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Antibiotic Resistant Bacteria Isolated from Michigan Birds - A High School Learning Experience

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Antibiotic Resistant Bacteria Isolated from Michigan Birds

Learning Objectives

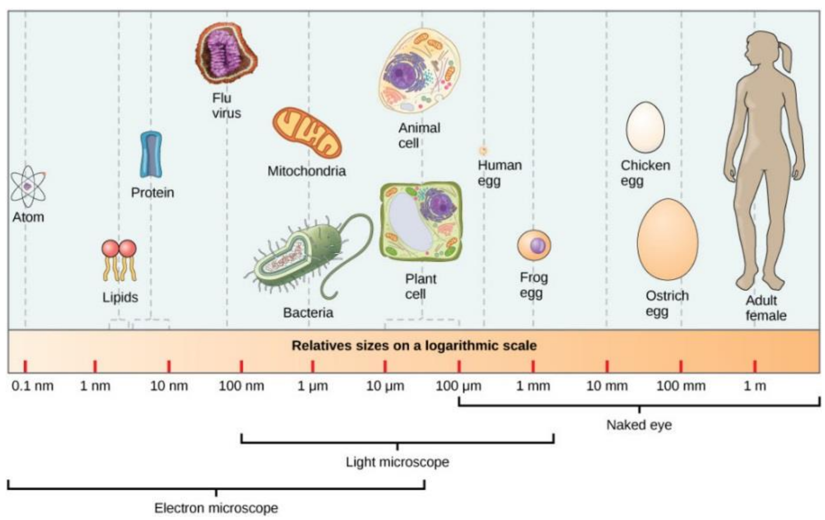
By the end of this exercise, students should be able to do the following:

- Explain what bacteria are and what they do.
- Describe how bacteria can become resistant to antibiotics.
- Explain the different ways that antibiotics can enter the environment.
- Discuss the ways that antibiotic resistant bacteria be carried by birds.
- Identify different classes of antibiotics and how they interact with bacterial cells to result in cell death.
- Design a scientific hypothesis that can be tested through experimentation.
- Interpret bacterial growth data in the presence of antibiotic discs.
- Make conclusions based on antibiotic resistance measurements in terms of presented and self-created hypotheses.

Introduction

What are bacteria?

Bacteria, extremely small prokaryotic cells, are everywhere throughout the world. One striking thing about bacteria is their size: comparing a 2-micrometer bacterial cell to a 6-foot-tall human is similar in scale to comparing a 6-foot-tall human to the distance between Chicago, IL and Denver, CO! Despite their small size, bacteria are found in almost every ecosystem and environment imaginable. From deep sea hydrothermal vents to the clouds miles in the sky, there are trillions of bacteria flourishing in all of Earth's ecosystems, performing many important functions in global nutrient cycles. While the majority of bacteria are beneficial to humans, such as the ones living in your intestines that help with digestion, some can cause devastating diseases. Certain types of bacteria produce toxic compounds, and if these bacteria colonize your body, life-threatening ***infections*** can result.



A size comparison showing many different items. Notice that bacteria cannot be seen with the naked eye.

https://courses.lumenlearning.com/biology1/chapter/comparing-prokaryotic-and-eukaryotic-cells/

What happens if you become infected by a dangerous bacterium?

Up until the 1930s, there was nothing you could do except stay in bed and hope your immune system would fight off the bacteria. This method oftentimes failed, and many people died from bacterial infections; in the early part of the 20th century, the average life expectancy for men and women was 46 and 48 years, respectively (Adedeji, 2016). Deadly bacterial infections ran rampant, and typhoid fever, cholera, tuberculosis, and gonorrhea were among the leading causes of death and disease worldwide. Acquisition of deadly bacteria occurred from everyday activities; for example, a small sip of contaminated water could introduce thousands of deadly bacteria into a person's body.

Bacterial invasion of the bloodstream.

https://howshealth.com/bacteremia-symptoms-causes-pathophysiology-treatment/

Penicillin, the first discovered antibiotic, is now being used to treat infections across the globe.

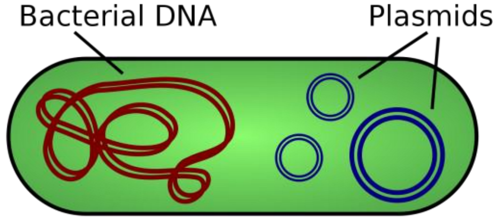
https://www.verywellhealth.com/whats-pencillin-1124178

What has science done to help cure infections?

