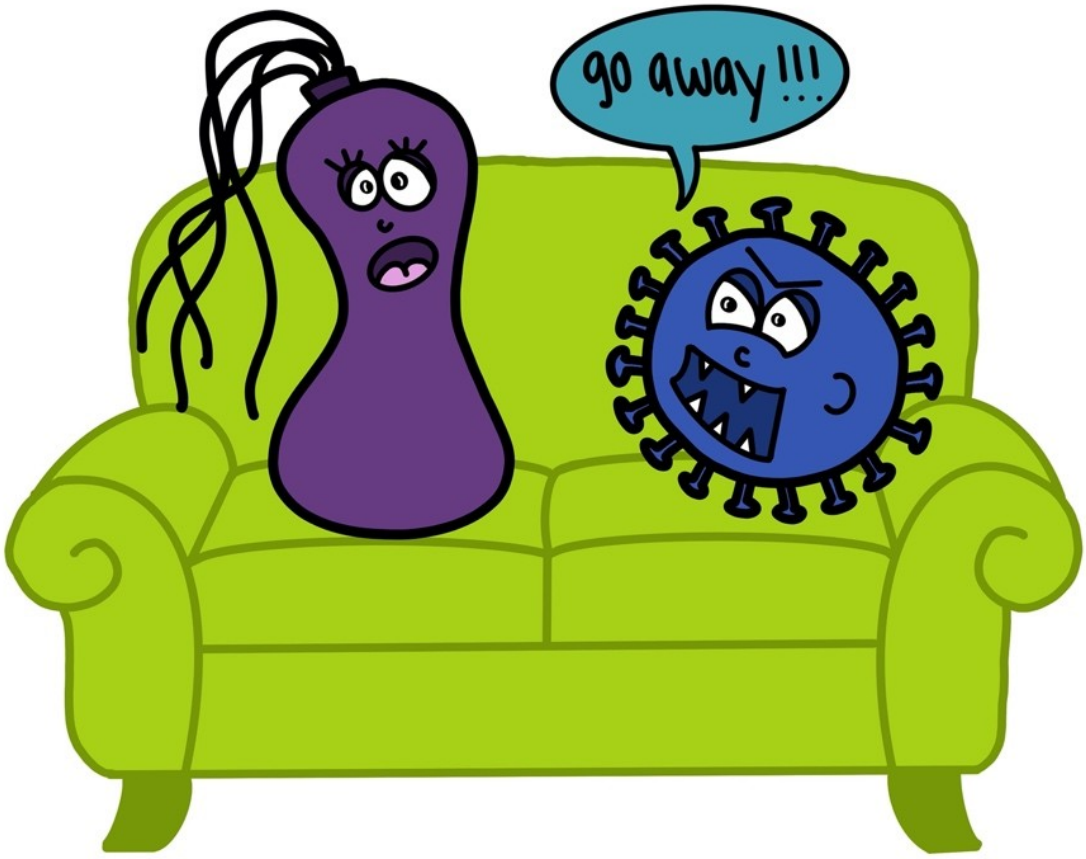
Competition and Microbial Weaponry

Henry: My sister and I are always fighting even though we are close relatives. Do bacteria experience sibling rivalry?



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Storyline

All siblings fight. Whether it is arguing over a favorite toy, the last cookie, or the best seat on the sofa, these competitions for space and resources also happen in the microscopic world. **Microbes** use many different types of molecules for their own version of sibling rivalry, as they steal food from each other and fight to occupy the most desirable spaces. Although we are largely unaware of these microscopic battles, they can have significant impacts on our lives as humans. In fact, we have learned how to use these molecules to keep unwanted bacteria away, allowing us to make humans healthier, increase production of food and energy sources, and protect important ecosystems that sustain our planet.

The Microbiology and Societal Context

The microbiology: microbial competition; microbial inhibitors; resource capturing systems; infections; antibiotic treatment; probiotics; microbial biotechnology. *Sustainability issues:* health; food production; global warming.

