ScienceNews
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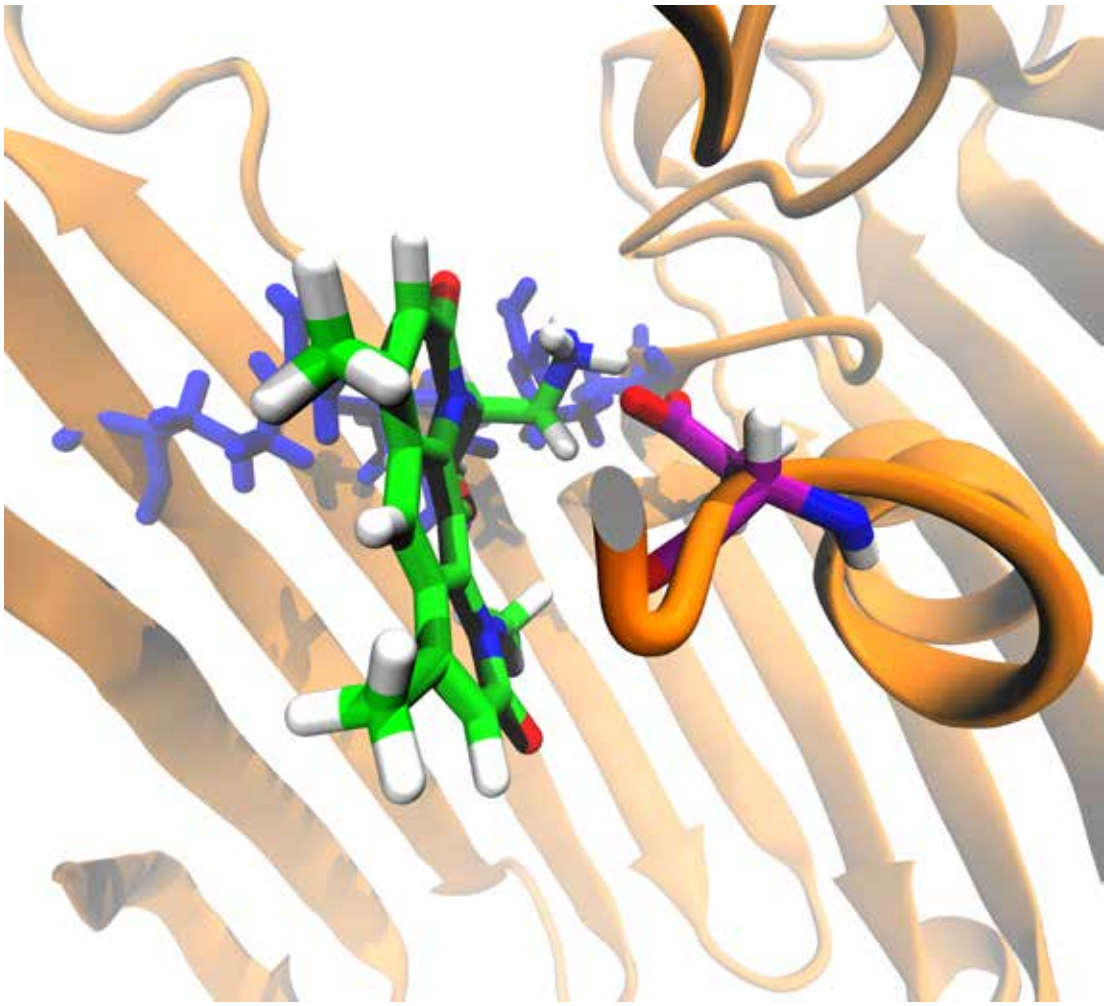

M.F. RICHTER *ET AL/NATURE* 2017

June 10, 2017

New 'Rules' for Finding Antibiotics

SOCIETY FOR SCIENCE & THE PUBLIC

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About this Issue

The article "New 'rules' for finding antibiotics" (12.4 readability score) proposes a new strategy for designing antibiotics for gram-negative bacteria. Students can focus on details reported in the article, follow connections to earlier articles about drug-resistant bacteria and antibiotics, and explore cross-curricular connections to other major science topics. In a related activity, students can test how susceptible yogurt bacteria are to antibiotics and determine whether the bacteria are gram-positive or gram-negative. *Science News for Students* provides another version of this article written at a lower Lexile level (8.3 readability score): "New 'rules' for finding antibiotics." Power Words are defined at the end of the *Science News for Students* article.

Connections to Curricula:

- Prokaryotes/bacteria
- Antibiotics
- Antibiotic resistance
- Biotechnology
- Microscopy
- Genetics
- Pharmacology
- Epidemiology
- Macromolecules

Want to read more about drug-resistant bacteria? Check out "Scientists watch germs evolve into super bugs" (7.6 readability score), from *Science News for Students*.

Looking for antibiotic resistance research conducted by students? Check out "Teen studies how germs resist our drugs" (8.5 readability score), from *Science News for Students*.

What's in this Guide?