In 1928, a groundbreaking discovery was made that changed the lives of everybody on Earth. Alexander Fleming, in his laboratory at St. Mary's Hospital in London, discovered that mold growing within close proximity to bacterial colonies halted bacterial growth. After many tests and assays, Fleming concluded that the mold was releasing some type of chemical that was able to effectively inhibit the bacterial cells from surviving. Over time, this chemical became known as penicillin, the first ***antibiotic*** - a drug that can be used to treat an infection. Since that time, many other antibiotics have been discovered, and each new discovery has saved a countless number of lives from previously lethal infections. Since the discovery of penicillin, bacterial infections that would have previously resulted in life-threatening diseases can now be cured by taking an antibiotic pill for a few days. Along with other healthcare advances and improved hygiene, this drastically increased the life expectancy of the human population; post-antibiotic discovery, the life expectancy rose to 78.8 years (Adedeji, 2016). Despite this, the discovery and commercialization of antibiotics has presented some negative, life-altering consequences for future human populations.

How have bacteria responded to human antibiotic use?

A full arsenal of effective antibiotics is extremely important to maintain ***public health***. Despite this, certain bacteria are able to become ***resistant*** to antibiotics and thus are not killed by an administered antibiotic. Through the process of evolution by natural selection, resistance mechanisms can be passed on to other bacterial cells, limiting the effectiveness of many antibiotics. ***Plasmid DNA*** is the main way that antibiotic resistance is passed among different bacteria. Plasmids are tiny pieces of DNA that can be passed from one bacterium to another. They carry a collection of genes that are not necessary for bacterial life, but their presence can give bacteria certain special capabilities; one of the major genes carried on plasmids is for antibiotic resistance. Through a process called ***horizontal gene transfer***, one bacterial cell can pass its plasmid DNA to another bacterial cell, ultimately resulting in two bacteria that are now resistant to a certain antibiotic. As these bacterial cells continually divide and reproduce, all of their progeny will have the resistance gene as well. Also, these progeny cells can perform horizontal gene transfer with other bacteria as well, so the resistance genes that bacteria have developed can spread extremely rapidly. Bacteria divide extremely quickly - some bacteria, such as *Escherichia coli*, can divide in as little as 17 minutes! (Todar, 2012) Over a 10-hour period, a single resistant bacterial cell can result in 34 billion resistant bacteria that are ready to infect humans or other animals if given the opportunity. Some species of bacteria can even carry resistance genes to many different antibiotics, so humans have little ability to treat these types of infections simply because our knowledge of discovered antibiotics is finite.



Plasmid DNA is separate from chromosomal DNA.

https://en.wikipedia.org/wiki/Plasmid

How do antibiotics enter the environment, and what happens when they get there?

One of the main factors that results in a drastic increase of antibiotic resistant bacteria is overuse of antibiotics. ***Anthropogenic*** (human activity related) activities such as agriculture, livestock rearing, veterinary practices, and over-prescription are resulting in the widespread distribution of antibiotics into the environment. Despite being in low concentrations, these antibiotics are killing ***susceptible*** bacteria while leaving resistant ones unharmed. Overuse of antibiotics can result in the uptake of the drugs by wildlife, both on land and in water. For example, when antibiotics are used in farming practices (crop production or animal raising), antibiotic-resistant

Wild birds can be exposed to antibiotics, antibiotic resistant bacteria, or both depending on their behaviors.

https://blog.nature.org/science/2015/06/10/consider-catbird-surprising-secrets-common-backyard-birds/

bacteria within these organisms can survive and increase in number. When the animal/plant is then consumed (by humans or other animals in nature), the resistant bacteria will be transferred to them; this transfer by ingestion mechanism can continue indefinitely until the bacterium is housed in an unfavorable environment (Chang et al, 2014). Also, when antibiotics are used as crop fertilizers, run-off can accumulate within bodies of water and subsequently select for antibiotic-resistant bacteria; these species, which will be living in close contact with other organisms in the aqueous environment, can easily infect them and begin to transfer resistance genes through plasmids (Carroll et al, 2015). There are two main ways that antibiotic-resistant bacteria can enter an animal. First, the animal can directly consume the bacterium; when this occurs, the bacterium can start living within the animal's intestines. Secondly, the animal may consume antibiotics within the environment, and these ingested antibiotics will select for resistant bacteria already present within their intestines.

Can antibiotic-resistant bacteria in birds cause problems?