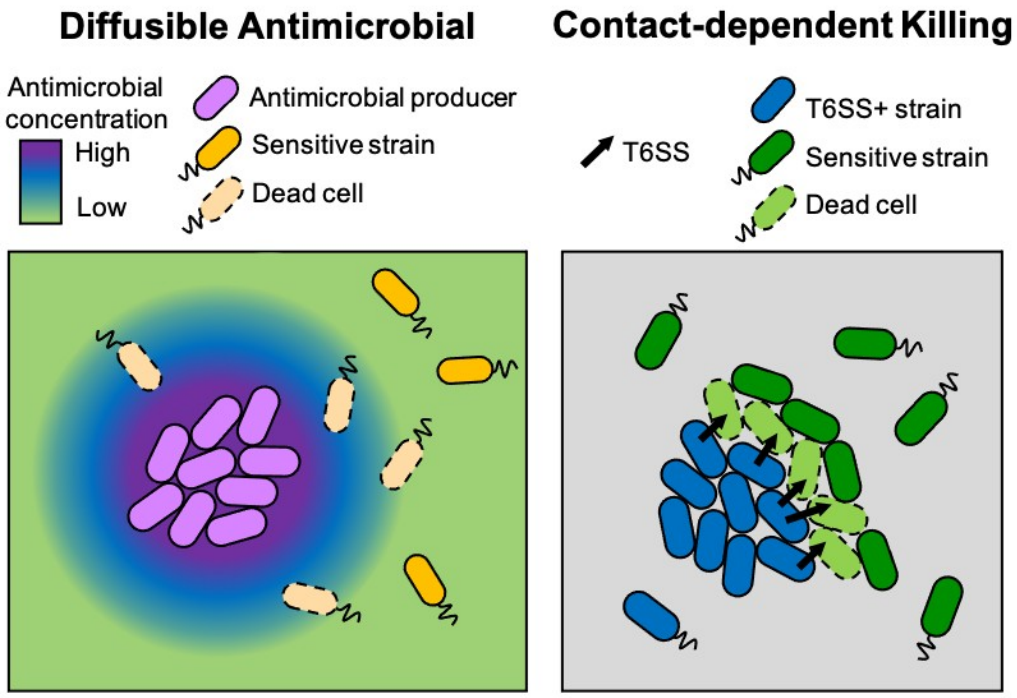
Competition and Microbial Weaponry: The Microbiology

1. ***Bacteria make many different kinds of weapons to eliminate competitors***: Bacteria use many different types of **microbial weapons** to compete with one another for limited resources, such as space and nutrients. Bacterial weapons range from tiny **diffusible** molecules that are released into the environment and travel far distances to kill competing bacterial cells, to **contact-dependent** nano-machines composed of many proteins that remain attached to the cell and kill nearby competitors by stabbing them with toxins (see box 1).

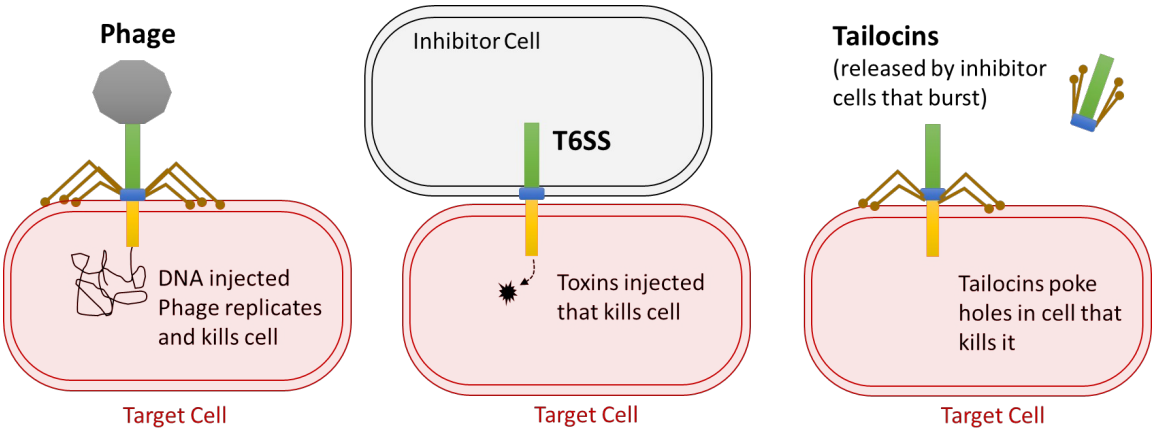
Most diffusible bacterial weapons are classified as **secondary metabolites**, which are molecules produced by bacteria that are not required for normal cell growth but provide the cell some other competitive advantage. Secondary metabolites that kill other microbes are referred to as **antimicrobials**. These molecules are produced in a bacterial cell, released into the environment, and enter a target cell either by diffusing across the cell membrane or entering through gateways made of proteins on the cell surface called **porins. Antibiotics** are a specific type of antimicrobial that are used to treat infections in animals and humans. Because antibiotics only act on structures specific to bacterial cells, they can safely be used to eliminate unwanted bacteria without harming the patient. Some microbes can also produce small peptides called **bacteriocins**, which are diffusible molecules with antimicrobial functions.