Article-Based Observation**:** These questions focus on reading and content comprehension by drawing on information found in the article "New 'rules' for finding antibiotics." Questions focus on student understanding of gram-negative bacteria and the bacteria's response to antibiotics.

Quest Through the Archives**:** With Internet access and your school's digital access to *Science News*, your students can use this short section to explore other articles about bacteria and antibiotic development as reported by *Science News* since 1924.

Cross-Curricular Discussion**:** These questions and extension prompts connect to the article "New 'rules' for finding antibiotics" and encourage students to think in more detail about scientific areas related to the article. The section is divided roughly by science subdiscipline for educators who would like to focus on one particular topic area. The extension prompts are either more topic-specific or more conceptually advanced. **Biological and Chemical Sciences** questions range from the nature of bacteria to types of antibiotics. **Engineering and Experimental Design** questions focus on methods of dealing with antibiotic resistance and useful applications of bacteria.

Activity: Students can culture yogurt bacteria with and without antibiotics to test the bacteria's sensitivity. Students can also stain microscope slides of yogurt bacteria to determine if they are gram-positive or gram-negative. After completing this activity, students will better understand how yogurt is made by bacterial fermentation and how antibiotics can affect this process.

Standards Alignment

Next Generation Science	Common Core
Matter and Its Interactions: HS-PS-1-5, HS-PS-1-6	ELA Standards: Reading Informational Text (RI): 1, 2, 4, 5, 7
From Molecules to Organisms: Structures and Processes: HS-LS1-1, HS-LS1-2, HS-LS1-3, HS-LS1-6, HS-LS1-7	ELA Standards: Writing (W): 1, 2, 3, 4, 6, 7, 8, 9
Heredity: Inheritance and Variation of Traits: HS-LS3-1, HS-LS3-2	ELA Standards: Speaking and Listening (SL): 1, 2, 4, 6
Biological Evolution: Unity and Diversity: HS-LS-4-2, HS-LS-4-3, HS-LS-4-4	ELA Standards: Reading for Literacy in Science and Technical Subjects (RST): 1, 2, 3, 4, 5, 7, 8, 9
Engineering Design: HS-ETS1-1, HS-ETS1-2	ELA Standards: Writing Literacy in History/Social Studies and Science and Technical Subjects (WHST): 1, 2, 4, 6, 7, 8, 9

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Article-Based Observation

Directions: Read the article "New 'rules' for finding antibiotics," and then answer these questions:

1. **Why are bacteria called gram-negative or gram-positive? What is special about a gram-negative bacterium's structure that affects its interaction with an antibiotic?**

2. **What has been the traditional way to learn how antibiotics cross the bacterial barrier? What situation has provided extra motivation for advances in drug development?**

3. **What is a porin, and why are they important for antibiotic design?**

4. **What did chemical biologist Paul Hergenrother and his group do to learn about porin passage?**

5. **Once Hergenrother's group was finished with its initial study of the 100 compounds, what additional studies did the team perform?**

6. **What antimicrobial did the researchers use to test the new "rules" discovered by their previous experiments? Why did they choose it initially, how was it altered and what was the outcome?**

7. **Explain what microbiologist Kim Lewis means when he says this research could "revive the failed effort to rationally design antibiotics." What fields of science are needed to create designer antibiotics?**

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Responses to Article-Based Observation

1. **Why are bacteria called gram-negative or gram-positive? What is special about a gram-negative bacterium's structure that affects its interaction with an antibiotic?** Possible student response: Unlike gram-positive bacteria, gram-negative bacteria do not retain a violet color when exposed to a dye called a Gram's stain. Whereas gram-positive bacteria have only one membrane, gram-negative bacteria have a cell membrane and cell wall. The outer membrane is impermeable to most antibiotics, so even if an antibiotic has the ability to kill gram-negative bacteria, it might not be able to get through the outer membrane to do so.

2. **What has been the traditional way to learn how antibiotics cross the bacterial barrier? What situation has provided extra motivation for advances in drug development?** Possible student response: Traditionally, scientists have studied the bacterial barriers to learn how compounds might cross them. Breaking the tradition, the research summarized in this article focused on the properties of molecules that could help them get across the barrier. Some of today's research is motivated by the fact that bacteria are becoming resistant to antibiotics, and all of the most critical bacteria on a list created by the World Health Organization are gram-negative bacteria.

3. **What is a porin, and why are they important for antibiotic design?** Possible student response: A porin is a protein that dots the outer membrane of a gram-negative bacterium and acts like a channel, or pore, to allow the cell to take in nutrients. Passage through a porin is typically how an antibiotic can enter gram-negative bacteria.

4. **What did chemical biologist Paul Hergenrother and his group do to learn about porin passage?** Possible student response: Hergenrother's group synthesized 100 compounds that have molecular characteristics similar to those of naturally occurring antimicrobials and then incubated each with *E. coli* bacteria in a tube for 10 minutes. Researchers then measured how much of each compound got inside the cells. Next, they analyzed the molecules that had the most success in penetrating the cells, discovering that an amine group was a shared feature.