As antibiotics accumulate within the environment, resistant bacteria can proliferate, even when large quantities are observed.

https://medicine.wustl.edu/news/bacterias-appetite-may-be-key-to-cleaning-up-antibiotic-contaminated-environments/

Of particular importance for this study was the introduction of antibiotic-resistant bacteria to birds. If a wild bird carries an antibiotic-resistant bacterium, then close contact with human populations or livestock can result in an easy route of transmission and subsequent infection across different animal species (Dolejska et al, 2008). Also, infection of migratory birds can lead to a larger zone of infection.
For certain species of birds, global migratory patterns can expose an exponentially large number of organisms to the resistant bacteria housed within their saliva and fecal deposits (Foti et al, 2011). The means by which birds become exposed to antibiotic-resistant bacteria can vary greatly, but in this study, land usage will be examined in depth.

How does environmental antibiotic presence differ between land use types?

In ***rural*** environments, such as farmlands and crop fields, the main source of antibiotics is from plant agriculture and animal healthcare. The combination of miniscule, non-deadly doses of antibiotics along with resistant bacteria can result in a spike of antibiotic-resistant bacteria present in rural communities (Meek et al, 2015). Within ***urban*** environments, excess antibiotics are present due primarily to human healthcare and veterinary practices; proximity to dense human populations increases the number of resistant bacterial species recovered from the environment (Allen et al, 2010). Thus, one of the objectives of this study was to determine if different land areas hosted different resistant bacteria, and if this was the case, information could be learned about which antibiotic distribution practice is the most harmful. With more information known about this topic, prevention methods can come into play, which can include limiting the usage of antibiotics and decreasing the concentrations used for each practice. Overall, antibiotic-resistant bacteria are a growing concern, and information about their dispersal patterns and host organisms can be extremely useful when attempting to remedy the problem.

Depending on where a bird was captured, different bacteria may be present within their gut.

https://keydifferences.com/difference-between-urban-and-rural.html

<u>Antibiotics</u>

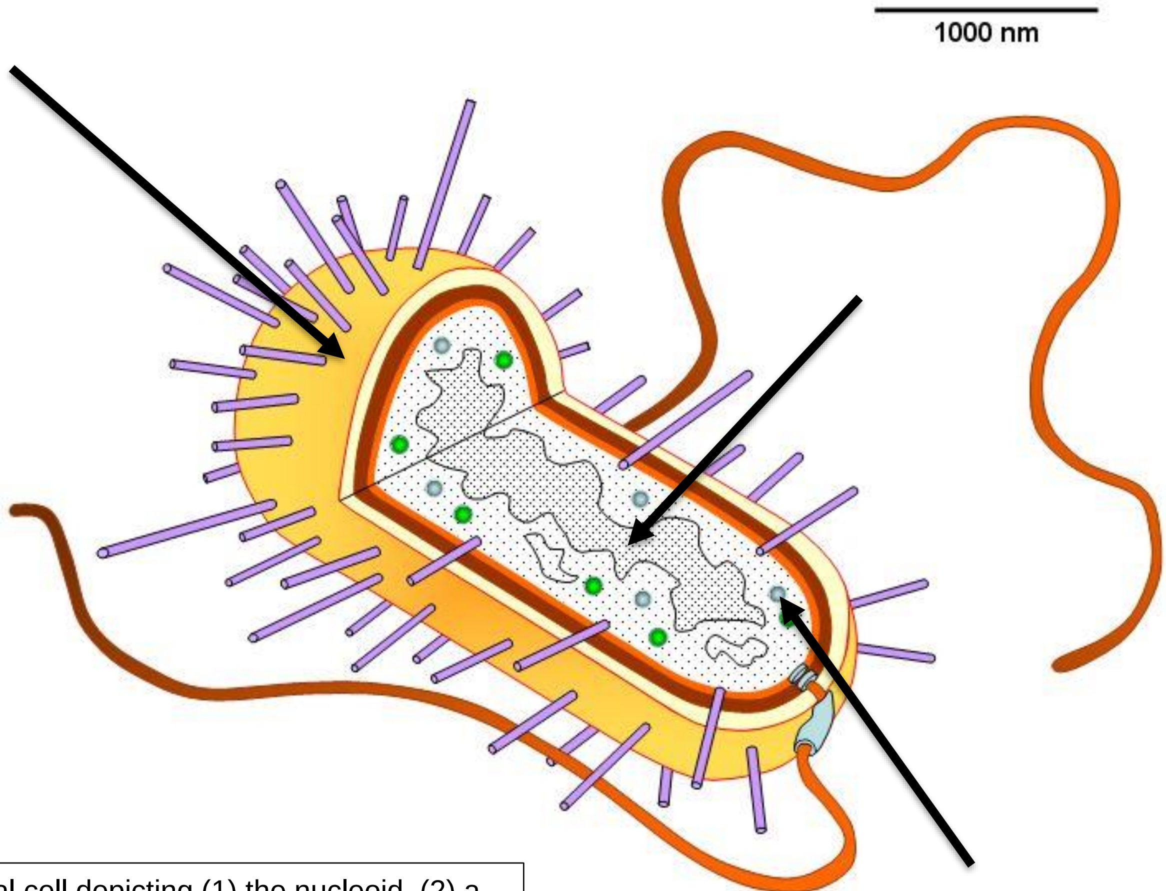
Throughout this lesson, you will be examining the effects of 8 different antibiotics on many different cultures of bacteria collected from birds living in southwest Michigan.