However, when bacteria live in aquatic or liquid environments, these diffusible bacterial weapons can quickly become diluted in the environment, making them ineffective at eliminating a competitor. Under these conditions, it is better for bacteria to use contact-dependent weapons to kill competing bacterial cells. The **type VI secretion system** (T6SS) is a common contact-dependent microbial weapon that shares structural similarities with **phage** (viruses that infect bacterial cells, see box 2) is built within bacterial cells using many different protein parts to construct what resembles a molecular syringe. When these bacteria bump into another cell, they

can use the T6SS to inject its neighbor with toxins, causing them to burst. Once the competitor cell has disappeared, the T6SS producing bacterium can take over the space and food that the other cells were using. Tailocins are a class of bacteriocins that resemble phage tails, which are released by cells through their own lysis. Tailocins diffuse through the environment until they make contact with a target cell. The molecular syringe of the tailocins pokes holes in the bacterial cell, resulting it its death.



Box 1: Image of diffusible vs contact-dependent; t6ss



Box 2: Several microbial weapons, including the T6SS (middle) and tailocins (right) are thought to have evolved from phage (left). This figure illustrates the conserved structures including the outer sheath (green), inner tube (yellow), baseplate (blue), and tail fibers (brown). Attack of a target cell by any of these

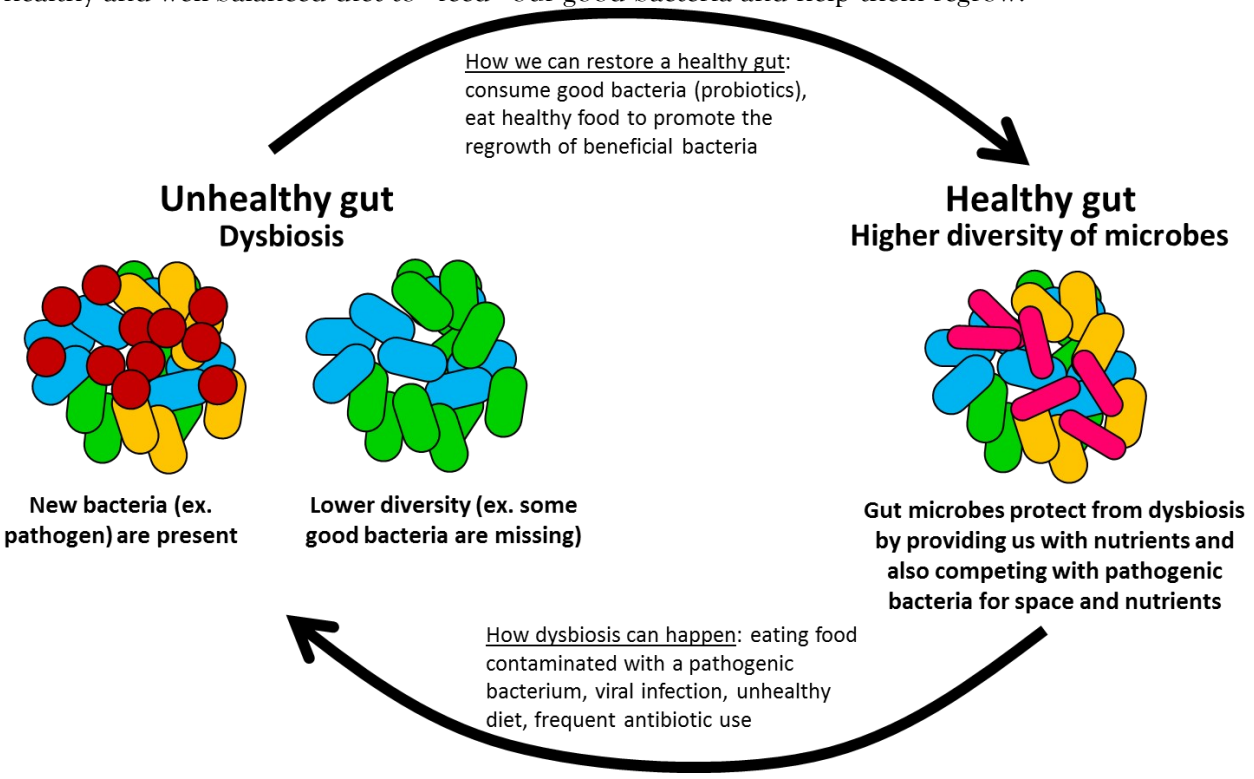
mechanisms results in the death, and often lysis, of the cell, which make them efficient weapons for competition.