5. **Once Hergenrother's group was finished with its initial study of the 100 compounds, what additional studies did the team perform?** Possible student response: After identifying that an amine group might aid in a molecule's passage across a bacterial barrier, Hergenrother's group used a larger set of amine-containing compounds and performed the *E. coli* incubation test. Using a computer program, the researchers also learned that a rigid, flat structure was more likely to pass through porins than a flexible or spherical one.

6. **What antimicrobial did the researchers use to test the new "rules" discovered by their previous experiments? Why did they choose it initially, how was it altered and what was the outcome?** Possible student response: Researchers used an antimicrobial called deoxynybomycin that is effective only against gram-positive bacteria. Deoxynybomycin is flat and rigid, so it already has "the right geometrical parameters," said Hergenrother. The researchers synthesized a deoxynybomycin derivative containing an amine group and tested it on gram-negative pathogens. It was effective on all but one pathogen tested.

7. **Explain what microbiologist Kim Lewis means when he says this research could "revive the failed effort to rationally design antibiotics." What fields of science are needed to create designer antibiotics?** Possible student response: This research shows that existing gram-positive antibiotics may be chemically altered to be effective on gram-negative bacteria. Many biological fields such as microbiology and immunology, as well as chemical fields, such as synthetic organic chemistry and instrumental analyses, are involved in building designer antibiotics.

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Quest Through the Archives

Directions: After reading the article “New ‘rules’ for finding antibiotics,” use the archives at www.sciencenews.org to answer these questions:

1. **Search for the oldest article in *Science News* that mentions antibiotics. What bacteria are discussed, and what does the article say about fighting disease caused by the bacteria?**

2. **Viruses, like Zika and Ebola, cause public alarm. Search for an article focused on bacterial infections that could have widespread implications for public health. What does it say?**

3. **Penicillin may be the most famous antibiotic of all, yet many people can’t use it because they are allergic to it. Search for an article about the connection between penicillin and allergies. What does it say?**

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Responses to Quest Through the Archives

1. **Search for the oldest article in *Science News* that mentions antibiotics. What bacteria are discussed, and what does the article say about fighting disease caused by the bacteria?** Possible student response: The article "Phage against dysentery," published 5/13/1944, discusses a bacteriophage that protects mice against eight types of dysentery. Conflicting ideas about how the bacteriophage works are attributed to the type of experimentation performed. Scientists are not sure whether this bacteriophage is a living organism, an enzyme or a ferment, but they believe that it kills germs by lysis or dissolution.

2. **Viruses, like Zika and Ebola, cause public alarm. Search for an article focused on bacterial infections that could have widespread implications for public health. What does it say?** Possible student response: The article "Doctors enlisted to turn the tide on antibiotic resistance," published 9/19/2014, discusses antibiotic resistance in well-known bacteria that have widespread public health implications — such as *Staphylococcus*, *Streptococcus*, *Escherichia coli*, *Salmonella* and *Neisseria gonorrhoeae*. Overuse of antibiotics is increasing the likelihood that these bacteria become drug resistant. Educating doctors and patients on appropriate use of antibiotics may help combat resistant microbes.

3. **Penicillin may be the most famous antibiotic of all, yet many people can't use it because they are allergic to it. Search for an article about the connection between penicillin and allergies. What does it say?** Possible student response: The article "Penicillin allergy? Think again," published 12/11/2016, discusses the idea that about 10 percent of the U.S. population believes they have a penicillin allergy, but researchers are learning that about 90 percent of those people aren't actually allergic. Rashes that doctors or parents think are caused by a reaction to penicillin, especially when babies receive the antibiotic, may actually be symptoms of the infection being treated. Furthermore, the prescription of broad-spectrum antibiotics in place of penicillin when there is a suspected allergy may be contributing to the prevalence of antibiotic-resistant bacteria.

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Cross-Curricular Discussion

After students have had a chance to review the article "New 'rules' for finding antibiotics," lead a classroom discussion based on the questions that follow. You can copy and paste only the questions that apply to your classroom into a different document for your students.

Biological and Chemical Sciences
Discussion Questions:

1. **What are some important differences between bacteria and other microorganisms?**
   - [*Bacteria are prokaryotic cells, which lack a membrane-bound nucleus and organelles. Some bacteria live independently in the environment. Other bacteria hang out in our bodies, in the space outside of our cells, where they may cause disease or even help us (as with the bacteria that aid digestion in our intestines). Some of the nastier bacteria actually invade our cells and replicate inside our cells, for example in tuberculosis lung infections.*
   - *Fungi are eukaryotic (complex) cells. Fungal cells are surrounded by a thick cell wall and can cause athlete's foot, for example. The mushrooms on pizza are fungi.*
   - *Protozoa are eukaryotic cells that are similar to our own — they are basically single-celled animals. However, some of the more vicious ones (like malaria) can invade our cells. Other protozoa just spend their whole lives harmlessly floating around in ponds.*
   - *Viruses are rogue genes that invade host cells, command cells to make more copies of the viral genes and usually (but not always) kill the host cells before spreading to infect more host cells. Viruses include the common cold, for example, and Ebola.*]