How do antibiotics work?

Antibiotics work in many different ways. What makes antibiotics such powerful drugs are that they usually act upon a process that only occurs in bacteria but does not occur in humans. This reduces side-effects to the infected patient. Some antibiotics are chemically engineered in laboratories (***synthetic***), but other antibiotics are naturally produced by bacteria and fungi that live in the environment. The 8 antibiotics used in this study work in many different ways.

Antibiotic	**How it works**	**Date first administered**	**Original source**	**Bacterial Susceptibility**

Ciprofloxacin (CIP)	Prevents DNA replication	1987	Synthetic	High
Nalidixic Acid (NA)	Prevents DNA replication	1967	Synthetic	Medium
Tetracycline (TE)	Prevents protein synthesis	1978	Natural	Medium
Doxycycline (D)	Prevents protein synthesis	1966	Synthetic	Medium
Streptomycin (S)	Prevents protein synthesis	1944	Natural	Medium
Vancomycin (VA)	Prevents cell wall formation	1964	Natural	Low
Ampicillin (AM)	Prevents cell wall formation	1961	Synthetic	Low
Penicillin (P)	Prevents cell wall formation	1942	Natural	Low



A bacterial cell depicting (1) the nucleoid, (2) a ribosome, and (3) the cell wall.

http://cronodon.com/BioTech/Bacteria.html

Throughout this lesson, you will be examining bacteria isolated from four different bird species. Fecal samples were collected from birds located in southwest Michigan in either rural or urban environments. Depending on a bird's behavior, we can hypothesize whether they will have certain bacteria resistant to specific antibiotic present within their guts. All of these bird species have different foraging, migratory, and roosting behaviors that ultimately affect which bacteria will and/or won't be present within their guts. Also, based on the bird's behaviors, we can determine if certain bacteria are more prevalent in certain land use types. Lastly, at the conclusion of this exercise, you should be able to determine if birds from urban or rural areas carry more antibiotic resistant bacteria.

American Robin

Song Sparrow

Black-Capped Chickadee

Gray Catbird

What are the behavioral traits of the birds in this study? (Cornell Lab of Ornithology, 2019)

Bird	**Foraging behavior**	**Migratory behavior**	**Roosting environment**	**Preferred living environment**	**Food preference**	**Social behavior**
American Robin	Walking on ground	Mostly stay within the United States – some go to Mexico	Tree branches surrounded by dense leaves	Rural (forests) or urban (parks)	Earthworms, snails, insects, and wild fruits	In winter/fall, they assemble in large flocks

		during winter				
Song Sparrow	Walk/hop on ground	Remain in the United States year-round.	On the ground in dense brush	Open/exposed areas	Insects, seeds, and wild fruits.	Young birds stay in flocks, but older birds find a mate and become more isolated
Black-Capped Chickadee	Identify food by walking on ground or flying, but fly away with food and eat it in tree/other area	None – stays in northern United States	Within small, natural cavities of trees	Deciduous and mixed forests	Insects, seeds, and wild berries	They live in flocks, are very active, and become curious with age
Gray Catbird	Fly low to ground in search of food	Trans-continental: some birds migrate as far south as South America for the winter months	Within dense shrubs – remain very close to the ground	Dense, shrubby areas.	Insects and wild fruits	Mostly independent, but occasionally gather in flocks

How were the birds sampled for this study?

As previously mentioned, the bacteria used in this study were isolated from Michigan birds across a gradient of land use. You may be wondering how this task was performed; after all, it is not easy to catch birds! To begin, sampling sites were selected based on being urban or rural. Since most of southwest Michigan is rural, major cities were used as sampling sites for urban environments (Kalamazoo, Grand Rapids, Jackson, Lansing, etc.). In total, 10 sites were selected that

> Mist netting is an effective way to capture birds without injuring them.
>
> https://jillianclemente.com/2015/10/23/mist-netting/

were rural, and 8 sites were selected that were urban. Upon arrival at the sites, ***mist nets*** were used to capture birds; after capture, the birds were placed in a sterile paper bag until they produced a fecal sample. This sample contained thousands of different bacteria, and it was quickly transferred to a tube containing bacterial growth nutrients and was placed on ice until arrival to the laboratory.