2. ***Bacteria can also make molecules that allow them to steal essential nutrients to enhance their own growth while competitors die***: Just like we sometimes take a vitamin every day to make sure we have all the nutrients we need, bacteria also have a requirement for elements like iron and other small molecules required for their growth. Iron is often very scarce in most environments, yet too much of it can be toxic. To get enough iron to grow, bacteria use different ways to grab the iron that is around them, but they are all competing for the same limited resource. Think of what would happen if there is a whole classroom of kids and only three cookies. There will likely be a battle over who gets the cookies, and the children might employ some creative, and possibly aggressive, strategies to get what they want. Bacteria produce and release molecules called **siderophores** that bind to the iron in the environment. Then the bacteria use special protein structures on their surface to swallow the iron-bound siderophore. In this way, microbial cells can suck up the iron they need from the environment. But not all bacteria make these iron vacuum cleaners. Some bacteria "cheat" by stealing the siderophores of other bacteria, without having to go through the trouble of producing it themselves. Competition for essential nutrients like iron can decide which bacteria grow and which ones die: the bacteria that are best at getting the iron will win, and if these bacteria are important for promoting a healthy gut, or perhaps can cause disease, the winner of this competition can greatly impact our own health.

3. ***Our bodies use the same strategy to reduce infections by microbes.*** Human cells and those of other animals also need iron for growth. In fact, we use iron-containing haemoglobin to ferry oxygen around our bodies in red blood cells, from our lungs to the organs needing it. However, there is very little free iron in our blood because it is tightly bound to various iron-binding proteins we produce, like transferrin and lactoferrin, so microbes that manage to enter our bodies cannot grow. This is one of our major "first line" defenses against infection by microbes. However, some microbes – the successful, specialised pathogens – have evolved so-called "high affinity" iron capture systems that can even steal iron from our iron binding proteins to enable them to grow in our bodies and cause disease. Moreover, if bacteria that are accustomed to low iron levels encounter an environment where iron is plentiful, they can grow quickly. For people who have certain health conditions, like liver disease, their blood tends to be more iron rich and they are at a higher risk for microbial infection. These individuals should not eat raw meat or shellfish, to reduce their risk of infection.