2. **What is the difference between gram-positive and gram-negative bacteria?**
   [*Different categories of bacteria have different types of cell walls. Gram-positive bacteria have a thick peptidoglycan (linked protein + sugar) cell wall. Gram-negative bacteria have a thinner peptidoglycan cell wall, then another membrane, then an outer layer of lipopolysaccharide. Thus gram-negative bacteria are protected by more walls made of more materials than are gram-positive bacteria.*
   The Gram's staining procedure, which was invented by the Danish bacteriologist Hans Christian Gram, makes gram-positive bacteria appear purple and gram-negative bacteria appear pink to red under the microscope.]

3. **How do antibiotics kill bacterial cells but not human cells?**
   [*The trick to developing antibiotics is to find a structural difference between the bacterial cells and human cells, and then create drugs that target the feature that bacteria but not human cells possess. For example, one major difference between bacterial and human cells is that bacteria have a cell wall around their plasma*

membrane, whereas human cells have only a plasma membrane. Antibiotics often attack the thick cell walls of bacteria. If a drug interferes with bacterial cell walls, the bacterial cells can be killed but the drug won't affect human cells.
Another major difference between bacterial and human cells is their ribosomes. Both cell types use ribosomes to produce proteins, but there are important differences between bacterial (prokaryotic) and animal (eukaryotic) ribosomes. Thus, it is possible to develop drugs that bind to bacterial ribosomes and stop their protein production, yet don't affect ribosomes or protein production in human cells.]

4. **What is an amine functional group? Explain the chemical and physical properties of an amine group based on its molecular structure. If a molecule without an amine functional group were synthesized to contain an amine, how might the properties of the molecule change?**
[*An amine is a nitrogen- and hydrogen-containing functional group with a trigonal pyramidal shape. Amines fall into different categories depending on how many hydrogens are bonded to a nitrogen atom. A primary amine is a nitrogen atom bonded to two hydrogen atoms and one hydrocarbon (RNH2 structure). A secondary amine is a nitrogen bonded to one hydrogen and two hydrocarbons (R2NH). Finally, a tertiary amine is a nitrogen bonded to three hydrocarbons and no hydrogen atoms (R3N). Because of the extreme polarity of the nitrogen to hydrogen bond, the trigonal pyramidal molecular shape of the functional group and the small size of the nitrogen and hydrogen atoms, both primary and secondary amines will interact through hydrogen bonding with other molecules. If primary or secondary amine groups were synthesized onto molecules that did not originally contain them, the new molecules would likely have a higher boiling point because of their newly created region for hydrogen bonding and stronger intermolecular attraction. The new molecules would also probably have a greater attraction to other polar or partially charged molecules.*]

Extension Prompts:

5. **What are the major categories of existing antibiotics? Pick an antibiotic and research how it works.**
[*Antibiotics can be divided into groups based on what targets they attack in bacterial cells:*
- *Many antibiotics attack the bacterial cell wall and are called broad-spectrum antibiotics. The structures of these drugs generally mimic part of the bacterial cell wall, such as peptidoglycan. That mimicry allows the drugs to get taken up by enzymes that synthesize the cell wall (such as transpeptidases), bind irreversibly to the enzymes or to the incomplete cell wall, and prevent the cell wall from being finished. These antibiotics cause osmotic lysis for actively growing, new bacteria. Some major types of antibiotics that target the cell wall include ß-lactams, or molecules that contain a beta-lactam ring in their chemical structure. ß-lactams include penicillins (such as penicillin and amoxicillin), cephalosporins, carbapenems, monobactams, glycopeptides and bacitracin (which specifically blocks transport of peptidoglycan components).*
- *ß-lactamase inhibitors block bacterial ß-lactamase enzymes that would otherwise destroy ß-lactam antibiotics, so they are often mixed in with ß-lactam antibiotics.*

- *Antibiotics that target unique features in bacterial versus animal cell plasma membranes include: polymyxins and cyclic lipopeptides.*
- *The large ribosomal subunit is made up of 31 proteins and two ribosomal RNAs (rRNAs) in prokaryotes, but 50 proteins and three rRNAs in eukaryotes. Many antibiotics attack these key features that make the prokaryotic large (50S) ribosomal subunit different from the eukaryotic large ribosomal subunit, thereby inhibiting bacterial protein synthesis: macrolides (such as erythromycin and azithromycin), chloramphenicol, lincosamides, oxazolidinones and streptogramins.*
- *Likewise, the small ribosomal subunit is composed of 21 proteins and one rRNA in prokaryotes, but 33 proteins and a larger rRNA in eukaryotes. A number of antibiotics attack these key differences in the prokaryotic small (30S) ribosomal subunit, again inhibiting bacterial protein synthesis: tetracyclines (such as tetracycline and doxycycline), aminoglycosides and nitrofurans.*
- *In a similar fashion, some antibiotics selectively inhibit prokaryotic but not eukaryotic transfer RNAs (tRNAs), including mupirocin and furanomycin.*
- *Some antibiotics selectively target prokaryotic DNA synthesis enzymes, such as metronidazole.*
- *Similarly, antibiotics that inhibit prokaryotic but not eukaryotic DNA gyrases include quinolones (such as ciprofloxacin and levofloxacin).*
- *Some antibiotics inhibit prokaryotic RNA polymerases but not eukaryotic RNA polymerases: rifampin, streptovaricins and actinomycin.*
- *Finally, some antibiotics inhibit prokaryotic enzymes involved in essential folic acid synthesis: trimethoprim and sulfonamides.*]