How can we identify unknown bacteria from the environment?

Once back in the laboratory, the collected bacteria are grown in petri dishes and identified. Bacteria have specific genes within their DNA that allow us to identify which species we have isolated. It is with molecular methods that we can identify the DNA sequence and determine which bacteria we have. Each bacterial species has a different DNA sequence for a specific gene, so the bacteria can be identified in this fashion. In total, 31 bacteria were isolated in this study. By using molecular methods, we were able to identify each bacterium; once we found the specific DNA sequence, an online database can be used to determine the specific species of bacteria that was isolated.

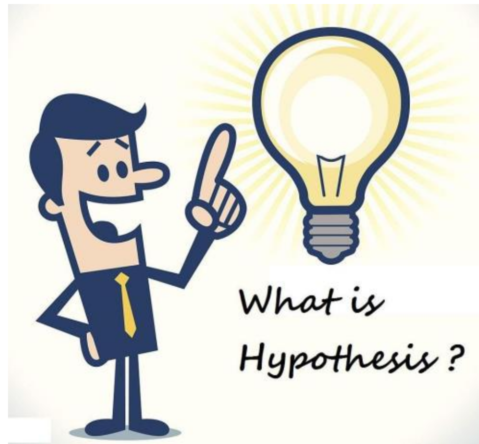
How do we test for bacterial antibiotic resistance?

Once we have isolated and identified a bacterium, we can then test to determine if the bacterium is resistant to antibiotics. To do this, the Kirby Bauer assay is used. The Kirby Bauer assay is a microbiological procedure that uses antibiotic-infused discs to test the resistance of bacteria to an assortment of antibiotics. In short, the bacteria are added to a plate that contains pre-made growth media, antibiotic-infused discs are applied to the surface of the media, and then the plate is incubated at 37°C for 24-48 hours. After incubation, each antibiotic disc will have a certain ***zone of inhibition*** surrounding it. Later in this lesson, you will be measuring the zones of inhibition produced by all of the antibiotics from bacteria isolated in this study. If a bacterium is sensitive to an antibiotic, then a large zone of inhibition will be present because the bacterial cells cannot grow near the antibiotic disc. On the other hand, if a bacterium is sensitive to an antibiotic, then a small zone of inhibition will be present because the bacterial cells can grow in the presence of that particular antibiotic.

Hypothesis Generation

What is a *hypothesis* and what must it include?

Each scientific study that is conducted is always done with a larger context in mind. This end goal can be to cure cancer, regenerate neurons, identify antibiotic resistant bacteria, or demonstrate that climate change is occurring. To help orient their research, scientists develop hypotheses that can be supported or refuted through experimental trials. Importantly, these hypotheses must be both testable and falsifiable. So, what exactly does it mean to have a testable and falsifiable hypothesis? Let's look at a few examples.



Having a thorough understanding of hypotheses is crucial when designing experiments.

https://www.thesisscientist.com/blog/how-write-hypothesis-examples-2018

What does a testable, falsifiable hypothesis look like?

A well-developed hypothesis can be tested by a scientific study. If a hypothesis is not testable, then any given study will not be able to support or fail to support it. On a similar note, if a hypothesis is not falsifiable, then no matter what research is conducted, we cannot physically gather enough evidence to fail to support it. To explain the process of proper hypothesis generation, let's look at an example related to the sun. A scientist observes that people who spend all day in the sun oftentimes get sunburns, but people who sit inside all day rarely get sunburns. This observation interests the scientist, so he pursues further research in the field of sunlight and discovers the following: sunlight contains many wavelengths of light, and UV-B radiation in sunlight has been shown to cause sunburns. Now the scientist has two observations and is ready to generate a hypothesis. His hypothesis is the following: Physically blocking UV-B radiation by wearing clothes that cover the skin while a person is in direct sunlight will prevent sunburns. Does this hypothesis seem testable and falsifiable? Yes! The scientist knows that spending time in direct sunlight causes sunburns, and he also knows the UV-B radiation in the sunlight is responsible for the sunburns. Thus, the scientist suspects that covering the skin with clothing, which will block the UV-B radiation from contacting skin, will prevent sunburns. An experiment can easily be set up to test this hypothesis, and the results will either support or fail to support the scientist's claim.

What does a hypothesis that cannot be tested with an experiment look like?