4. ***Doctors use microbial weapons to treat infections in humans***: Although bacteria make antimicrobials to kill or stop the growth of other bacteria that would otherwise try to use the space or food they need, doctors use some of these molecular weapons (antibiotics) for treating infections in humans, but they must be used carefully. By taking antibiotics when we are sick, we can eliminate the bad bacteria that may be causing infections in the ear, nose, throat, or even a cut. However, these antibiotics often kill lots of different bacteria in our bodies, including the good bacteria in our gut that help keep us healthy. Therefore, antibiotics should only be taken if we have a bacterial infection, not a viral infection, for which it does nothing. Because a healthy gut has a high diversity (many different kinds) of good bacteria, taking antibiotics can kill some of these good bacteria or the gut bacteria can be upset by the presence of a new bacterium (ex. a

pathogen). Both of these examples are cases of **dysbiosis**, or the imbalance in microbial populations that are unhealthy for our guts (see box 3). However, we can take steps to help our good bacteria recover from dysbiosis and restore a healthy gut by eating food like yogurt, kefir, and other fermented foods, which contain a **probiotic** that has good bacteria in it, and also eat a healthy and well-balanced diet to "feed" our good bacteria and help them regrow.



Box 3: Cycle of healthy to unhealthy and healthy microbiome

5. ***Veterinarians use bacterial weapons to keep animals healthy***: It is a common misconception that *people* can become resistant to a certain type of antibiotic, when in fact it is *bacteria* that live in and on our bodies that evolve antibiotic resistance. Antibiotic resistance happens when bacteria survive exposure to drugs that are meant to kill them. Instead of being eliminated by antibiotics, a few bacterial cells will continue to grow, making infections caused by antibiotic-resistant germs difficult or impossible to treat.

Antibiotic-resistant bacteria can be spread to humans in a number of different ways, including through food consumption or contact with animals. Just like people, pets and livestock sometimes get sick and need to be treated with antibiotics. There are three main uses for antibiotics in animals: (1) treating infections in sick animals, (2) controlling diseases for a group of animals when only some animals are sick, and (3) preventing disease in populations of animals that are at risk for becoming sick. In fact, antibiotic use in animal husbandry accounts for about 80 % of the estimated antibiotic use in the United States. Food animals like pigs and chickens are often kept in very close quarters, which makes them stressed and especially vulnerable to infection, and which promotes transmission of infections that arise. Antibiotics are used frequently in these populations to prevent the spread of sickness when it does occur. For example, if one chicken becomes sick, a farmer may treat the entire flock with **prophylactic antibiotics** so that the rest of

the animals don't become infected. However, bacteria that infect chickens can become resistant to antibiotics in the same ways that we described above. And when humans consume undercooked

chicken infected with antibiotic- resistant bacteria, we can be infected by those same bacteria; however, we may not be able to treat the infection because those bacteria no longer respond to antibiotics (see box 4).

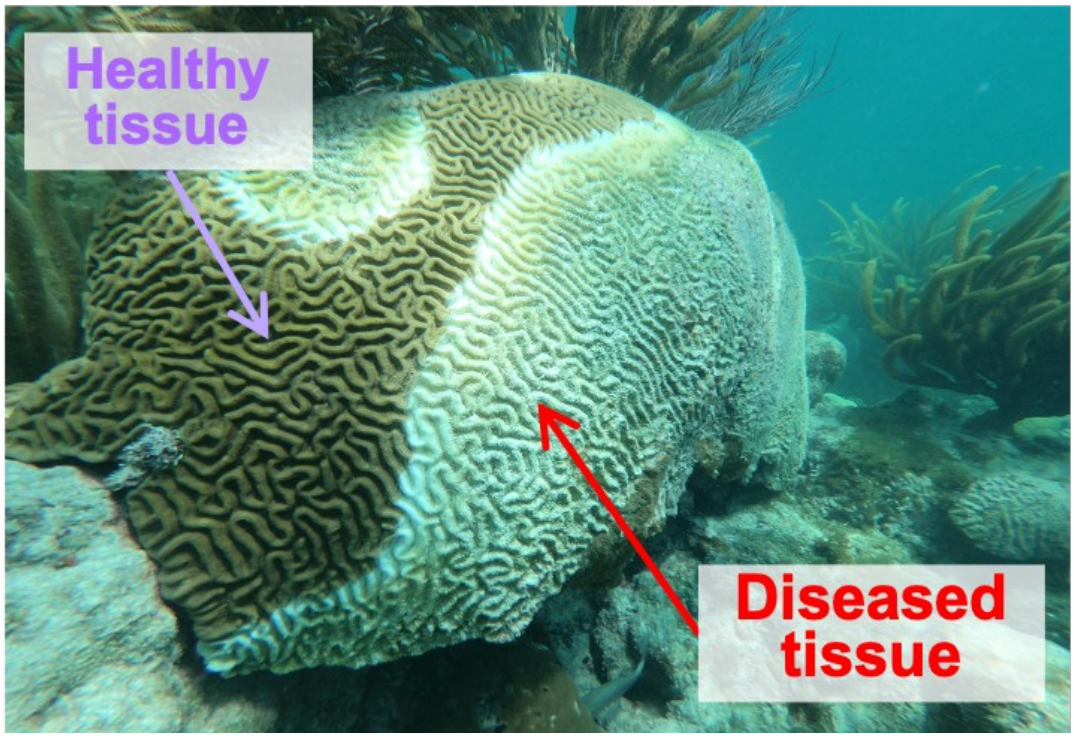
Box 4. Use of antibiotics in food animals.

