri dishes containing only nutrient agar and labeled "No Amp" on the bottom
- 2 petri dishes containing nutrient agar and the antibiotic ampicillin. The dishes should be labeled "Amp" on the bottom. (Petri dishes provided by the Office of Biotechnology)
- 2 copies of the laboratory instructions, one for each student

The following supplies can be shared by three students:

- 1 petri dish containing colonies of E. coli (MM294)
- 1 microcentrifuge tube (1.5 ml), labeled “P”, containing 4 drops of plasmid DNA that is placed on ice to keep cold until used. The tube should be labeled "DNA".
- 1 container for used toothpicks

The teacher should have available for the entire class:

- 1 incubator for the petri dishes set at 37°C or less. It is difficult to maintain the temperature precisely unless a research incubator is used. Prolonged temperatures above 40o C will kill the bacteria. Temperatures lower than 37°C will result in slower growth of the bacteria, but will not kill them.
- 1 Sharpie marking pen
- Containers for placing tubes on ice after DNA has been added, such as a Styrofoam cup.
- Containers for the 42°C water bath, such as a Styrofoam cup.

Preparation of Supplies

1. Sterilization of packets of toothpicks, and paper clips can be accomplished by wrapping each item in aluminum foil, labeling the contents with a marking pen, and
    a. baking them in an oven at 350°F for 15 minutes
    b. putting them in a pressure cooker at 15 pounds for 15 minutes
    c. placing them in an autoclave for 15 minutes.

    After the packets have cooled, they should be stored unopened at room temperature. The students should be instructed when opening the packets to touch only that part of the object that will not come in contact with the solutions or petri dishes.

2. Sterilization of the 1.5 ml microcentrifuge tubes can be accomplished by wrapping in aluminum foil all of them needed by the teacher to prepare the supplies for the students. The tubes can be:
    a. baked at 250°F (they melt at 350°F) for 30 minutes
    b. put in a pressure cooker at 15 pounds for 15 minutes
    c. placed in an autoclave for 15 minutes.

3. Calcium chloride. Dissolve 0.75 g of $CaCl_2$ into 50 ml of distilled water in a labeled 100-ml glass bottle with a cap. Keep the cap loose and place it in:
   a. boiling water for 30 minutes
   b. a pressure cooker at 15 pounds for 15 minutes
   c. an autoclave for 15 minutes.

   Allow the bottle to cool until it is comfortable to hold, cap it tightly, and store in a refrigerator until used.

4. Ampicillin solution. For each 1,000 ml of Amp agar to be prepared, dissolve 50 mg of ampicillin (sodium salt) in 1 ml of cool sterile distilled water. The water can be sterilized by placing it in a glass bottle that is not more than half full, putting the cap on loosely, and using one of the procedures described for the calcium chloride. The sterile water should be stored in the refrigerator until it is used to make the ampicillin solution. The ampicillin solution should not be prepared and stored in advance for an extended period. The solution should be prepared and put in the refrigerator immediately before the nutrient broth solution (Item 6) and the agar plate solution (Item 7) are prepared.

5. Plasmid DNA solution. The plasmid DNA used in the laboratory has a gene for ampicillin resistance. The plasma DNA is obtained from the supplier in a concentrated solution, which has to be diluted to 0.005 ug/ul for the DNA transformation experiment. The DNA should be distributed to the students in tubes kept on ice. Any unused 0.005 ug/ul DNA can be stored in the freezer for future use. In a self-defrosting freezer, the DNA should be put on ice in an insulated container, such as a Thermos jar.

6. Nutrient broth solution. Calculate the amount of nutrient broth that is to supplied to the students and add extra for spillage and other factors. Weigh 25 mg of LB premix /ml of distilled water into a bottle and label it. Add the appropriate volume of distilled water to the bottle. The bottle should not be more than half full so that it does not boil over during sterilization. With the cap of the bottle loose, use one of the sterilization procedures described for the calcium chloride (Item 3). After the LB has cooled and is comfortable to hold, cap it tight and store in a refrigerator until it is dispensed to the class.