6. **How do bacteria become resistant to antibiotics?**
   - [*In some cases, bacteria decrease the permeability of their cell wall or their channels, and antibiotics can no longer penetrate the bacteria. Some bacteria have used that method against vancomycin and ß-lactams.*
   - *In other cases, bacteria actually use pumps to spit out any antibiotic that gets in. Some bacteria have used that method against tetracyclines, quinolones, aminoglycosides, ß-lactams and macrolides.*
   - *A variety of bacterial enzymes can interfere with or destroy antibiotics. Some bacteria have used that method against ß-lactams, aminoglycosides, macrolides, rifamycins, chloramphenicol, tetracyclines and vancomycin.*
   - *Finally, bacteria mutate so that the shape of an antibiotic target changes and the antibiotic will no longer attack that target. Some bacteria have used that method against macrolides, quinolones, aminoglycosides, penicillins, vancomycin and rifamycins.*]

7. **What are plasmids and why are they important for antibiotic resistance in bacteria?**
   [*Plasmids are typically small circular pieces of DNA that replicate independently from the host's chromosomal DNA. They are infectious from one bacterium to others. If an antibiotic-resistance gene is in the main bacterial chromosome, it gets passed down to the direct descendants of that bacterium. But if an antibiotic-resistance gene is in a plasmid, it can rapidly spread to other bacteria.*]

Biological and Chemical Sciences Question Bank

What are some important differences between bacteria and other microorganisms?

What is the difference between gram-positive and gram-negative bacteria?

How do antibiotics kill bacterial cells but not human cells?

What is an amine functional group? Explain the chemical and physical properties of an amine group based on its molecular structure. If a molecule without an amine functional group were synthesized to contain an amine, how might the properties of the molecule change?

What are the major categories of existing antibiotics? Pick an antibiotic and research how it works.

How do bacteria become resistant to antibiotics?

What are plasmids and why are they important for antibiotic resistance in bacteria?

Engineering and Experimental Design

Discussion Questions:

1. **Why is it important to take a full course of antibiotics and not just stop as soon as you start to feel better?**
   [*Not finishing the full course of antibiotics can promote antibiotic resistance. The first few doses of an antibiotic will wipe out most of the bacteria, but any mutant bacteria that are slightly resistant to the antibiotic will survive. If you stop taking the antibiotic then, those slightly resistant bacteria will reproduce, and all of the resulting bacteria will be slightly resistant to that antibiotic. After a few more rounds of taking a little of the antibiotic, but not enough, other mutated bacteria may be highly resistant to the antibiotic. In contrast, if you take the complete course of antibiotic without a break, eventually even the slightly resistant bacteria will likely die before they can reproduce.*]

2. **How can you deal with bacteria that are antibiotic resistant, or can easily mutate to become antibiotic resistant?**
   [*For bacteria that can mutate very easily (such as tuberculosis), doctors usually prescribe a cocktail, or mixture, of several antibiotics. That way even if the bacteria mutate to become resistant to one of the antibiotics, another antibiotic in the cocktail will likely kill them.*]

Extension Prompts:

3. **What are the advantages and disadvantages of routinely keeping farm animals on antibiotics?**
   [*Antibiotics can prevent bacterial infections in the animals, making it cheaper and easier to raise them, even in poor conditions where bacterial infections could easily spread. On the other hand, farms that use a lot of antibiotics are a training ground for bacteria to develop resistance, and antibiotic-resistant bacteria can be passed to humans who handle meat from the animals before it is fully cooked.*]

4. **Many antibiotics are naturally produced by certain microorganisms, and were discovered in these organisms in nature. Why would microorganisms produce antibiotics?**
   [*If a microorganism can kill off other types of microorganisms that might consume the same nutrients, it gives that microorganism a resource advantage.*]