In regard to the scientist's sun research, he could have made the following hypothesis as well: Sunburns occur when a person is exposed to UV-B radiation from sunlight only in the presence of oxygen for long periods of time. Can this hypothesis be tested? No! To test this hypothesis, the scientist would have to expose people to sunlight without oxygen present, and this is an unethical experiment. There is no experimental design that would result in the scientist being able to explicitly test this hypothesis. Thus, this is a bad example of a hypothesis. Another bad example of a hypothesis is the following: aliens exists on a planet one million light years away from Earth. We cannot travel one million light years away, so this statement could be true or false. Without having the power to prove this statement in any way, we must understand that it is a bad scientific hypothesis because it isn't falsifiable.

Complete the section below on your own before the in-class exercise.

So far, we know there are three total variables for this study:

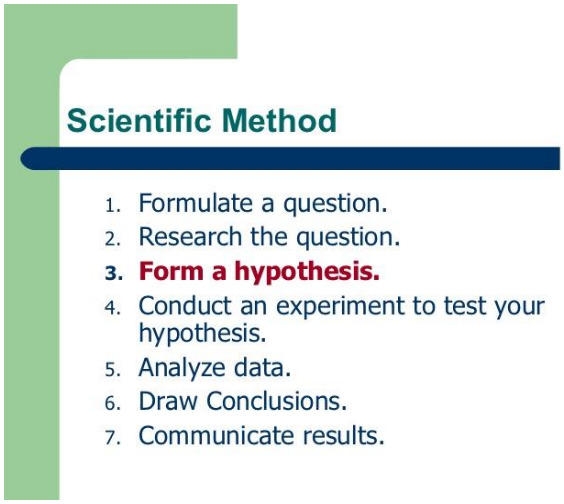
- Antibiotics
- Bird Species
- Land Use

Try writing out good scientific hypotheses for each of the three variables in this study. At the conclusion of this lesson, we will revisit your hypotheses while analyzing the results.

Antibiotics:

Bird Species:

Land Use:



The scientific method involves a step where hypotheses must be formed.

https://www.slideshare.net/mrmularella/developing-a-hypothesis-and-title-for-your-experiment

So, what is an example of some hypotheses that are both testable and falsifiable in regard to this lesson? Gray Catbirds will contain bacteria that are resistant to a greater number of antibiotics than will American Robins, Black-Capped Chickadees, or Song Sparrows. This hypothesis is testable because an experiment can be designed that scientifically allows a researcher to test this claim. Also, if the experiment is designed properly, then this statement can be proved false. Thus, this hypothesis is both testable and falsifiable, and after you complete this lesson, you should be able to determine if this hypothesis is supported by evidence or refuted.

One last example of a satisfactory hypothesis is the following: Birds taken from urban environments will have gut bacteria that are resistant to a greater number of antibiotics than birds taken from rural environments. By the end of this lesson, you should also be able to determine if this hypothesis is supported or not by the evidence. Lastly, we could also make a hypothesis with regard to specific antibiotics based on the year that they were introduced to the market. Try to design a testable, falsifiable hypothesis surrounding an antibiotic based on the year it was first administered. After you have completed this lesson, look through the evidence and see if your hypothesis is supported or not.

Complete this section in class, after reviewing results

Results Interpretation Section

Based on the graphs and data presented during the lecture, please add your results interpretations to the boxes below:

Antibiotic Susceptibility by Antibiotic:
Antibiotic Susceptibility by Bird Species:
Antibiotic Susceptibility by Land Use:

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Thesis Justification

Throughout my entire educational career, science has always been my favorite subject; learning about life processes in biology, chemical reactions in chemistry, the human body in anatomy and physiology, and the brain’s function in psychology have been on the forefront of my education. Unfortunately, many students do not get as excited for these courses as I do. Over the past 20 years, the number of students who chose to study science has been decreasing (Osborne et al, 2010). Due to this, I wanted to create a lesson plan and exercise that high-school students will look forward to and hopefully enjoy. When designing this plan, the actual subject of the lesson had to be chosen carefully; in-depth scientific data and complex processes will quickly result in the loss of student’s attention. Thus, I had to make my lesson plan interesting, relatable, and exciting. If done correctly, students should be extremely engaged with the lesson, presentation, and exercise, and ultimately student’s will have enjoyed a science lesson.

Microbiology, the study of microorganisms, has interested me for the past few years. One of the most shocking discoveries that I made was that there are more bacterial cells on and within a human body than there are human cells. This fact interests me for several reasons, most notably because we are a living “ecosystem” that trillions of “residents” call home. Another thing that I find interesting about microbiology is when things go awry; in humans, dangerous bacteria are able to cause disease and death on continental scales! Throughout history, there have been many diseases that have caused widespread destruction, and many of them were bacterial: diphtheria, cholera, and bubonic plague are some of the most notable. For high school students, I think microbiology is a very interesting subject, but can be difficult to teach due to the lack of laboratory, inexperienced students, or potential dangers. Thus, I decided to create a lesson plan that evolves around microbiology and pertinent laboratory skills.