   Before the class, put 2 ml of the LB into glass test tubes, leave the caps loose, and place them in an appropriate rack in boiling water for 30 minutes to sterilize them. After the 30 minute-period, remove the tube rack from the boiling water, let the tubes cool, then tighten the cap. Unused broth can be re-boiled and stored in the refrigerator for future use.

7. Agar plates. Two types of agar plates should be prepared: Without ampicillin " No Amp", and with ampicillin "Amp". Prepare separate solutions for the "No Amp" and the "Amp" plates. For each type of plate, 25 ml of agar solution will be required per plate. Label the plates on the underside, not the lid, before they are poured.

   "No Amp" plates: Prepare 3 "No Amp" plates for each group of 3 students; one for preparation of the starter culture and 2 for each pair of students to use for transformation. It is best to prepare about 5 extra plates for the entire class in case contamination occurs in one or more of them. Place the required volume of distilled water in one or more glass bottles with caps. The bottles should not be more than half full. Add 25 mg of LB premix and 15 mg of agar /ml of distilled water. With the caps loose, sterilize the solution by one of the methods described for the calcium chloride (Item 3).
   After sterilization, the bottles should be swirled to mix the solution and cooled at room temperature to 55°C, which is when the bottles can be held without an insulated glove. The petri dishes labeled "No Amp" should be poured immediately. The bottom of the dish should be covered with the agar. Agar

begins to solidify at about 45°C, therefore, it is important to pour the plates as rapidly as possible. If the "No Amp" agar does solidify, it can be re-boiled and used again. Rinse the bottle with a large amount of tap water immediately after use so that the agar does not solidify in it or in the sink.

"Amp" plates: Prepare 2 "Amp" plates for each group of 3 students. Follow the same procedure as for the "No Amp" plates until the agar has cooled to 55°C. Add 1 ml of the ampicillin solution (Item 4) per liter (1,000 ml) of solution, swirl to mix, and pour immediately the plates labeled "Amp". If the agar solidifies, it cannot be reheated because the ampicillin will be destroyed above 60°C.

Allow the "No Amp" and "Amp" plates to harden for about 30 minutes or until the agar has a milky or opaque appearance, then turn the dishes upside down (lid down, agar up). If they are to be kept for more than 2 days, store them upside down in a refrigerator. The plates can be kept refrigerated for a month.

8. Note: People differ in their sensitivity to temperature and a teacher may prefer to measure the temperature of the agar to determine when 55°C is reached, particularly for the solution to which ampicillin is added. It is not possible to put a thermometer into the heated agar solution because it will become contaminated. There are two alternatives that can be used.

    a. The bottle of agar can be put into a container with the same volume of cool tap water as the volume of the medium inside the bottle. When the temperature of the tap water reaches 55°C, the contents inside the bottle should be at a similar temperature.

    b. The bottle of agar can be put into a hot water bath at 55°C and allowed to stand for 30 minutes.

Preparation of the E. Coli Starter Plate

One petri dish containing live E. coli is needed for each group of four students. A strain of E. coli should be used that does not have resistance to ampicillin. E. Coli MM294 is provided by the Biotechnology Outreach Education Center, if requested.

Use a sterilized transfer loop, a paper clip bent into a loop and sterilized, or a sterilized toothpick. Use the device to touch a colony of bacteria from a petri dish or test tube. Spread the bacteria on the plate in a zig-zag pattern to obtain individual colonies as the concentration of bacteria on the transfer device becomes less. Incubate the plates at 37°C for 24-36 hours. Colonies should grow to the size of this 0 for use in the lab procedure.

Clean Up After the Laboratory

Sterilize used toothpicks and 1.5 ml microcentrifuge tubes before placing them in the regular trash.
Sterilize the pipettes before washing them. Sterilization can be achieved by placing them in boiling water for 30 minutes, autoclaving for 15 minutes, or putting them in a pressure cooker at 15 pounds for 15 minutes.

Wash glass bottles, pipettes, and paper clips for future use.

Petri dishes can be burned, if convenient. If not, freeze the plates overnight or allow them to dry out in the refrigerator for 1 month, then wrap them securely in a plastic bag and place them in the regular trash.