5. **What are some ways that bacteria can be useful?**
   - [*Normal human microbiota. Your body actually contains as many bacterial cells as human cells, many on your skin and in your gastrointestinal tract. The relationship is mutually beneficial, since people provide the bacteria with an environment to live in and the good bacteria keep harmful bacteria away. Bacteria in the gastrointestinal tract aid with digestion, and they may aid (or in some cases hinder) the immune and endocrine systems.*
   - *Nitrogen fixation. Bacteria in the soil or water absorb nitrogen gas (N_2) from the air and convert it into ammonia (NH_3) or other nitrogen-containing molecules that can be readily taken up by plants and used to build larger biomolecules. Without this nitrogen fixation, there would be no plants and so no animals that eat plants.*
   - *Making yogurt and cheese. Milk can be turned into yogurt, a variety of different cheeses and other products by adding specific types of bacteria and treating the mixture under the right sorts of conditions. In general, the bacteria convert lactose (milk sugar) to glucose (simple sugar), then use fermentation to convert that glucose to lactic acid or lactate. Some bacteria can also ferment sugar to ethanol or ethyl alcohol plus carbon dioxide.*
   - *DNA and protein production. Bacteria are great at making lots of copies of themselves, which requires copying their DNA and proteins. If you are a biology researcher in need of lots of copies of a new gene or new protein, a simple solution is to stick the gene into a harmless strain of* E. coli*, let them reproduce for a while, then extract the copies of the gene or the protein made by the gene from all of the reproduced* E. coli.
   - *Bacterial degradation and remediation involves finding natural bacteria or genetically engineering bacteria to eat, or at least to surround and hide, substances we don't want in the environment. This includes oil spills, for example, and uranium ions.*
   - *Bacterial pesticides can be used instead of chemicals as a sometimes more environmentally benign method to kill certain insects. For example,* Bacillus thuringiensis *spores or their components (often sold as gardening supplies) can kill insect larvae before they develop into adult insects.*]

6. **How might antibiotic-resistant plasmids be useful for genetically engineering bacteria?**
   [*If a plasmid contains an antibiotic-resistance gene and also some new gene of interest, you can introduce it into a herd of bacteria, then add that antibiotic to eliminate any bacteria that did not take up the plasmid. Thus all of the surviving bacteria should have the gene of interest. You have to be sure you are using an antibiotic that wouldn't treat bacterial infections in humans, because you are making more bacteria resistant to that antibiotic.*]

Engineering and Experimental Design Question Bank

Why is it important to take a full course of antibiotics and not just stop as soon as you start to feel better?

How can you deal with bacteria that are antibiotic resistant, or can easily mutate to become antibiotic resistant?

What are the advantages and disadvantages of routinely keeping farm animals on antibiotics?

Many antibiotics are naturally produced by certain microorganisms, and were discovered in these organisms in nature. Why would microorganisms produce antibiotics?

What are some ways that bacteria can be useful?

How can antibiotic-resistant plasmids be useful for genetically engineering bacteria?

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Teacher Guide: How is Yogurt Made?

Class time: 30 to 50 minutes during two class periods. (Plus two to three days between class periods for the milk to solidify.)

Purpose: Students can grow safe yogurt bacteria in sterilized milk, observe that the bacteria solidify the milk into new yogurt and test how antibiotics affect the bacteria.

Materials:

- Student instructions: Blackline Master 3
- Small boxes of Parmalat or Lil' Milk ultra-high-temperature (UHT) pasteurized milk (typically sold in the non-refrigerated baking aisle of the grocery store)
- Cup(s) of yogurt (plain yogurt is simplest)
- Tube(s) of triple antibiotic ointment
- 15 ml graduated sterile plastic test tubes
- Lab markers (preferably alcohol-resistant)
- Paper towels
- 70% ethyl or isopropyl alcohol in squirt bottles (rubbing alcohol)
- Non-latex gloves
- Safety goggles
- Lab coats or lab aprons
- Alcohol burners or Bunsen burners
- Flame-sterilizable inoculation needles or loops (straightened paper clips taped to pencils can also be used as long as the paper clips have no plastic coating)
- Water bath (optional): Styrofoam cooler, aquarium heater, thermometer, plastic test tube racks, paper clips, nearby sink

Notes to the teacher: Yogurt contains harmless live bacteria (typically *Lactobacillus* and non-pathogen *Streptococcus* species). When a drop of these bacteria is added to fresh milk, they can acidify and solidify the milk to make new yogurt.

Triple antibiotic ointment contains three antibiotics (bacitracin, neomycin and polymyxin B). When a small amount of antibiotic ointment is added to fresh milk along with the yogurt bacteria, the antibiotics will kill the bacteria and prevent the milk from solidifying into new yogurt. This lab activity is a good demonstration to show that antibiotics kill bacteria.

This activity is also a good opportunity to discuss and demonstrate the importance of using sterile techniques in science experiments. In this experiment, we want only yogurt bacteria to grow in the milk,

not other bacteria from the classroom, students' hands or elsewhere. To minimize or avoid introducing other bacteria, students should:

- Wear gloves, sterilize gloves with alcohol by pouring some alcohol on the gloves and rubbing it around the gloves. After sterilizing the gloves with alcohol, students should avoid touching anything with their gloves that is not absolutely essential for the experiment.
- Use UHT milk, in which all of the bacteria have already been killed by high heat (unlike in regular milk). Because of this, UHT milk can be stored at room temperature (until it is opened and is exposed to new bacteria from the environment).
- Use sterile test tubes, keep them tightly capped except when adding something to them and be very careful handling the test tube caps.
- Sterilize an inoculation needle by passing its end through a flame for a few seconds, then let it cool in the air for a few seconds before using it to transfer a drop of yogurt or antibiotic ointment.
- Use separate inoculation needles for yogurt and antibiotic, and wipe and flame them between uses to avoid cross-contamination.