6. ***Farmers use bacterial weapons to enhance crop production***: Since the 1950s, antibiotics have been used in agriculture to help prevent the spread of crop-destroying bacterial infections. Antibiotics such as **streptomycin** and **oxytetracycline** are mixed with water and sprayed on crops over prolonged periods of time. Many scientists have argued that antibiotic use in food plant production should not be allowed because their use could contribute to the development and spread of antibiotic resistance among human pathogens that can also reside in and on crop plants. Although the use of antimicrobials in the food agriculture industry is only a fraction (~0.5%) of all the antimicrobials used in the United States, it can still contribute to the spread of antibiotic-resistant bacteria. For example, **fire blight** is a plant infection caused by the bacterium *Erwinia amylovora*, which is known to cause disease in rosaceous plants, including apple and pear plants all around the world. Streptomycin has been very effective in preventing fire blight, but streptomycin-resistant *E. amylovora* have begun to emerge over the last several decades. Antibiotic resistance can be hard to detect in crop plants before an entire field is destroyed, at which point the resistant strain is widespread. This happens because, at first, a population of bacterial pathogens contain only a few antibiotic-resistant cells, and the majority are sensitive to the treatment. However, when an infected crop is treated with antibiotics, resistant cells will survive and continue to cause infection. Over many cycles of antibiotic treatment, the antibiotic-resistant strain will eventually become the dominant strain type because it is not killed off by the antibiotic. At this point, crop farmers have no way to kill off the infection-causing bacteria and their food plants may be destroyed by infection.

This leads us to an important question: why can't crop farmers just start using a different antibiotic if streptomycin is no longer effective? Different antibiotics are more or less effective at killing types of bacteria, so crop farmers can't try just any antibiotic. In the case of fire blight, other antibiotics have not been as effective as streptomycin in protecting crops against *E. amylovora* infection. More importantly, the emergence of antibiotic resistance is outpacing the rate at which we discover new antibiotics. Most of the antibiotics we use today were discovered in the 1950s, however there is much potential for identifying new antimicrobials in bacteria that live in the soil,

as well as marine habitats. The spread of antibiotic resistant strains of *E. amylovora* and our limited antimicrobial resources might make a strong case for eliminating the use of antibiotics in plant crops. However, without them we would have no way of controlling fire blight infections in economically important food crops such as apples and pears. One counterargument is that because plant agriculture accounts for such a small percentage of global antibiotic use, eliminating antibiotics in agriculture would have little effect on the current antibiotic-resistance crisis in human medicine, but could be detrimental to the apple and pear industries.