For my thesis project, I decided to have the topic of antibiotic resistant bacteria isolated from Michigan bird species. With this topic, I could have taken my research into many different directions, and I was extremely excited to work with living organisms from a natural environment. The twist of antibiotic resistance also played a large part in my topic selection; antibiotic resistant bacteria are a serious threat to humans, and I specifically chose this topic because I have legitimate concerns about the future of the medical field without some form of limitation on antibiotic use, misuse, and overuse. By disseminating this information to high school students, my hope is that antibiotic use can be curtailed and only provided when absolutely necessary.

Within the realm of science, proper hypothesis development skills are key to success. At first, creating sound scientific hypotheses can be a difficult task; most introductory-level students develop vague, confusing hypotheses that oftentimes do not contain measurable concepts or are too narrow in their scope (Strangman & Knowles, 2012). To help remedy this problem, I included a section within my lesson plan that allows students to attempt to develop their own hypotheses. Through this process, which is guided by the instructor, students can understand exactly what should and should not be included in a scientific hypothesis. At the end of the lesson, students can also compare their hypotheses to the results and determine if their hypothesis was supported or not by evidence. Lastly, a component of my lesson plan involves hands-on measurements and data input. This was specifically chosen because, according to research, student's who perform hands-on activities learn and remember more than student's who do not (Dahnapal & Shan, 2014). Thus, my lesson was specifically designed to encourage high school student's participation in science, help create better, smarter scientists for the future, and communicate information about antibiotics and antibiotic resistant bacteria to a younger population in hopes that the threat of resistant bacteria will decrease.

Thesis Goals

Having the broad topic of antibiotic resistant bacteria allowed me to select a more personalized "niche" for my thesis. Thus, I chose to create a lesson plan for high school students. My ultimate goal was to create an all-inclusive lesson that could be performed in about 2 hours – not too long, but not too short either. For 2 hours, I wanted students to be fascinated and (slightly) disturbed by some of the information that they are learning. To make this a reality, I set out on a mission to create a background informational handout, a detailed lecture presentation, in-class activity exercises and student engagement opportunities, pre/post quizzes to assess student learning, and feedback forms to continually improve my lesson over time. Before my thesis defense, I also wanted to pilot my lesson in a high school classroom to assess my lesson's applicability, nuance, and overall suitability for a wide range of students.

Over the past several months, I have worked diligently to create a lesson plan that spreads awareness for the potential threat of further antibiotic resistance. Bacteria can evolve at astounding rates, and the medical community could face disaster if more deadly bacteria evolve resistances to antibiotics. While this point is certainly stressed throughout my thesis project, I also wanted to teach high school students about microbiology. Throughout my high school career, I was never exposed to prokaryotic cells and the microscopic world that surrounds us all. The extent of my high school "microbiology" experience was using light microscopes to see small cells in samples of pondwater. Looking back, I am extremely saddened that I was unable to learn more about bacteria and viruses, but I aimed my thesis project to change this for future high school students. My lesson plan expands on the basic microbiological techniques of cell culturing and media preparation, and after reading through the information I prepared, students will develop an enhanced understanding of microbiology as a whole. Overall, I wanted my lesson plan to be useable for a wide range of classrooms, so I made it as broad as possible so that many different teachers can incorporate some aspect of my thesis into their classrooms.

To summarize, the background information of my thesis contains paragraphs on bacteria in general, infections and their consequences, antibiotic discoveries, antibiotic resistance methods, antibiotic modes of action, bird information, antibiotic resistance testing protocols, a lengthy section of proper scientific hypothesis generation, and a results interpretation section for students to use to sharpen their science and critical thinking skills. The goal of this entire project was to create a lesson that encompasses many different aspects of microbiology, natural elements, and scientific techniques, and I feel that my lesson plan is widely applicable and is a "fun" way to learn about future public health threats. Through lectures, classroom discussions, and hands-on activities, students are able to understand more about the world around them while systematically developing useful ways of medication use and dissemination with regard to prescribed antibiotics. In the end, I want my lesson to be insightful, entertaining, and serious, and if all three of these elements come together perfectly, students should genuinely enjoy learning about antibiotic resistance.