The yogurt bacteria will solidify a tube of milk overnight at 37° C, or in two to three days at room temperature. If you have a heated water bath, incubator or a warm room (like a boiler room), you can put the sealed tubes there to incubate. If you would like to create a water bath, you can easily make one by filling a large Styrofoam cooler with water, dropping an aquarium heater and a thermometer into it and adjusting the thermostat on the aquarium heater until it heats and maintains the water at around 37° C. Using paper clips like hooks, you can attach plastic test tube racks to the inner walls of the cooler, so that the racks will keep test tubes mostly immersed in the water with just their tops sticking out of the water.

Please make sure students follow all applicable safety rules in working with flames and using inoculation needles. Students should not consume any of the yogurt or milk before, during or after the experiment.

If you have enough time, your students can dilute a drop of yogurt in water, streak it on a microscope slide, Gram's stain the slide and observe the yogurt bacteria at 400x or 1000x under a microscope. *Lactobacillus* bacteria look like chains of tiny sausages linked end-to-end, and *Streptococcus* bacteria look like chains of tiny spheres. Both types of bacteria are gram-positive and should appear purple when Gram's stained. Students could test for bacteria from a cup of fresh yogurt and/or from their milk tubes after the samples have been incubated.

Materials for observing bacteria under a microscope:

- Student instructions: Blackline Master 4
- Gram's stain (such as this Gram's stain kit from Home Science Tools)
- Pre-cleaned glass microscope slides
- Pre-cleaned thin glass cover slips
- Optional: Canada balsam or microscope slide mounting cement
- Microscopes with 400x (or 1000x and immersion oil)
- Squirt bottles with water

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Student Guide: How is Yogurt Made?

Yogurt contains harmless live bacteria (typically *Lactobacillus* and non-pathogen *Streptococcus* species). You can observe how these bacteria affect milk, and you can test what happens when you add antibiotics. Throughout this lab it will be very important to practice good sterile techniques to avoid adding bacteria from the environment or from your skin to the experiment.

Notes: Wear gloves, goggles and other appropriate safety gear. To kill any environmental bacteria on your gloves, sterilize gloves with alcohol by pouring some alcohol on and rubbing it around the gloves until it dries. Do not touch anything with your gloves that you do not need to touch for this experiment. If necessary, alcohol your gloves again during the experiment.

The test tubes are sterile, and you should keep them tightly capped except when you need to add something to the tubes. When you remove the test tube cap, try to hold it with your alcohol-sterilized glove instead of setting it down on a surface that might have bacteria.

The box of ultrahigh-temperature (UHT) pasteurized milk is sterile. Any bacteria in the milk were killed when it was heated to a high temperature. This means the milk can be stored at room temperature until it is opened and exposed to environmental bacteria. (Normal milk still has some bacteria, so it must be refrigerated and will still go bad within a week or so as those bacteria multiply in the milk.)

Procedure:

1. Label four test tubes with your or your group's name and one each of the following labels:
   - –Yogurt –Antibiotic
   - +Yogurt –Antibiotic
   - –Yogurt +Antibiotic
   - +Yogurt +Antibiotic

2. Carefully remove the sterile straw from its plastic on the side of the UHT milk box and insert it into the box. The straw should form a tight seal as it passes through the foil opening. Carefully use the straw to put 10 ml of milk into each of the four test tubes (measure the amount added on the tube). If the straw dribbles milk on the table, clean up the mess with paper towels and ask your teacher for help.

3. You will use one inoculation needle to transfer yogurt bacteria and another to transfer antibiotic. To avoid cross-contamination, do not get the needles confused and wipe them off thoroughly between uses. Before each use of an inoculation needle, pass its tip through a flame for a few seconds to

kill any bacteria; then let it cool for a few seconds without setting it down (it might pick up more environmental bacteria from the table or other surfaces).

4. Dip a flame-sterilized inoculation needle into a freshly opened cup of yogurt, get a tiny blob of yogurt on the tip and add that yogurt blob to one of the +Yogurt tubes. Clean and flame sterilize the inoculation needle again and use it to add a tiny yogurt blob to the other +Yogurt tube.

5. Use another flame-sterilized inoculation needle to add a small blob of antibiotic ointment to one of the +Antibiotic tubes. To get most of the antibiotic off the needle, you may need to swish the needle around in the milk in the tube and/or gently scrape the needle against the inside wall of the tube. Clean and flame the inoculation needle again and use it to add a small antibiotic blob to the other +Antibiotic tube.