7. ***Conservationists use microbial weapons to help diseased coral***: Corals are keystone species, which are organisms that help hold an ecosystem together by providing habitats for other organisms. Just like humans, pets, and plants, corals can contract diseases that can make them sick (see box 5), which can be detrimental to the health of the entire ecosystem. Similar to our human guts, corals maintain a multitude of beneficial symbiotic microbes including bacteria, algae, and archaea. However, exposure to **biotic stress** (living or biological) or **abiotic stress** (non-living chemical or physical factors) can cause an imbalance (dysbiosis, see box 2) as pathogens invade or the number of beneficial bacteria decreases. Indeed, global climate change is predicted to increase environmental stress for corals through increased temperatures, **ocean acidification**, storms, and pollution, just to name a few, and scientists are already observing an increase in coral disease throughout the world's oceans, due in part to a resulting dysbiosis.



Box 5. Image of *Pseudodiploria strigosa* (symmetrical brain coral) that appears to have Stony Coral Tissue Loss Disease (SCTLD), with healthy and diseased tissue indicated. The causative agent(s) of SCTLD is not yet known but it is widely thought to be a bacterial disease that can pass from one coral to another through water circulation and currents, and has a high mortality rate in hard corals in the Caribbean. Image taken in the Florida Keys, courtesy JP Rippe.

Coral diseases are challenging for scientists to study and treat because they often manifest in very similar ways, making it difficult to diagnose the exact cause in sick corals. It can also be difficult to determine whether a microbe is a pathogen or a symbiont simply by how a bacterial cell looks or its species identification because coral microbiomes are very complex, and are typically composed of hundreds of species of bacteria. To further complicate the matter, some microbes can act as opportunistic pathogens, where they do not harm the coral when it is healthy, but can become pathogenic when corals transition to a state of dysbiosis or are stressed. Similar to how certain regions of the human can be infected by a pathogen (ex. ear or throat infection), sometimes only one side of a coral may be diseased, while the other side may be healthy. Conservationists are now using antimicrobials to try and treat coral diseases. Marine biologists mix antibiotic treatments with epoxies and other sticky substances to treat diseased tissue on a coral colony, much like how medical doctors treat human skin wounds. These methods allow the antimicrobial to stay on the coral, rather than diffuse through the environment. Such treatments are showing promising results and scientists are acting quickly to implement them to save these important ecosystems from collapse.

8. ***Biotech and industry continue to look for new microbial weapons:*** Humans have used antimicrobials to treat disease for centuries. Before the development of modern pharmaceuticals, extracts from molds and plants were commonly used to treat infections. The first antibiotic, penicillin, was accidentally discovered by Dr. Alexander Fleming in 1928. He noticed that a green mold, *Penicillium notatum,* had contaminated his petri dishes and was killing the bacteria he was trying to grow. Eventually, scientists were able to grow, purify, and distribute penicillin, which changed the way we treat illnesses and wounds. Today, scientists have taken advantage of the natural products created by microbes and have developed additional methods to make synthetically- produced antimicrobials (produced by chemical synthesis).

To identify naturally-produced bacterial weapons, research scientists first identify bacteria that produce molecules with a possible antimicrobial function. This identification process can take years to complete, as many organisms have the capability to produce bacterial weapons but only make them under very specific growth conditions, which can be challenging to identify. When a new potential antimicrobial is found, it is tested against a range of organisms, such as known pathogens, to determine its potential therapeutic use to treat bacterial infections that cause disease in plants, animals, and humans. Many antimicrobials do not continue beyond this point to mass production because the new molecule may be similar to a product that is already available, or is not effective against the desired pathogens. If the antimicrobial appears to be novel, then the organism producing the compound is grown in large quantities in order to isolate the compound. Once the compound is isolated and identified, further testing is performed to determine whether it can kill bacterial pathogens in live plants and animals, and whether it is safe for the diseased plant or animal. To produce these compounds at large scales, scientists can continue to grow the natural producer in very large **bioreactors**, or they can engineer easy-to-grow bacteria to produce the compound, or biochemists can synthetically produce the compound using a series of chemical reactions to build the molecule one piece at a time.

Pupil Participation

1. ***Class discussion of the issues associated with competition and microbial weaponry.***

2. ***Pupil Stakeholder awareness***
    a. When you feel sick, do you ever take antibiotics to get better?
    b. Should you take an antibiotic if you have a viral infection?
    c. We have already documented the spread of antibiotic resistance in plant agriculture, although it makes up a small percentage of our overall antimicrobial use. Do you think that we should continue using antimicrobials on crops? Why or why not?