Literature Review

With the limited timeframe for this thesis project, I wanted to base my lesson plan on a previous study done at Western Michigan University. Diana Carter, a master's student alumni, wrote her thesis on antibiotic resistant bacteria isolated from songbirds in southwest Michigan. This study used three antibiotics to test for resistance, and the main goal was to look for different antibiotic resistant bacteria from four different bird species over gradients of land use. Luckily for me, I was able to use the same bacterial isolates that Carter used in her study. Thus, I was using 31 bacteria isolated across urban and rural environments from four different birds: Gray Catbirds, Song Sparrows, American Robins, and Black-Capped Chickadees. Also, instead of using three antibiotics, I expanded this project by using eight: ampicillin, penicillin, ciprofloxacin, nalidixic acid, tetracycline, doxycycline, vancomycin, and streptomycin.

My thesis, while using the same bacterial samples as the Carter study, was still extremely unique in that the end goal was a lesson plan for high school students. Despite this, the Carter study was the pioneering research that I exploited to create my thesis, and I am grateful to have had access to all of the bacterial isolates. My lesson plan includes a full description of the methods detailed in the Carter study, and I was able to use the sample collection information to enrich my lesson and make it more enjoyable for students to learn about.

Methods

To complete my desired thesis project, I began by researching all of the different antibiotics and learning about their modes of action. Also, I began researching the many different behavioral traits of the four birds. With newly discovered knowledge, I began to synthesize an “informational” handout for students to read before being lectured. This informational handout is meant to serve as background information that the students should know before coming to a lecture and completing an in-class activity. Included in this informational handout was general descriptions of bacteria, their ability to infect humans, antibiotic discovery information, bird behavioral traits, and a section on hypothesis creation. After creating this handout, I began working on my in-class activity.

To create an in-class activity, I wanted to make something that students can perform “hands-on.” Using the Kirby Bauer assay to test for antibiotic resistance, I knew I could make an exercise that allowed students to measure the zones of inhibition produced by each antibiotic. Thus, using the 31 bacterial isolates from the Carter study, I began performing Kirby Bauer assays with each one. To do this, I used Mueller-Hinton microbiological media in 125 mL petri dishes. Once the media was prepared and poured, I added each bacterium isolate to 10 mL of tryptic soy broth and allowed incubation at 37°C for 48 hours. After this time, I added 250 µL of the broths to their own petri dish, spread the liquid with a sterile plastic spreader, and used a sterile cotton swab to ensure the entire media surface was covered. After allowing the liquid to dry, I added eight antibiotic disks to the surface of the media, all at equal distances apart from each other to allow appropriate zones of inhibition to form. At this point, I incubated the plates at 37°C for 48 hours. In total, I plated each bacterial isolate four times, which gave a total of 124 final plates.

After the bacteria grew into a defied lawn with prominent zones of inhibition, I photographed each plate, scaled the pictures to actual size, and labeled each antibiotic disk. All pictures were then printed out and laminated, and this gave students the ability to measure zones of inhibition without actually growing the potentially harmful bacteria. To facilitate some sort of data collection, I created a Google Form that allows students to input their measurement data. The output of the Google Form was programed to be graphs so that students could do data acquisition and result interpretations on their own.

This in-class exercise was incorporated into the lecture that was also created. The goal of the lecture was to touch on some of the material presented in the background informational handout, walk students through the in-class activity, help students form hypotheses, and allow guidance when students are interpreting the graphs and discussing results.

To assess student learning for this lesson, a pre quiz was also created; before students read the background information handout or participate in the lecture, they all need to take the quiz in order to gauge how much is already known about antibiotic resistance. After the lecture, students are asked to take the same quiz, and then their pre and post answers can be compared to assess learning. Also, after the entire lesson is concluded, students are asked to fill out a feedback form; this is used to gauge student excitement about the lesson and see if anything needs to be changed for future presentations.

Student Feedback

On June 4, 2019, I piloted this lesson at Van Buren Technology Center to a group of 11th grade students. Students took the pre quiz, read the background information, participated in a discussion-based lecture, performed the in-class activity, took the post quiz, and filled out the feedback forms. Overall, the lesson went excellent, and the students seemed to enjoy the lecture itself. Based on their feedback, I decided that I needed to streamline the data collection process and make the lecture more interactive/discussion-based. Other than that, the students (and teacher) loved the hands-on measuring activity and seemed to find the topic of the lesson very interesting. Some students were surprised to find out that antibiotic resistant bacteria are in the environment right now!

After listening to the students and reviewing their feedback, I made substantial edits to the lecture, background information, and data collection system. Now, the lesson is ready to be piloted again, and as time goes on, I hope to see more classrooms using my lesson in an attempt to limit the chances of an antibiotic resistant bacteria epidemic. Antibiotics are an excellent tool that advances human healthcare and public health as a whole, but their overuse can bring devastating consequences if something isn’t done to prevent that from occurring.

References

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