6. Seal the tubes tightly, and shake and swirl them around to mix their contents thoroughly. Give the tubes to your teacher to incubate at 37° C overnight, or at room temperature for two to three days.

7. What do you predict will happen to the milk in each of the four tubes after they have been incubated? Record your thoughts below.

   Tube 1: –Yogurt –Antibiotic
   Tube 2: +Yogurt –Antibiotic
   Tube 3: –Yogurt +Antibiotic
   Tube 4: +Yogurt +Antibiotic

8. After the tubes have been incubated and you get them back from your teacher, keep the tubes sealed while closely observing the color and texture of the milk in each tube. Try slowly leaning the tubes to one side or the other. Try gently turning the tubes upside down. Record your observations for each tube:

   Tube 1: –Yogurt –Antibiotic
   Tube 2: +Yogurt –Antibiotic
   Tube 3: –Yogurt +Antibiotic
   Tube 4: +Yogurt +Antibiotic

Answer the following questions to analyze your results:

1. Did the results match your predictions for each test tube? Explain.

2. If the results matched your predictions, what do you think happened in each tube?

3. If the results did not match your predictions, what do you think happened that you did not expect? What additional factors might have influenced the results?

4. How might the experiment have turned out differently if you had not practiced sterile techniques?

5. How might the experiment have turned out differently if you had used the same inoculation needle for transferring both yogurt and antibiotic, and did not clean or flame the needle between uses?

6. What variations of this experiment could you do to test other conditions, or a range of conditions?

7. Relatively speaking, how effective is antibiotic ointment on *Lactobacillus* and non-pathogen *Streptococcus*? What are the antibiotics in the ointment used in your experiment? Research to find out how each antibiotic kills *Lactobacillus* and non-pathogen *Streptococcus*. What other types of bacteria could these antibiotics be useful for?

8. Knowing what you do now, how do you feel about eating yogurt? Research and explain how yogurt is made. How does this relate to the experiment you just performed?

9. Research *Lactobacillus* and non-pathogen *Streptococcus* bacterial species. Are they gram-positive or gram-negative bacteria? What color would you expect them to turn if dyed with Gram's stain? In what environment do they thrive? How do they affect humans?

SN IN HIGH SCHOOLS June 10, 2017

New 'Rules' for Finding Antibiotics

Student Guide: Gram Staining

Use the following set of instructions to stain your cultured bacteria, prepare a microscope slide and view it under a microscope. Write down your observations and explain how they compare with your prediction about the color of the Gram's stain for each type of bacteria.

Procedure:

1. Fill a 15-ml plastic test tube with 5 ml of water, add a drop of yogurt and put the cap on the tube. Seal the tube tightly, and shake and swirl it vigorously to mix it well.
2. Use a clean inoculation needle to put a drop from the tube on a clean microscope slide. If necessary, use the needle or the edge of another clean slide to smear the liquid into a very thin film.
3. Let the bacteria on the slide air dry for about two minutes, then hold the slide with forceps (tweezers) and move it back and forth through the alcohol lamp flame three to four times. Do not let the slide get too hot.
4. Put one to two drops of crystal violet stain on the sample and let it sit for 60 seconds.
5. Rinse off the stain with a gentle squirt from the water bottle.
6. Put one to three drops of Gram's iodine stain on the sample and let it sit for 60 seconds.
7. Rinse off the stain with a gentle squirt from the water bottle.
8. Tilt the slide and add ethyl alcohol one drop at a time so that the alcohol runs over the entire sample. Stop adding alcohol drops as soon as the liquid dripping off the slide becomes colorless. That might take about five seconds or so.
9. Rinse off the alcohol with a gentle squirt from the water bottle.
10. Put one to three drops of safranin stain on the sample and let it sit for 60 seconds.
11. Rinse off the stain with a gentle squirt from the water bottle.
12. Very gently blot (but do not rub) the slide with a paper towel to dry it.
13. Put a clean cover slip on the slide. If necessary, use another slide or a paper towel to very gently mash it down or move it into position. If instructed by your teacher, add a tiny drop of Canada balsam or microscope slide mounting cement before you put the cover slip on the slide.

14. Observe the slide under the microscope. Start at low power (40x), focus back and forth until you find a reddish-purple colored layer and then work your way up to higher powers, refocusing each time as necessary. (Be careful not to focus on other, non-colored layers that might just be dust, bubbles or scratches above or below the actual sample.)

15. Describe what bacterial shapes and colors you see. The bacteria will be tiny, even at 400x or 1000x magnification. You may also see clumps of protein, blobs of fat or other fairly large debris from the yogurt. If you see something photogenic, you can hold a cell phone camera up to the eyepiece and adjust the camera position and focus to take a photo.

Notes:

- If the bacteria are colorless, you may have used too little stain or left the stain on too briefly, or you may have rinsed with too much water or ethyl alcohol.
- If the bacteria are too dense, you can dilute a drop of yogurt with more water and make a new slide.
- If the bacteria are too scarce, you can dilute a drop of yogurt with less water and make a new slide.