3. **Exercises**
    a. Finding new antimicrobials will be important to keep humans healthy. Can you think of ways we could try and discover new antimicrobials? Where might we look? How would we know if we found a new antimicrobial?
    b. Some antimicrobials will eliminate good bacteria along with the bad bacteria. What can you do to help your good bacteria after taking antibiotics to get rid of bad bacteria that are making you sick?
    c. Activity: Stopping Superbugs!

https://www.sciencebuddies.org/teacher-resources/lesson-plans/stopping-superbugs#lesson

The Evidence Base, Further Reading and Teaching Aids

CDC things do know about antibiotic resistance:
https://www.cdc.gov/drugresistance/about/5-things-to-know.html
WHO antibiotics used in healthy animals cause the spread of antibiotic resistance:
https://www.who.int/news-room/detail/07-11-2017-stop-using-antibiotics-in-healthy-animals-to-prevent-the-spread-of-antibiotic-resistance
Overuse of antibiotics in food animals and how to responsibly use antibiotics:
https://foodprint.org/issues/antibiotics-in-our-food-system/
The role of agriculture in preventing the development of antimicrobial resistance:
https://youtu.be/d3YXW_gWNz4
What causes antibiotic resistance:
https://www.youtube.com/watch?v=znnp-Ivj2ek
Penicillin: Who found this functional fungus
https://kidsdiscover.com/quick-reads/penicillin-found-functional-fungus
How products are made: Antibiotics
http://www.madehow.com/Volume-4/Antibiotic.html
Scientists are putting antibiotics into the ocean – on purpose. And it's our only hope:
https://www.popsci.com/coral-antibiotics-science/
Antibiotics for the Reef:
https://keysweekly.com/42/antibiotics-for-the-reef-bocc-hears-coral-disease-treatment-update/
About Stony Coral Tissue Loss Disease: http://www.perryinstitute.org/sctld/

Glossary

Microbe – microscopic organisms including bacteria, viruses, protists, and fungi

Microbial weapons – molecules produced by microbes that are used to kill or stop the growth of other competing microorganism
Diffusible weapons – molecules that kill other microbes by diffusing through the environment
Contact-dependent weapons – molecules or muilti-protein nano-structures that kill other microbial cells when physical contact is made between the producer and target cells
Secondary metabolites – Molecules that are produced by bacteria that are not required for basic cell growth
Antimicrobials – an agent (chemical or biological) that kills or stops the growth of microbes; an example of a diffusible microbial weapon
Porins – protein "channels" that allows molecules to cross the cell membrane
Antibiotics – medicines that fight bacterial infections in animals (including humans)
Bacteriocins – small peptides produced by bacteria with antimicrobial properties
Type VI secretion system – a nano-syringe made of many protein parts that can be used by bacteria to inject toxins into target cells; an example of a contact-dependent microbial weapon
Phage – viral particles that specifically infect and kill bacterial cells (also called bacteriophage)
Tailocins – a class of bacteriocins that resemble phage tails
Siderophores – molecules made by bacteria that are released into the environment to bind iron and transported back into the cell
Probiotic – live microbes that are beneficial to our bodies and are consumed to promote a healthy gut
Prophylactic antibiotics – medication taken to prevent a bacterial infection
Streptomycin – an antibiotic medication used to treat a variety of bacterial infections
Oxytetracycline– an antibiotic medication used to treat a variety of bacterial infections
Fire blight – the disease caused by a bacterial infection of certain fruit trees
Biotic stress – disturbances to living things that are from biological sources
Abiotic stress – disturbances to living things that are from non-living sources (ex. dramatic changes in the physical or chemical environment)
Dysbiosis – an imbalance in the microbes present in the gut resulting in disease
Ocean acidification – with increasing carbon dioxide in the atmosphere, more carbon dioxide is absorbed by seawater and the resulting chemical reactions reduces the pH of the seawater to make it more acidic
Bioreactor – a specially-built vessel that allow for growth of organisms to produce chemical substance to be purified for industrial or medical use